ISBN 978-86-82431-73-2

11th
INTERNATIONAL
SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

P R O C E E D I N G S

11[th] - 13[th] October 2017 - Belgrade, Serbia

ISBN 978-86-82431-73-2

INSTITUTE FOR ANIMAL HUSBANDRY
BELGRADE - SERBIA

11th
INTERNATIONAL
SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION

P R O C E E D I N G S

11th - 13th October 2017 - Belgrade, Serbia

Publisher
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Editor-in-Chief
Milan M. Petrović, PhD, Principal Research Fellow
Director of the Institute for Animal Husbandry, Belgrade-Zemun

Address of the Editor's office

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

Circulation 200 copies.

The publication of this Proceedings is sponsored by the Ministry of Education and Science of the Republic of Serbia.
The Proceedings is printed by the Institute for Animal Husbandry, Belgrade, 2017

ISBN 978-86-82431-73-2

PATRON

Ministry of Education, Science and Technological Development of the Republic of Serbia

ORGANIZER

Institute for Animal Husbandry
Autoput 16, P. Box. 23,
11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611; +381 11 2670 121;
+381 11 2670 541;
Fax: + 381 11 2670 164;

biotechnology.izs@gmail.com
www.istocar.bg.ac.rs

EDITOR

INSTITUTE FOR ANIMAL HUSBANDRY
For Editor - Milan M. PETROVIĆ, Ph.D
Editor in Chief - Zdenka ŠKRBIĆ, Ph.D

INTERNATIONAL SCIENTIFIC COMMITTEE

CHAIRMAN

Prof. Dr. **Martin Waehner,**
Anhalt. University of Applied Sciences, Bernburg, Germany

SECRETARY

Dr. **Milan P. Petrović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Milan M. Petrović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Giuseppe Bee,**
Agroscope Posieux, Posieux, Switzerland
Dr. **Vlada Pantelić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. **Dr. Yusup A. Yuldashbaev,**
Russian State Agrarian University, Moscow Timiryazev Agricultural Academy,
Faculty of Animal Science and Biology, Russia
Dr. **Vesna S. Krnjaja,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Giacomo Biagi,**
Department of Veterinary Medical Sciences, - University of Bologna, Italy
Prof. Dr. **Marjeta Čandek-Potokar,**
Agricultural Institute of Slovenia, Slovenia
Dr. **Zdenka Škrbić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. **Dr. Elena Kistanova,**
Institute of Biology and Immunology of Reproduction
„Kiril Bratanov", Sofia, Bulgaria
Prof. Dr. **Marina I. Selionova,**
FSBSI-All-Russian Scientific Research Institute of Sheep and Goat Breeding,
Stavropol, Russia
Dr. **Dragana Ružić-Muslić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **María Muñoz,**
Centro de I+D en Cerdo Ibérico, Dpto.
Mejora Genética Animal, INIA, Zafra, Badajoz, Spain
Prof. Dr. **Rui Miguel Carracha Charneca,**
Universidade de Évora, Escola de Ciências e Tecnologia,
Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM),
Évora, Portugal

INTERNATIONAL SCIENTIFIC COMMITTEE

ORGANIZING

CHAIRMAN

Dr. **Milan M. Petrović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SECRETARY

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Dr. **Dušica Ostojić Andrić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Zoran Ilić,**
Faculty of Agricultural Sciences, Lešak, Serbia
Dr. **Violeta Caro Petrović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Aleksandar Simić,**
Faculty of Agriculture, University of Belgrade, Serbia
Dr. **Violeta Mandić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Prof. Dr. **Milun D. Petrović**
University of Kragujevac, Faculty of Agronomy, Čačak, Serbia
Prof. Dr. **Slavča Hristov,**
Faculty of Agriculture, University of Belgrade, Serbia
Dr. **Aleksandar Stanojković,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Nikola Stanišić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Nevena Maksimović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Veselin Petričevič,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Dr. **Dragan Nikšić,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

SYMPOSIUM SECRETARIAT

CHAIRMAN

Dr. **Čedomir Radović,**
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

MEMBERS

Slavko Maletić, grad.econ.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Olga Devečerski, grad. prof.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Stanislav Marinkov, grad.arch.
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nikola Delić, DVM,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Marija Gogić, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Maja Petričević, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Marina Lazarević, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Nenad Mićić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Bogdan Cekić, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Miloš Marinković, MSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia
Vladimir Živković, BSc,
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

CONTENTS

INVITED PAPERS

ORALLY PRESENTED PAPERS

POSTER SECTION I

POSTER SECTION II

POSTER SECTION III

INVITED PAPERS

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

TRENDS AND CHALLENGES IN THE GENETIC IMPROVEMENT OF FARM ANIMALS

Milan P. Petrovic[1], Dragana Ružic Muslic[1], Violeta Caro Petrovic[1], Nevena Maksimovic[1], Bogdan Cekic[1], Yusup A. Yuldashbaev[3], Marina I. Selionova[2]

[1]Institute for Animal Husbandry, Belgrade, Serbia
[2] FSBSI -All-Russian Research Institute for Sheep and Goat Breeding, Stavropol, Russia
[3] Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Faculty of Animal Science and Biology, Moscow, Russia
Corresponding author: milanppet@yahoo.com
Review paper

Abstract: Animal breeding, in essence, dealing with the variable, primarily genetic, looks at, above all, the genetic improvement of populations. In many methods, it is about selecting the parents of the next generation of high production skills and well acclimatized with appropriate environmental conditions. With these methods, it is important to carefully collect all the more important information on the production capabilities of animals and their origin, and this is ensuring by keeping the basic birth record. Despite the desires of the breeder through history to improve the desirable properties of animals as soon as possible, the development of genetics in the twentieth century has opened the perspective of more effective genetic improvement. The development of molecular biology and DNA analysis methods over the last two decades opened the door to a new era of faster and more accurate selection of domestic animals. Aside from a favorable increase in production, animals in a population that have selected for high production efficiency seems more at risk for behavioral, physiological and immunological problems. Genetic selection may lead to loss of the homeostatic balance of animals, resulting in the occurrence of pathologies and consequently in impaired animal welfare. There are opinions that physiological and immunological features should be incorpotated in the selection programs, taking into account their correlation with the production characteristics. DNA technologies can be useful for identifying loci responsible for some physiological and immunological characteristics, or animal health.

Key words: genetic improvement, selection, animal, challenges, animal health

Introduction

The livestock sector globally is highly dynamic. In developing countries, it is evolving in response to rapidly increasing demand for livestock products. In developed countries, demand for livestock products is stagnating, while many production systems are increasing their efficiency and environmental sustainability. Historical changes in the demand for livestock products have been largely driven by human population growth, income growth and urbanization and the production response in different livestock systems has been associated with science and technology as well as increases in animal numbers *(Thornton, 2010).*

Genetic improvement of domestic animals is undoubtedly based on the basic genetics laws as the science of heredity and adaptation of species, because the characteristics of domestic animals are determined by their genotype that is subject to hereditary and adaptable variability (*Petrovic, 2000*). Animal breeding, in essence, dealing with the variable, primarily genetic, looks at, above all, the genetic improvement of populations. In many methods, it is about selecting the parents of the next generation of high production skills and well acclimatized with appropriate environmental conditions. With these methods, it is important to carefully collect all the more important information on the production capabilities of animals and their origin, and this is ensured by keeping the basic birth record (*Vidovic, 2013*). Bearing all this in mind, it is normal that the application of statistics in the field of biological sciences becomes more and more important. This is because this is a large number of data, i.e. Observation, farm and system of pyramidal model of selection and production of genetic changes from praded to commercial throat. All data from test, sighting or production needs to be agreed, assessed, attributed to a particular importance to individual influences, to direct the tests to an efficient path, as only useful solutions can be obtained. Despite the desires of the breeder through history to improve the desirable properties of animals as soon as possible, the development of genetics in the twentieth century has opened the perspective of more effective genetic improvement (*Petrovic et al., 2001, 2005, 2011). *The development of molecular biology and DNA analysis methods over the last two decades opened the door to a new era of faster and more accurate selection of domestic animals (*Pearson, 2006; Pennisi, 2007; Teneva and Petrovic, 2010; Petersen et al., 2013; Petrovic and Pantelic, 2015*). Improvement of production traits using DNA technology has been named "Genomic Selection".
However, aside from a favorable increase in production, animals in a population that have selected for high production efficiency seems more at risk for behavioral, physiological and immunological problems. Genetic selection may lead to loss of

the homeostatic balance of animals, resulting in the occurrence of pathologies and consequently in impaired animal welfare.

Regarding of Grandin (1999), several long-term selection studies using a variety of small animals have clearly shown that overselection for a single trait may have adverse or unexpected effects on other traits. Future application of modern reproduction and DNA-techniques in animal breeding may increase production levels even faster than at present, which may result in more dramatic consequences for behavioural, physiological and immunological traits (*Rauwa et al., 1998; Sbardella, and Gaya, 2010).*

Demand for livestock products in the future could be heavily moderated by socio-economic factors such as human health concerns and changing socio-cultural values. There is considerable uncertainty as to how these factors will play out in different regions of the world in the coming decades *(Thornton, 2010).*

The aim of this paper is to review the methods of genetic improvement of domestic animals, emphasize the importance of selection in animal husbandry and the consequences of its over-intensification. This would contribute to the development of awareness of the necessity of balancing between desires and possibilities, in order to preserve the biological balance and health of animals and humans in the future.

Genetic improvement by traditional selection

Changing traits in animals, to produce desired characteristic, have resulted in common food species that are now genetically different from their ancestors. After domestication, animals were selected in different environments and for different traits, leading to the modern breeds.

The basics of genetic improvement of domestic animals lie on changes in the frequency gene, the goal of which is to achieve more productive populations. In essence, this type of genetic improvement represents a man's deliberate action to favor or eliminate certain individuals because of their interests.

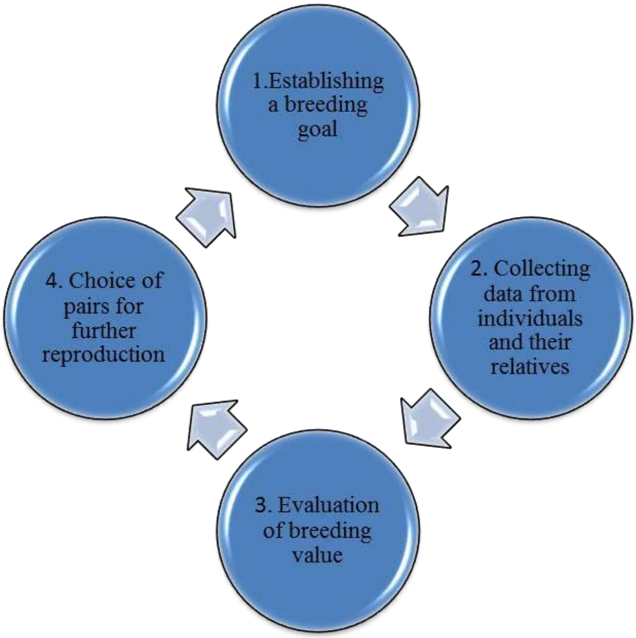
Selection plays an important role as a method for increasing productivity in modern animal breeding, since the selected individual of both sexes reproduces and gives the progeny of the desired type (*Petrovic et al., 2009; 2011; 2012; 2014; Petrović and Pantelić, 2015*).

Depending on the breeding goals, in the history of livestock breeding, until now, there have been various breeding programs. Breeding program is the combination of recording selected traits, the estimation of breeding values, the selection of potential parents and a mating program for the selected parents including appropriate (artificial) reproduction methods *(Oldenbroek and Van der Waaij, 2015).*

Most traits of interest in livestock are multifactorial in nature, affected by a large number of individual genes along with environmental factors. Quantitative genetic theory has become the primary basis for the development of methods to develop model- Quantitative Genetic Model, and evaluate alternative breeding programs (*Falconer and Mackay, 1996).*

Genetic improvement of animals by selection takes place in several major phases:

1. Establishing a breeding goal
2. Collecting data from individuals and their relatives
3. Evaluation of breeding value
4. Choice of pairs for further reproduction



The assessment of the breeding value is crucial for the success of the selection. Prediction of breeding values is a fundamental component of modern breeding programmes, as those with the highest values should be selected. The most important models for estimating breeding values, Best Linear Unbiased Prediction (BLUP), is due to *Henderson (1950, 1984)* and incorporates both fixed (environmental) effects and random (genetic) effects in a mixed model *(Lynch & Walsh 1998; Sorensen and Gianola 2002).*

Selection used the animals with the highest value for the selection criteria. Regardless of the fact that the selection reaches an optimal or selective limit, in livestock production, the trend of further increase in production traits continues with the introduction of new variability (*Vidovic, 2013; Petrovic and Pantelic, 2015).*

Genetic improvement by genomic selection

Traditional genetic improvement of livestock, using information on phenotypes and pedigrees to predict breeding values, has been very successful. However, breeding values should be able to predict more accurately by using information on variation in DNA sequence between animals (*Goddard and Hayes, 2007*).

Genomic selection - is a powerful tool for future use. Currently, the effectiveness of genomic selection is limited by various interactions between the locus of quantitative traits, the variability of quantitative features, and the influence of environmental factors. However, research results in many countries have confirmed that the use of standard statistical methods along with genomic selection increases reliability in predicting breeding values of animals (*Petrovic i Pantelic, 2015*). *Meuwissen et al., (2001*) proposed a novel approach where the breeding value could be estimated from markers spanning the entire genome.

Genomic selection should be able to at least double the rate of genetic gain in the dairy industry (*Hayes et al. 2009),* as it enables selection decisions to be based on genomic breeding values, which can ultimately be calculated from genetic marker information alone, rather than from pedigree and phenotypic information. Genomic selection is not without its challenges, but it is likely to revolutionize animal breeding.

In the last decade, low-and high-density genomic tools have been broadly used to study and characterize the genetic diversity and population. During the last 25 years, a number of Quantitative Trait Loci (i.e. regions of the genome responsible for a fraction of the genetic variance of a trait) have been mapped with genetic markers, paving the way to marker-assisted selection -MAS (*Boichard et al., 2016).* Despite progress, the MAS did not give the best results in the selection.

Reason of that is small numbers of QTL, and also the low association between markers and QTL at the population level.

Developing chips for genotyping over 50,000 single nucleotide polymorphisms (SNP) created a new era in the genomic selection of animals.

Genomic selection can have a major impact on animal breeding programs, especially where traits that are important in the breeding objective are hard to select for otherwise. Genomic selection provides more accurate estimates for breeding value earlier in the life of breeding animals, giving more selection accuracy and allowing lower generation intervals. From sheep to dairy cattle, the rates of genetic improvement could increase from 20 to 100 % and hard-to-measure traits can be improved more effectively (*Van Der Werf, 2013*).

According to *Boichard et al.(2016), g*enomic selection is a very recent innovation. Strong evolutions have started, including reduction in genotyping costs, phenotyping strategies for new traits, approaches for the creation or the replacement of reference populations, increase in robustness and persistency of genomic predictions using causal mutations identified from genome sequences, or genomic prediction of genetic-environment interactions.

In practice, genomic selection will be a reliable "prognosis" for livestock production and will accelerate the process of selecting the most valuable animals. *Van Der Werf (2013)* stated that reference populations for genomic selection need to be large, with thousands of animals measured for phenotype and genotype. The smaller the effective size of the breeding population, the larger the DNA segments they potentially share and the more accurate genomic prediction will be. The relative contribution of information from relatives in the reference population will be larger if the baseline accuracy is low, but such information is limited to closely related individuals and does not last over generations.

The advancement of molecular genetics has enabled the sequencing of the genome of several species of domestic animals in the last few years, partly or completely. Information on the whole animal genome becomes more interesting for researchers and breeders because they provide the ability to identify genetic variations that produce different production performances (*Bai et al., 2012*). This could also increase the chances of resistance to pathogens that slow down the production of animals and can also provide useful information in the production of healthy food for human consumption (*Bai et al., 2012*).

The first genome sequencing was done in poultry (*Burt, 2005*), followed by pigs (*Archibald et al., 2010*), cattle (*Zimin et al., 2009*), horses (*Wade et al., 2009*) and sheep (*International sheep Genomics, 2010, Bai et al 2012*).

Challenges and negative effects in genetic improvement

Negative effects of animal breeding can arise as a result of the desire to force only one trait. The experience in genetic improvement has shown that the selection does not only affect one trait but also changes some other features that are not planned. This phenomenon is a negative correlation response to the selection *(Van Eerden et al., 2004; Greer, 2008).* It is especially important that the results of this process can not be seen immediately, but gradually from generation to generation, which practically means that negative effects can be seen when the process is already moving away.

High variability in domestic animal populations is necessary both for the continuous genetic improvement of economically important traits, and for adapting changes in climatic conditions. Unfortunately, an increasingly intense selection method causes a trend in reducing genetic variability, both within and between the breeds. From this comes two problems:

1. Greater intensity of selection means the selection of relatively few animals for breeding. A small animal for breeding results in a greater number of inbreeding, and therefore a loss of genetic diversity.
2. Loss of small, local populations. Lower genetic potential and lower production are a key driver for the loss of small breeds, since they replace them with international high productivity breeds

This trend of genetic improvement of animals is critical for the long-term survival of small local breeds *(Biscarini et al., 2015).*

All living organisms and even domestic animals are complex biological systems. All molecular, biochemical and physiological processes interact with the inner and outer environment. In addition, most of the biological processes in the body are in a smaller or higher correlation. In order to better understand the effects of genetic improvement of domestic animals, it is necessary to bear in mind the so-called principle of homeostasis or balance, which implies self-regulatory return to the state before some changes in its composition or functioning have occurred.

For the purpose of genetic improvement of domestic animals, we are conducting the selection. If the selection is more intensive, the selection pressure is also greater. It should be known that strong selective pressure can interfere with the regular biological functions of the abilities, and the individuals can interact internally during breeding. This influence depends on this selection pressure and heritability of the selection criterion, and mainly depends on the genetic correlation between both properties *(Pereira, 1999).*

There is a different experience regarding the negative effects of the selection. According to *Ferreira et al.(2005)* Selection of high production impedes reproduction as well as cause Insufficient activity of metabolism Endangering welfare and health of animals. Therefore, a compromise between the level of production and animal health is necessary *(Van Eerden et al., 2004).*

Oldenbroek and Van der Waaij (2015) stated that selection for large offspring has resulted in a high fraction of difficult births, sometimes requiring caesarean sections in the Texel sheep, and even almost as a standard way of delivering in the beef cattle breeds Belgian White-and-Blue cattle and the Dutch Improved Red-and-White. In the Texel sheep selection against difficult births has resulted in a decreasing fraction of birth requiring assistance. In this case the process could be reversed. But in the Belgian White-and- Blue and the Dutch Improved Red-and-White cattle the situation is more problematic and the repair process will take many generations.

In selection of high milk yield of cattle production, animals have to be fed with a diet richer than their normal grass diet

in easily digestible nutrients with a lower content of effective fibres (*Rauwa et al., 1998; Ferreira et al., 2005).* However, this leads to disorders in food digestion. Their
physiology is not well adapted to this new diet, and they were not co-selected for physiological traits *(Rauwa et al., 1998; Sbardella and Gaya (2010).*

Selection of high production performance in all types of domestic animals leads to a reduction in the reproduction power *(De Vries and Veerkamp, 2000; Dechow et al., 2002; Norman et al., 2007).*

The progress made in dairy cattle in the selection of genes for productively desirable traits was not accompanied at the same speed by improvements in foot and leg stability (*Sbardella and Gaya, 2010).* According to *Madalena (2008),* selection for high milk production of cattle, associated with a reduced heat tolerance. Selection of the growth rate has a negative effect on the power of fertilization in pigs. *Robinson and Buhr (2005)* informed that, males with a higher growth rate are less able to mate a sow in oestrus than males with an intermediate growth rate, and the sperm quality is inferior in males with a bigger growth rate.

Animals that are selected on a high gain have weaker bones and here is a genetic association found between length of productive life and leg soundness score (*Serenius et al., 2006*). Selection for muscle deposition increase might be associated with the arising of the PSE (pale, soft, exudative) phenomenon in meat, which is caused by recessive homozygosis for the Halothane gene. Pigs with greater growth rate exhibit high incidences of gastric, cardiac, renal and pulmonary problems such as pneumonia and pleuritic (*Sbardella and Gaya, 2010).*

It has long been established that in poultry farming, the selection at high yields can lead to metabolic problems, reduce reproductive performance, and increase the number of infertile eggs *(Barbato et al., 1984).* Many health problems have been identified as a result of excessive selection and this has been the trend in all farm animal species.

Conclusions

Selection is an inevitable method for the genetic improvement of domestic animals. However, selection is also necessary in order to preserve the genetic structure of indoor populations, such as domestic animals. Without selection, all kinds of cultivated animals tend to return to their original "wild" form. The problem arises when the selection is used to achieve the maximalist demands of farmers and businessmen. In other words, when we want an animal to produce several times more milk than the logic of biological reality. For example, milk is a cubic food and a cow with a production of 10 liters a day can feed its calf. Through selection, a man got a cow that gives 5-10 times more milk because of business. It's similar to other animal species. Studies have shown genetic correlation Between productive traits and undesirable effects. In many cases selection for high productive efficacy in livestock species is accompanied by undesirable side effects for some physiological and reproductive traits, endangering the welfare of animals. There are opinions that physiological and immunological features should be included in the selection programs, taking into account their correlation with the production characteristics. DNA technologies can be useful for identifying loci responsible for some physiological and immunological characteristics, or animal health. In any case, the future of livestock farming must take into account the harmony between biological reality and the desire for genetic progress in order to achieve as much profit as possible.

Trendovi i izazovi u genetičkom unapređenju domaćih životinja

Milan P. Petrovic, Dragana Ružic Muslic, Violeta Caro Petrovic, Nevena Maksimovic, Bogdan Cekic, Yusup A. Yuldashbaev, Marina I. Selionova

Rezime

Odgajivanje životinja u suštini je bavljenje promenama, odnosno varijacijama proizvodnih osobina, prvenstveno genetskim, radi poboljšanja populacije. U mnogim metodama se radi o odabiru roditelja sledeće generacije visokih proizvodnih osobina i dobro aklimatizovanim na uslove sredine. Važno je pažljivo sakupljati sve bitnije informacije o proizvodnim potencijalima životinja i njihovom poreklu, a to se osigurava matična evidencija. Uprkos želji odgajivača kroz istoriju da poboljšaju poželjna svojstva životinja što je pre moguće, razvoj genetike u dvadesetom veku otvorio je perspektivu efikasnijeg genetskog unapređenja. Razvoj molekularne biologije i metoda analize DNK tokom poslednje dve decenije otvorio je vrata novoj eri brže i preciznije selekcije domaćih životinja. Pored povećanja proizvodnje, životinje u populaciji koja ima visoku efikasnost izložene su većim rizikom bihevioralne, fiziološke i imunološke prirode. Intenzivna selekcija može dovesti do gubitka homeostatske ravnoteže životinja, što dovodi do pojave patoloških problema, a time i smanjenja dobrobiti životinja. Postoje mišljenja da bi fiziološke i imunološke osobine trebale biti ugrađene u programe selekcije uzimajući u obzir njihovu korelaciju sa proizvodnim karakteristikama. DNK tehnologije mogu biti korisne za identifikaciju lokusa odgovornih za neke fiziološke i imunološke karakteristike, ili za zdravlje životinja.

Ključne reči: genetsko unapređenje, selekcija, životinja, izazovi, zdravlje životinja

References

ARCHIBALD A.I., BOLUND I., CHURCHER C., FREDHOLM C., GROENEN M.A., HARLIZIUS B., LEE K.T., MILAN D., ROGERS J., ROTHSCHILD M.F., UENISHI H., WANG J., SCHOOK L.B. (2010). Pig Genome Sequence – analysis and publication strategy. BMC Genomics, 11, 438.

BAI, Y., SARTOR, M., CAVALCOLI, J. (2012). Current status and future perspectives for sequencing livestock genomes. Journal of Animal Science and Biotechnology, 3(1), 1-6.

BARBATO G..F., SIEGEL, P.B., CHERRY, J.A., NIR, I. (1984): Selection for body weight at eight weeks of age. 17. Overfeeding. Poult. Sci., 63: 11-18.

BISCARINI F., NICOLAZZI E.L., STELLA A., BOETTCHER P.J., GANDIN G. (2015): Challenges and opportunities in genetic improvement of local livestock breeds. Front Genet., 6: 33.

BOICHARD D., DUCROC V., CROISEAU P., FRITZ S (2016): Genomic selection in domestic animals: Principles, applications and perspectives. C. R. Biologies 339 274–277.

BURT, D. W. (2005). Chicken genome: current status and future opportunities. Genome Research, 15(12), 1692-1698.
DE VRIES, M.J. AND VEERKAMP, R.F. (2000): Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci., 83: 62-69.
DECHOW, C.D., ROGERS, G.W. AND CLAYT, J.S. (2002): Heritability and correlations among body condition score loss, body condition score, production and reproductive performance. J. Dairy Sci., 85: 3062-3070.
FALCONER, D.S.; MACKAY, T.F.C. (1996): Introduction to quantitative genetics. Harlow, UK: Longman. xii, 438 p. p.
FERREIRA P.M., CARVALHO A.U., FACURY-FILHO E.J., FERREIRA M.G., FERREIRA R.G. (2005): Afecções do sistema locomotor dos bovinos. *Proceedings of 2nd Simpósio Mineiro de Buiatria*, Belo Horizonte, MG. 26p
GODDARD M.E., HAYES B.J. (2007): Genomic selection. J. Anim. Breed. Genet. 124 (2007) 323–330.
GRANDIN T., DEESING M.J., (1998**):** Genetics and Animal Welfare. Genetics and the Behaviour of Domestic Animals, GRANDIN T.(Editor) Academic Press, San Diego, California, pp 319-341(1998), with 1999 updates.
GREER A.W. 2008. Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. *Parasite Immunol.*, 30: 123-132.
HAYES B.J., BOWMAN P.J., CHAMBERLAIN A.J., GODDARD M.E. (2009). Genomic selection in dairy cattle: Progress and challenges. Journal of Dairy Science. 92, 2, 433–443.
HENDERSON C. R. (1950): Estimation of genetic parameters. Ann. Math. Stat. 21, 309–310.
HENDERSON C. R. (1984): Applications of linear models in animal breeding Guelph, Ontario: University of Guelph.
INTERNATIONAL SHEEP GENOMICS CONSORTIUM[1], ARCHIBALD AL, COCKETT NE, DALRYMPLE BP, FARAUT T, KIJAS JW, MADDOX JF, MCEWAN JC, HUTTON ODDY V, RAADSMA HW, WADE C, WANG J, WANG W, XUN X. (2010): The sheep genome reference sequence: a work in progress. Anim Genet., 41 (5):449-53.
LYNCH M., WALSH B. (1998): Genetics and analysis of quantitative traits Sunderland, MA: Sinauer Associates.
MADALENA, F.E. (2008):.Efeitos colaterais da genética de alta produção. In: Milk point.UFMG.[online]http://www.milkpoint.com.br/?noticiaID=42598andactA=7andareaID=61andsecaoID =171 (15-2-2008).
MEUWISSEN T.H., HAYES B.J,, GODDARD M.E. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics, 157(4),1819-1829.

NORMAN, H.D., HUTCHISON, J.L., WRIGHT, J.R., KUHN, M.T. AND LAWLOR, T.J. (2007): Selection on yield and fitness traits when culling Holsteins during the first three lactations. J. Dairy Sci., 90: 1008-20.

OLDENBROEK K.,VAN DER WAAIJ L (2015): Textbook Animal Breeding and Genetics for BSc students. Centre for Genetic Resources The Netherlands and Animal Breeding and Genomics Centre , 2015.

PEARSON H. (2006):"Genetics: what is a gene?". Nature, 441 (7092): 398–401.

PENNISI E. (2007): "Genomics. DNA study forces rethink of what it means to be a gene". Science 316 (5831): 1556–1557.

PEREIRA, J.C.C. 1999. Melhoramento genético aplicado à produção animal. Belo Horizonte: FEPMVZ.

PETERSEN J.L., MICKELSON J.R., RENDAHL A.K., VALBERG S.J., ANDERSSON L.S., AXELSSON J., ET AL. (2013): Genome-Wide Analysis Reveals Selection for Important Traits in Domestic Horse Breeds. PLoS Genet 9(1): e1003211. doi:10.1371/journal.pgen.1003211.

PETROVIC P.M., PETROVIC M.M., CARO PETROVIC V., ZUJOVIC M., RUZIC MUSLIC D., TOMIC Z., MAKSIMOVIC N. (2011): Development of Sustainable Livestock Production Systems in Serbia. *Proceedings* of The 3rd International Conference on Sustainable Animal Agriculture for Developing Countries. July 26-29,2011, Nakhon Ratchasima. Thailand, 2-6.

PETROVIC P.M., PANTELIC V (2015): Savremena selekcija domaćih životinja (Modern selection of farm animals). Institute for Animal Husbandry, Belgrade, 320 pp.

PETROVIC P.M., SRETENOVIĆ LJ., RUŽIĆ MUSLIĆ D., MEKIĆ C., MAKSIMOVIĆ N. (2009). The effect of the level of application of selection and breedeng criteria as factor of sustainable sheep production on productive traits of sheep in extensive rearing system. Biotechnology in Animal Husbandry, 25(1-2), 111-119.

PETROVIĆ P. M., CARO-PETROVIĆ V., RUŽIĆ-MUSLIĆ D., ILIĆ Z., SPASIĆ Z., STOJKOVIĆ J., MILENKOVIĆ M. (2012). Genetic and phenotypic aspects of the body measured traits in merinolandschaf breed of sheep. Biotechnology in Animal Husbandry, 28(4), 733-741.

PETROVIĆ P. M., MEKIĆ C., RUŽIĆ MUSLIĆ D., ŽUJOVIĆ M. (2005): Investigation Of Heritability and Genetic Corelation of Milk Sheep Traits. Biotehnologija u stočarstvu, 21, 55-60.

PETROVIĆ P. M., ŽUJOVIĆ M., NEGOVANOVIĆ D, STRSOGLAVEC S., RUŽIĆ D. (2001): The Importance Of New Selection Methods In Modern System Of Sheep Breeding. VI International Symposium “Systems of Animal Breeding of the new millennium”. Biotechnology in Animal Husbandry, 2001; 17 (5-6) 159-167.

PETROVIĆ P.M, PETROVIĆ M. M., RUŽIĆ MUSLIĆ D., CARO PETROVIĆ V., MAKSIMOVIĆ N., ILIĆ Z., VUČKOVIĆ S. (2011): Opportunities and challenges for sustainable sheep production in Serbia. *Proceedings* of the 3rd International Congress-New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, October 5-7. Biotechnology in Animal Husbandry 27 (3), p 463-472

PETROVIĆ P.M. (2000): Genetic and Improvement of Sheep (Book-Monography). Naučna knjiga, Beograd, 2000; 365 p.

PETROVIĆ P.M. (2000): Genetic and Improvement of Sheep (Book-Monography). Naučna knjiga, Beograd, 2000; 365 p.p.

PETROVIĆ P.M., CARO PETROVIĆ V., ILIĆ Z., RUŽIĆMUSLIĆ D., PETROVIĆ M.M., STOJKOVIĆ J., MAKSIMOVIĆ N. (2014). Relationship Between Birth Weight and Body Growth Characteristics of Lambs. Biotechnology in Animal Husbandry, 30 (2), 193-201.

RAUWA W.M., KANISB E., NOORDHUIZEN-STASSENC E.N., GROMMERSC F.J. (1998): Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Production Science 56:15–33.

ROBINSON, J.A.B., BUHR, M.M. (2005): Impact of genetic selection on management of boar replacement. *Theriogenology*, 63: 668-678.

SBARDELLA, M. AND GAYA, L.G. (2010): Unfavourable side implications of animal breeding in ivestock species: a review. zootécnicas: uma revisão. Arch. Zootec. 59 (R): 157-168.

SERENIUS T., STALDER K.J., BAAS T.J., MABRY J.W., GOODWIN R.N., JOHNSON R.K., ROBISON O.W., TOKACH M., MILLER R.K. (2006): National pork producers council maternal line national genetic evaluation program: A comparison of sow longevity and trait associations with sow longevity. Journal of Animal Science 84, 2590-2595.

SORENSEN D., GIANOLA D. (2002): Likelihood, Bayesian and MCMC methods in quantitative genetics New York, NY: Springer 2002, XVIII, 740 p.

TENEVA A., PETROVIĆ P.M. (2010): Application of Molecular Markers in Livestock Improvement. Biotechnology in Animal Husbandry, 26, 135-154.

THORNTON P.K (2010): Livestock production: recent trends, future prospects. Phil. Trans. R. SOC. B BIOL SCI., 365, 2853–2867.

VAN DER WERF J (2013): Genomic selection in animal breeding programs. Methods Mol Biol. 1019:543-61.

VAN EERDEN E., VAN DEN BRAND H., PARMENTIER H.K., DE JONG M.C., KEMP B. (2004): Phenotypic selection for residual feed intake and its effect on humoral immune responses in growing layer hens. *Poult. Sci.,* 83: 1602-1609.

VIDOVIĆ V., NEMEŠ Z., POPOVIĆ-VRANJEŠ A., LUKAČ D., CVETANOVIĆ D., ŠTRBAC L., STUPAR M. (2013): Heritability and correlations of milk traits in the view of kappa-casein genotypes in Vojvodina Holstein-friesian dairy cattle. Mljekarstvo 63 (2), 91-97.

WADE C. M., GIULOTTO E., SIGURDSSON S., ZOLI M., GNERRE S., IMSLAND F., et al. (2009). Genome sequence, comparative analysis, and population genetics of the domestic horse. Science, 326(5954), 865-867.

ZIMIN A., DELCHER A., FLOREA L., KELLEY D., SCHATZ M., PUIU D., et al. (2009). A wholegenome assembly of the domestic cow, Bos taurus. Genome Biology, 10(4), R42.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

ANIMAL BREEDING IN FRONT OF HIGHER GLOBAL NEEDS AND RESOURCE AND ENVIRONMENT PROTECTION

Martin Wähner

Anhalt University of Applied Sciences Bernburg, Germany
Corresponding author: martin.waehner@hs-anhalt.de
Review paper

Abstract: In near future there are a lot of expectations of global plant and animal breeding.
- More efficient use of available resources (area, water, fertilizer, etc.)
- Optimal use of solar energy for photosynthesis
- Minimize the content of unwanted ingredients in plants
- Effective conversion of feed into food of animal origin (milk, meat, fish, eggs)

High performance animals produce more excretions in absolute quantity but lower excretion per unit of products (milk, meat, eggs). Knowledge about the needs of the animals is essential for excretion.
The consequence can be a lower number of high performance animals. But in present time this strategy cannot be right as well in the industrialized countries as in the developing countries generally. There are very high variations in supply of foodstuffs between different countries all over the world.
Higher performance in animals correlates with animal health and higher manual and technical effort. In Germany, as a special example, the positive development of milk yield per cow correlates with higher number of cows per stock and intensity of manual effort per cow and day positively.

Keywords: farm animal global needs, animal production of meat, milk, eggs, high performance animals, production of excretions in absolute quantity and per unit of products, global supply of foodstuffs

Introduction

In 2050 about 9 billion people will live on the earth. It is 20% more than in current time. In front of this background there are a lot of expectations of global plant and animal breeding.

- More efficient use of available resources (area, water, fertilizer, etc.)
- Optimal use of solar energy for photosynthesis
- Minimize the content of unwanted ingredients in plants
- Effective conversion of feed into food of animal origin (milk, meat, fish, eggs)

The global people development is a challenge to agriculture. In addition to this development a changes in the eating habits of the population in the developing countries are to be expected (Figure 1).

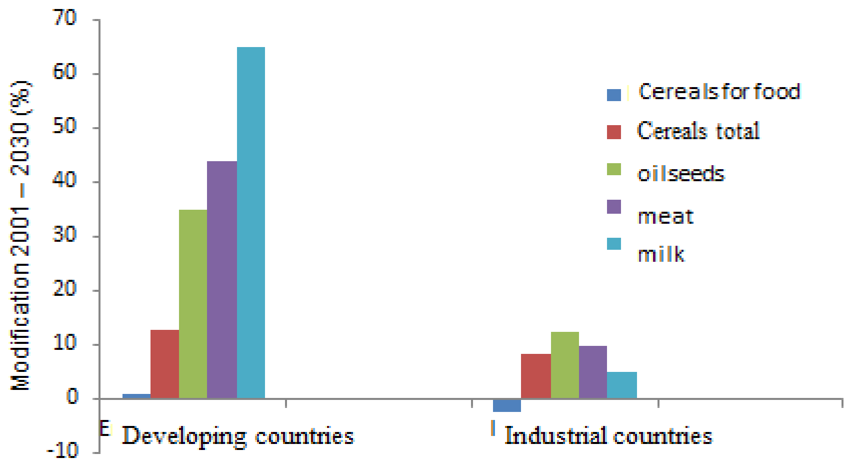


Figure 1. Expected change pn per capita consumption per inhabitant for specific food in developing and industrialized countries between 2001 and 20030 (IAAATD, 2007)

In front of this background there is a challenge for animal production or Livestock's long shadow (*Steinfeld et al., 2006*). Table 1 contains informations about this development.

Table 1. Challenge for animal production or Livestock's long shadow (STEINFELD et al., 2006)

	Currently (= 100%)	2050	growth (%)
People on earth (Billion)	6,5	9,0	138
meat production (Mio. t) Carcass weight	229	465	203
Milk production (Mio. t)	580	1043	130

Meat is is an important part in human diet. It is a preposition for more balanced nutrition (aminoacids, trace nutrients a.s.e), for higher hedonic value and meat represents social status. There is a closed correlation between income and meat consumption.

There is a very great global variability in the supply of animal protein. In table 2 extreme values are shown.

Table 2. Extreme values of the current protein supply (FAO 2009) and CF for milk (FAO, 2010)

	Minimum	Maximum
Milk (kg/ head and year) Meat (kg/head and year	3,3 (Burundi) 3,1 (Bangladesh)	367,7 (Sweden) 125,6 (USA)
Protein from animals (g/head and day	1,7 (Burundi)	69,9 (USA)
Carbon Footprints (kg Co/kg milk)	1,3 (Europe)	7,5 (Subsahara)

Generally, in past and in present time, the possession of farm animals demonstrated richnes.

Looking at the population development it is interesting to get informations about average consumption of raw materials for production of different foods (Table 3)

Table 3. Average consumption of raw materials for production of different foods

	Consumption of raw materials	
Foods	Water (l)	Area (m^2)
1 bun (50g)	70	0,1
1 glas milk (0,25 l)	100	0,2
1 grilled sausage (120g)	400	0,6

In recent years in the farm animals has a performance increase. This affects the milk output (Table 4), meat and eggs.

Table 4. Milk production in Germany

Year	Milk kg	Fat%	Protein %
1990	4.710	4,09	3,33
1995	5.427	4,25	3,40
2000	6.110	4,22	3,41
2010	7.085	4,16	3,42
2014	7.541	4,07	3,41

In Germany, the high levels of animal welfare and animal and environment protection lead to a reduction in the number of cows and dairy farms generally. Especially small farms will be closed. There is a contradiction between the political goal and the reality in dairy cattle farming. In Germany the small family farm agree on one with the political goal, but not the larger, specialized dairy farm. In front of this background following investigation is very interesting.

An investigation was carried out to determine the relationship between stock size and milk yield in Holstein cows. In figure 2 you can see a positive development of milk yield in farms with more cows per stock.

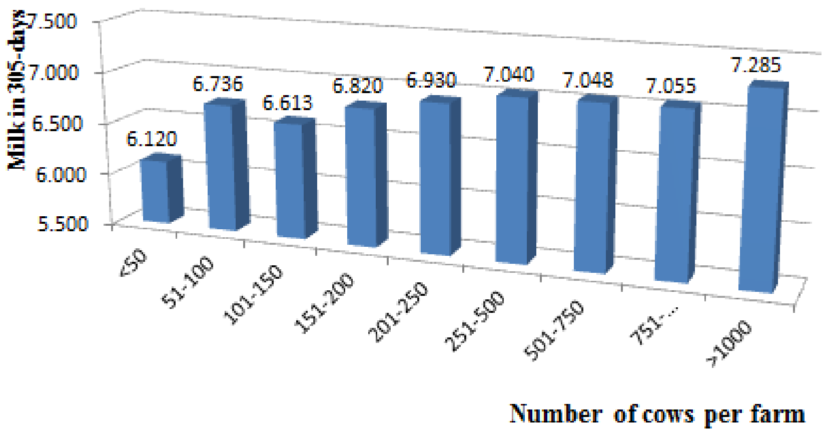


Figure 2. Milk performance in 305-days depend on number of cows per farm

Additionally an increase in livelihood in dairy cows in larger farms was observed (Figure 3).

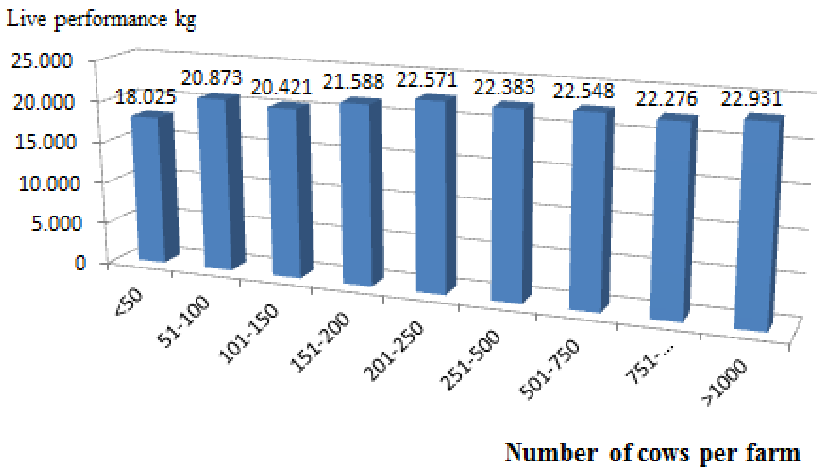


Figure 3. Milk production per life of cows depend on number of animals per farm

The better management, better health status and higher qualification of the persons in the larger farms are responsible for this development.

Very often the question is asked whether high performance correlates with lower animal health. Generally there are only low antagonism between milk production and traits for animal health (*Swalve, 1999*). Table 5 contains correlations between breeding values for milk production, somatic cell score and longevity.

Table 5. Correlations between estimated breeding values for black-and –white Holstein bulls (VIT-Proofs 8/1999; only bulls with birth years from 1989 onwards and with repeatabilities of EBV for RZM-Production, RZE-Type, RZS-Somatic Cells Score of >75% and >60% for RZN-Longevity; n=789 bulls) – SWALVE (1999)

	RZS (Somatic Cell Score)	RZE (Type)	RZN (Longevity)
RZM (Milk Production)	-0,11	0,21	-0,12
RZS (Somatic Cell Score)		0,15	0,39
RZE (Type)			0,07

The level of yield in plant production and animal production influences the need of arable land per people. In FAO-calculation (2006) is demonstrated influence of higher farm production in plants and animals on need arable land per person (Table 6).

Table 6. Influence of yield in plant production and performance in farm animals on requirement of arable land per inhabitant and year for production of milk and meat in 2050 (52 kg meat, 116 kg milk per inhabitant and year – FAO 2006)

Level of yield in plant production and performance in farm animals	Need of arable land/ people (m^2 / person)
2t DM cereals, 5t DM basic feed / ha 7,5 kg milk / cow & day, daily gain: meat cattle 500g; pig: 400g/day	1.500
4t DM cereals, 10t DT basic feed / ha 15 kg milk / cow & day, daily gain: meat cattle 900g; pig: 600g/day	600
8t DM cereals, 15t DM basic feed / ha 30 kg milk /cow & day, daily gain: meat cattle 1300g; pig: 800g/day	300

Higher performance of the animals results in increased excretion total, but in reduced excretion per unit with the consequence of a (limited) reduction in the number of animals. The effect of an increase in output on the issue decreases with increasing output. Disease incidence and expenses. Knowledge about the needs of the animals is essential for reducing excretion. The need for optimal ration design and feed additives can improve the nutrient utilization.

For climate relevant emissions in animal production are methane and nitrogen or laughing gas (N_2O). Here is the same situation. High performance cow produces more methane then low performance cow. But high performance cow produces less methane per unit milk then low performance cow (Figure 4).

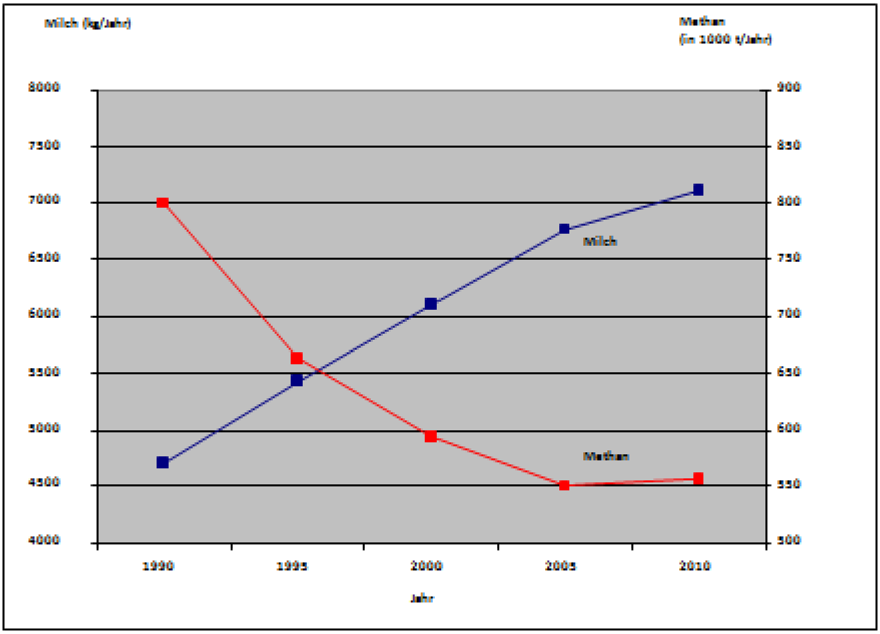


Figure 4. Development of production of methane and milk in Germany 1990-2010

Until now, there is a deficit of knowledge about potentials of feed additives to reduce methane production in the rumen.
There are some main topics for science.

- Reduction of the urine N-content of the N-excretions (scientific question!).
- Improved excrement management - reduction of the absolute gas emission, reduction of the gas concentration in the excrements
- Urgent improvement of the information situation of all climate-relevant emissions in the production of food - along the entire food chain.

New ways for the breeding of animals is shown by the genomic selection. Since the beginning of genomic selection, the increase in breeding progress has been estimated to be nearly 100%. The effect is based on the shortening of the generation interval. Functional features of cows and sows are particularly interesting. This also combines positive effects for robustness and health, life performance and longevity. In the end, this is very much important for the issue of "animal welfare".

Expections for plant and animal breeding are following. More efficient use of available resources (area, water, fertilizer a.s.e.). Optimal use of solar energy for photosynthesis. Minimize the content of unwated ingredients in plants and effective conversion of feed into food of animal origin (milk, meat, fish, eggs).

Conclusion

- Higher performance of the animals results in increased excretion but reduced excretion per unit with the consequence of a (limited) reduction in the number of animals.
- The effect of an increase in output on the issue decreases with increasing output. Disease incidence and expenses.
- Knowledge about the needs of the animals is essential for reducing excretion.
  The need for optimal ration design and feed additives can improve the nutrient utilization.
- Until now there is still lacking knowledge about the potential of feed additives to reduce methane production in the rumen.
- Reduction of the urine N-content of the N-excretions (scientific question!) Improved excrement management - Reduction of gases with a high concentration of gases
- Urgent improvement of the information situation of all climate-relevant emissions in the production of food - along the entire food chain.

Uzgoj domaćih životinja u svetlu rastućih svetskih potreba i zaštite resursa i životne sredine

Martin Wähner

Rezime

U bliskoj budućnosti postoji puno očekivanja od globalnog uzgoja biljnih i životinjskih vrsta.

- Efikasnije korišćenje raspoloživih resursa (zemljište, voda, đubrivo, itd.)
- Optimalno korišćenje solarne energije za fotosintezu
- Minimiziranje sadržaja neželjenih sastojaka u biljkama
- Efikasno pretvaranje hraniva u proizvode odn. Hranu životinjskog porekla (mleko, meso, riba, jaja)

Životinje sa visokim performansama proizvode više izlučevina u apsolutnoj količini, ali manje po jedinici proizvoda (mleko, meso, jaja). Poznavanje potreba životinja je neophodno u tom smislu.

Posledica može biti manji broj životinja sa visokim performansama. Ali u današnjem vremenu ova strategija ne može biti jednako ispravna i za industrijalizovane zemlje kao i za zemlje u razvoju. Postoje vrlo velike varijacije u snabdevanju prehrambenih proizvoda između različitih zemalja širom svijeta.

Veće performanse kod životinja su u korelaciji sa zdravljem životinja i većim ručnim i tehničkim naporima. U Nemačkoj, kao poseban primer, pozitivan razvoj odnosno povećanje prinosa mleka po kravi korelira pozitivno sa većim brojem krava po zapatu i intenzitetom rada po kravi i danu.

Ključne reči: globalne potrebe farmskih životinja, proizvodnja mesa, mleka, jaja, visoko proizvodne životinje, globalno snabdevanje prehrambenim proizvodima

References

FAO 2006, 2009, 2010: FAO Report

STEINFELD, H., GERBER, P., WASSENAAR, T., CASTEL, V., ROSALES, M., DE HAAN, C. (2006): Livestock's long shadow, FAO, Rome 2006.

SWALVE, H.H. (1999): Grenzen in der Zucht auf Milchleistung – Aus der Sicht der Züchtung. Züchtungskunde71, (6), 428 – 436.

DIGITAL IMAGE ANALYSIS FOR PREDICTION CARCASS WEIGHT OF SOME BEEF CATTLE BREEDS

Yalcin Bozkurt[1], Nazire Mikail[2], Cihan Dogan[1]

[1]Suleyman Demirel University, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey, 32260.
[2]Siirt University, Department of Animal Science, Faculty of Agriculture, Siirt, Turkey
Corresponding author e-mail:yalcinbozkurt@sdu.edu.tr
Original scientific paper

Abstract: This research aimed to develop prediction models for accurate estimation of performance and carcass measurements of beef cattle grown in feedlot beef system by using Digital Image Analysis (DIA). For this purpose, 37 animals were used in total and composed of 20 animals of the Brown Swiss breed and 17 animals of the Holstein breed with the age of about 4-5 months at the beginning of the experiment. Animals were fed the same dietary rations throughout the experimental period of 12 months. When the animals reached 500-550 kg bodyweights, they were slaughtered. Associations between carcass weights (CW) and some carcass measurements such as, carcass length (CL) and carcass depth (CD) were examined for prediction ability. The digital images of each live animal were taken and the same parameters digital carcass length (DJCL) and digital carcass depth (DJCD) were also determined from the images, using the data with 137 observations for each trait. Then, prediction models were developed by DIA.
The linear, quadratic and cubic regression models were performed to predict CW for both breeds and since there were no statistically significant differences ($P > 0.05$) in carcass measurements between breeds. Therefore, the data of these breeds were combined and found that DJCL would be the best possible traits in predicting CW (R^2=73.6, 81.3 and 83.5% for linear, quadratic and cubic terms respectively). All type of regressions showed that addition of quadratic and cubic terms contributed little benefit in predicting CW. Therefore, all linear terms of all digital carcass measurements by DIA were considered for analysis and they were significant and R^2 values for equation containing both DJCL and DJCD were 76%. However, DJCD was poorly performed in estimates with R^2 only 4.6%. It can be concluded that in cases where CW is not measurable, it can be predicted correctly by measuring DJCL and different models with different parameters such as digital carcass area may be needed to predict CW in different nutritional and environmental conditions.

Key words: prediction, carcass weight, carcass measurements, digital image analysis

Introduction

The decisions on agricultural activities are first and foremost dependent on trials and errors at the level of small agriculture in developing countries, particularly those characterized by small scale farming poor resources and investments. Ration preparations are based on the body weight of the animal and are particularly important for predicting body weight, including growth rate, body condition and conformation, especially for bovine body measurements for many purposes *(Wilson et al., 1997; Fourie et al., 2002)*

Estimation of meat products has been the focus of many studies in developed countries. The value of cattle cut obtained from the carcasses of different breeds of cattle is very important for marketing standards that emphasize the quality, uniformity and consistency of both consumers and meat. For this reason, an evaluation procedure for predicting the weight and yield of carcass and beef retail segments is of great importance for the beef industry *(Cross and Belk, 1994).*

In General, animal marketing among farmers is based on visual evaluation, especially in developing countries. Most veterinary medicines are prescribed according to live weight criteria. However, prescribing and prescribing drugs are often made with approximate estimates. The use of ration formulation, drug estimation, body condition score, and live weight criteria in marketing requires advanced facilities that are expensive and cost-effective for many small-scale farmers.

If scientists appreciate the importance of correctly estimating the body weight of an animal, then a simple and logical technique should be considered in management decisions. Some studies have indicated a relationship between some body measurements and body weight (*Peters and Ball, 1995; Nesamvuni et al., 2000*). It is also important to know the body weight of cattle, especially for various reasons related to breeding for selection, nutrition and health services.

The results of the most studies have recognized that the accuracy of estimating body weight from heart girth or other body traits that may be affected by breed, type, age, size and condition of the animal (*Heinrichs et al., 1992*) and as well as by different environmental conditions *(Enevoldsen et al., 1997).*

Therefore, the aim of this study was to gain further information about the relationship between carcass weight and some digital carcass measurements of different breeds such as Brown Swiss and Holstein cattle and also to determine the value of using one digital carcass measurement as a single variable entry to the model to predict carcass weight and to validate the potential of this method as a means of predicting carcass weight under small scale farming conditions by using DIA.

Materials and Methods

Animals

The animals carcass used in this study were composed of 20 Brown Swiss and 17 Holstein cattle and 37 animals were used in total, divided into two groups on the basis of breeds. The average carcass weights were 246 and 262 kg for Brown Swiss and Holstein groups respectively. The digital images of various measurements were collected using a digital camera (canon) and a reference card to eliminate the distance between the object and the camera. Data were collected starting from December 2012 to March 2013 from the animals experimented. The carcass measurements of the slaughtered cattle were collected at Gulkoy slaughterhouse near Isparta province. A total of 37 observations were used for each trait measured. Carcass weights were recorded to the nearest kilogram (kg) and the digital carcass measurements in centimetre (cm).

Digital Carcass Measurements

Digital images and digital carcass measurements were taken by the same person throughout the experimental period to avoid the experimenter error in measuring the digital parameters which are as follows:

1- Digital Carcass Length (DJCL) - was the distance from the point of the shoulders to the ischium; in other words, from the sternum (manubrium) to the aitchbone (*tuber ischiadicum*),

2- Digital Carcass Depth (DJCD) - from sternum area immediately caudal to the forelimbs to top of the thoracic vertebra.

Statistical Analysis

1. The best prediction equations for carcass weight as independent variables, including DJCL and DJCD were determined. Descriptive statistics regression analysis of CW on each of the independent variables was performed using the General Linear Models procedure of Minitab, 16 Inc. (Minitab, 2016).

Correlation coefficients were also obtained between digital carcass traits. Polynomial regression analysis of carcass weight on DJCL and DJCD were performed.

Linear, quadratic and cubic effects of independent variables on CW were included in the following model:

$y_i = b_0+b_1X_i+b_2X_i^2+e_i$

Where
y_i= CW observation of an i' th animal,
b_0= intercept, b_1 and b_2 = corresponding linear, quadratic and cubic regression coefficients I,
X_i = Digital carcass measurement (DJCL, DJCD) and
e_i = residual error term
Several different regression analyses were conducted;
1- Two digital carcass measurements, expressed as linear functions, were combined in CW prediction equation
2- Each digital carcass measurement was included separately in regression analysis as linear, quadratic and cubic expressions to predict CW; and
3- The linear regression of each other digital carcass measurement was then also added to the model as described previously.

Results and Discussions

There were no statistically significant differences in digital carcass measurements between breeds (P >0.05). Therefore, data of these breeds were combined for all statistical analysis.

Descriptive statistics of carcass weight and digital carcass traits are shown in Table 1.

Table 1. Descriptive statistics of carcass weight and digital carcass traits by weight means

Weight Means (s.e)	CW (kg)	DJCL (cm)	DJCD (cm)
	253.85 (4.59)	167.89 (1.60)	68.62 (2.75)

CW: Carcass Weights, DJCL: Digital Carcass Length, DJCD: Digital Carcass Depth, s.e: Standard Error

The average values for combined CW 253.85 kg. The corresponding ranges for DJCL and DJCD were 167.89 cm and 68.62 cm respectively.

Regressions models of animal carcass weight on various digital carcass measurements using individual observations are shown in Table 2.

Table 2. Prediction equations of carcass weight and the linear effects of digital carcass traits

Models with two variables	R^2 %
CW = -161+2.36DJCL+0.255DJCD	76
Models with one variable	
CW = - 148 + 2.393 DJCL	73.6
CW = 230 + 0.349 DJCD	4.6

Regression models of animal carcass weight on various digital carcass measurements using individual observations are shown in Table 2. As Table 2 shows models with one variable with determination coefficients it was found that DJCL would be the best possible traits in predicting CW (R^2=73.6%) the other digital carcass measurement. In other words, the R^2 values in the models with one predictor shows the proportion of variation in the dependent variable that is predictable from the independent variable. Therefore, in this study 73.6% of the variation in CW can be explained by DJCL.

It was observed that in every steps of regression analysis, inclusion of DJCL in the equation increased R^2 greatly. It was also found that when all variables were included in the regression DJCD was not significant while the rest gave significant slope values.

However, the highest R^2 values were obtained from the equation contained two digital carcass traits that included DJCL and DJCD (R^2=76 %). These results were in line with the findings of *Tuzemen et al. (1993), Ulutas et al. (2001), Bozkurt et al. (2007), Bozkurt et al. (2008).*

In addition, in this study the individual equations with one predictor CD had the lowest R^2 values as 4.6% (Table 2).

Results of regression analysis of carcass weight on the linear, quadratic and cubic effects of each digital carcass measurement are presented in Table 3.

Table 3. Regressions of carcass weight on the linear, quadratic and cubic effects of each digital carcass measurement[#]

Measurements	Model	Intercept	b_1	b_2	b_3	R^2 %
Digital Carcass Length (DJCL)	Linear	-148	2.39	-	-	73.6
	Quadratic	-1402	18.12	-0.04898	-	81.3
	Cubic	9319	-185.4	1.231	-0.00267	83.5
Digital Carcass Depth (DJCD)	Linear	230	0.349^{ns}	-	-	4.6
	Quadratic	-154.9	8.625	-0.03683	-	34.6
	Cubic	-1328	53.01	-0.5572	0.001786	38.2

[#]Only none significant regression coefficients had superscripts (ns), the rest were significant at P<0.05.

It was observed in this study that a 1 cm change in DJCL and DJCD resulted in almost 2.4 and 0.35 kg change in carcass weight respectively (Table 3).

Higher order polynomial equations were examined. The R^2 values from the regression models indicate that digital carcass length to be the most highly related to carcass weight considering all linear, quadratic and cubic coefficient terms. For all digital carcass traits, addition of the cubic term increased the R^2 slightly.

However, while all linear, quadratic and cubic terms of DJCL and DJCD were significant (P <0.05); only the linear terms of DJCD was not significant (P >0.05). However, *Heinrichs et al. (1992)* reported that none-significant cubic term for heart girth and significant term for wither height. In contrast *Heinrichs et al. (1992)* found that quadratic term of body length was significant. The results in this study also showed that linear, quadratic and cubic expressions of DJCL is the most useful predictors, and support the findings of *Wilson et al. (1997), Bozkurt (2006), Bozkurt et al. (2007) and Bozkurt et al. (2008).* These results were in line with *Heinrichs et al. (1992), Wilson et al. (1997), Ulutas et al. (2001), Bozkurt (2006), Bozkurt et al. (2007)* and *Bozkurt et al. (2008).*

It can be noted that, in the correctness of the carcass weight estimates, the additional digital carcass measurements of the equations provide a slight increase except DJCL alone. Correlation coefficients of the traits are shown in Table 4.

Table 4. Pearson correlations between digital carcass traits in both breed cattle

Variables	CW	DJCL
DJCL	0.86	
DJCD	0.22	0.07

Correlation value of DJCL was found to be statistically significant (P <0.05); but DJCD was found none significant (P >0.05). Amongst two the digital carcass measurements, the highest correlation was found between DJCL and CW (r=0.86). The second highest correlation was between DJCD and CW (r=0.22). In addition the correlation value between DJCL and DJCD (r=0.07) was lower than the correlation between the rest of the digital carcass traits. It was expected that DJCL would give higher correlation coefficient value than the other digital carcass measurements since the R^2 value between BW and DJCL was also high.

Conclusions

This study also indicated that digital carcass length can be used with high precision in predicting the carcass weight for Brown Swiss and Holstein cattle raised under small-scale farming condition. Digital carcass length exhibited the highest correlation with the carcass weight of the digital carcass traits examined.

When using any of the other carcass measurements in models with linear, quadratic, and cubic terms, DJCL usually makes the most significant contribution when compared to other carcass traits. DJCD can be considered as the second best predictor.

In conclusion, the use of digital carcass length provides a simple way of estimating the carcass weight. This is the general aim of applying the model into practice. However, there is always a need to develop different models in order to define different model parameters besides the investigations in this area as well as other breeds, and to predict the carcass weight in different management and environmental conditions. It is also important to be able to measure carcass dimensions to reduce experimental errors.

Analiza digitalnih slika za predviđanje mase trupa kod nekih tovnih rasa goveda

Yalcin Bozkurt, Nazire Mikail, Cihan Dogan

Rezime

Cilj ovog istraživanja bio je da se razviju modeli predviđanja za tačnu procenu performansi i merenje trupova tovnih goveda gajenih u sistemu tovilišta koristeći Digital Image Analisis - DIA (Analiza digitalnih snimaka). Za ovu svrhu ukupno je korišćeno 37 životinja, od kojih 20 grla braon švajcarske rase i 17 životinja rase holštajn, starosti od oko 4-5 meseci na početku eksperimenta. Životinje su hranjene istim obrokom tokom eksperimentalnog perioda od 12 meseci. Kada su dostigle 500-550 kg telesne težine, goveda su zaklana.

Veze između mase trupa (CW) i nekih merenja trupa, kao što su dužina trupa (CL) i dubina trupa (CD) su ispitane za mogućnost predviđanja. Napravljene su digitalne slike svake žive životinje, i isti parametri digitalne dužina trupa (DJCL) i digitalne dubine trupa (DJCD) takođe su određene iz slika, koristeći podatke sa 137 opservacija za svaku osobinu. Zatim, su razvijeni modeli za predviđanje pomoću DIA.

Linearni, kvadratni i kubični regresioni modeli izvedeni su da predvide CW za obe rase, pošto nije bilo statistički značajnih razlika (P> 0,05) u merenjima trupa između rasa. Zbog toga su podaci o ovim rasama kombinovani i ustanovljeno je da će DJCL biti najbolja moguća osobine u predviđanju CW (R^2 = 73,6; 81,3 i 83,5% za linearne, kvadratne i kubične termine). Sve vrste regresija pokazale su da dodavanje kvadratnih i kubičnih termina nije imalo koristi u predviđanju CW. Zbog toga su svi linearni izrazi svih digitalnih merenja trupa pomoću DIA razmatrani za analizu i bili su značajni, a R^2 vrednosti za jednačinu sa DJCL i DJCD-om su bile 76%. Međutim, DJCD je imao slabe rezultate u procenama sa R^2 od samo 4,6%.

Može se zaključiti da u slučajevima kada CW nije merljiv, može se pravilno predvideti merenjem DJCL, a različiti modeli sa različitim parametrima, kao što je područje digitalnog trupa, mogu biti potrebni za predviđanje CW u različitim nutritivnim uslovima i okolnostima.

Ključne reči: predviđanje, masa trupa, merenja trupa, digitalna analiza slike

Acknowledgements

This study was presented here as a part of project number 111O269 and project number 114O778 and the authors thank TUBITAK (The Scientific and Technological Research Council of Turkey) for financially supporting these projects.

References

BOZKURT Y. (2006): Prediction of body weight from body size measurements in Brown Swiss feedlot cattle fed under small-scale farming conditions. Journal of Applied Animal Research, 29-32.
BOZKURT Y., AKTAN S., OZKAYA S. (2007): Body weight prediction using Digital Image Analysis for slaughtered beef cattle. J. Appl. Anim. Res. 32:195-198.
BOZKURT Y., AKTAN S., OZKAYA S. (2008): Digital Image Analysis to predict carcass weight and some carcass characteristics of beef cattle. A. Journal of Animal and Veterinary Advances, 3(3):129-137.
CROSS, H.R., BELK, K.E. (1994): Objective Measurements of Carcass and Meat Quality, Meat Science, v.36, pp. 191–202
ENEVOLDSEN C., KRISTENSEN T. (1997): Estimation of body weight from body size measurements and body condition scores in dairy cows. J. Dairy Sci., 1988-1995.
FOURIE P.J., NESER F.W.C., OLIVIER, J.J., VONDER WESTHUIZEN C. (2002): Relationship between production performance visual appraisal and body measurements of young Dorper rams. S. Afr. J. Anim. Sci., 256-262.
HEINRICHS A.J., ROGERS G.W., COOPER J.B. (1992): Predicting body weights and height in Holstein heifer using body measurements. J. Dairy Sci., 3576-3581.
Minitab, 2016. Minitab User’s Guide. Release 13 for windows. Inc.

NESAMVUNI A.E., MULAUDZI J., ROMANYIMI N.D. TAYLOR G.J. (2000): Estimation of body weight in Nquni-type cattle under communal management conditions. S. Afr. J. Anim. Sci., 97-98.
PETERS A.R., BALL P.J.H. (1995): Reproduction in Cattle. Butterworths.London.
TUZEMEN N., YANAR M., AKBULUT O., OCKERMAN H.W. (1993): Prediction of body weights from body measurements in Brown Swiss calves reared in the eastern region of Turkey. World Review of Anim. Prod., 50-54.
ULUTAS Z., SAATCI M., OZLUTÜRK A. (2001): Prediction of body weights from body measurements in east Anatolian red calves. Ataturk Unv. Ziraat Fak. Dergisi, 61-65.
WILSON L.L., EGAN C.L., TEROSKY T.L. (1997): Body measurements and body weights of special-fed Holstein veal calves. J. Dairy Sci., 3077-3082.

ASSOCIATION OF *GHR* GENE POLYMORPHISMS ON BEEF CARCASS QUALLITY

Ante Ivanković, Valentino Držaić, Jelena Ramljak, Miljenko Konjačić

Department of Animal Science and Technology, University of Zagreb Faculty of Agriculture, Svetošimunska 25, 10 000 Zagreb, Croatia
Corresponding author Ante Ivanković, e-mail: aivankovic@agr.hr
Original scientific paper

Abstract: In last decades selection in cattle breeding use genomics and direct selection to reveal preferred variants of polymorphic genes to a significant extent. In competitive beef production an important factor is the optimization of growth dynamics, quality carcasses production and meat rich in flavor. The aim of this research was to determine the allele variants of the *GHR* gene in the population of fattening beef and their correlation with the growth characteristics, carcasses and meat quality. In the studied population, dominance of the allele *A* variant (0.588) of the *GHR* gene was determined. Correlation between *GHR* gene allele variants with growth characteristics, slaughter weight, hot and cold carcasses weight, and dressing percentage was not determined. Significant relation was observed between *AA* genotype of *GHR* gene with a higher fat content ($p<0.01$) and a lower content of muscle tissue ($p<0.05$). Also, significant correlation between *A* allele with the body fat coverage ($p<0.05$) was observed. The results suggests on potential of the *GHR* gene polymorphism in the immediate cattle selection assisted by genetic markers. Favouring the allele *G* of *GHR* gene, it is possible to reduce the proportion of fat tissue in young bulls and increase the proportion of muscles. It is desirable to continue monitoring allele variants effects of the *GHR* gene on phenotypic characteristics and interaction with other candidate genes.

Key words: polymorphism, *GHR* gene, carcass quality, beef quality

Introduction

Sustainable beef production is conditioned with genetic basis and numerous non-genetic factors which affects growth dynamics, body conformation and meat quality. Consumers, especially in economically developed countries, put greater emphasis on meat quality, its origin and respect the principles of good agricultural practice in production technologies. *Koknaroglu et al.* (2005) suggests that fattening performance and profitability are quite complex and are affected by housing type, season, initial weight, concentration level, sex, and pen cattle population. Inherited combinations of genes considerably determine the growth, conformation and carcass efficiency, and nutritive meat quality.

Therefore, increasing of the competitiveness in beef production is possible by adjusting non-genetic factors and production technologies (*available resources*) and selecting favorable genotypes and alleles of desirable genes. Cattle breeds and genotypes for meat production are significantly different in their dynamics of growth, development and accumulation of tissue, expected optimal carcass weight, dressing percentage and other important characteristics. Optimal slaughter ages and final weights vary widely among cattle breed types (*Alberti et al., 2008*). Improvement of the genetic characteristics of bovine breeds for meat production is carried out by selection measures, primarily using genetically more favorable variants. By the nineties of the twentieth century, cattle selection was based on the achievements of population genetics, measurement of phenotypes and using individuals of better breeding values. The development of molecular genetics has enabled direct insight into the gene structure, understanding their expression on proteins, and interaction of genetic variants with some of phenotype characteristics. Current selection methods in cattle breedig use precision genotyping of individuals at the nucleotide level using high-resolution HD Chip's (*genomic selection*) to a great extent. Also, a whole set of candidate genes has been noted with indications of direct interaction with fattening and/or dairy cattle phenotype. Observed gene polymorphisms together with genomic selection (SNP Chip methodology) can be utilized in genetic improvement of cattle production characteristics.

One of the candidate gene for which it is assumed to play a significant role in cattle metabolism is the gene encoding the growth hormone receptor (*GHR*, Gen Bank Acc.No: AF140284, *Ge et al., 1999*). *GHR* gene is in direct interaction with cattle growth hormone and thus affects the metabolic processes and growth of the organism (*Di Stasio et al., 2005; Waters et al., 2011*). *Ge et al. (1999; 2000)* determined on the chromosome 20 in codogenic sequence of the *GHR* gene several polymorphic positions. In the exon 10 there are four nucleotide (*SNP*) substitutions at positions 76 (T/C), 200 (G/A), 229 (T/C) and 257 (A/G). The polymorphism at position 257 in exon 10 induced serine/glycine substitution at protein position 555 (S555G) of the *GHR* gene (*Di Stasio et al., 2005*). The S555G polymorphism has been associated with performance traits (*Di Stasio et al., 2005; Sherman et al., 2008; Waters et al., 2011*) and meat quality (*Reardon et al., 2010*). *Ardicli et al. (2017)* did not find association between the *GHR* polymopphysms on 257 position and carcass/fattening performance traits. Since some research has point on potential influence of *GHR* gene polymorphism on cattle growth dynamics and meat quality, the aim of the study was to determine the polymorphism of the *GHR* gene in the fattening beef population and correlation of *A* and *G* allele variants with growth characteristics.

Material and methods

The research included sample of forty animals of both sexes (23 young bulls and 17 heifers). Given the genetic profile of fattening animals, fifteen (15) individuals were Simmental (11♂, 4♀) and twenty-five (25) were crosses of Simmental x Holstein (12♂, 13♀). Calves are weighted after calving and next day were placed in individual boxes where they were kept for two weeks. From the third to the tenth week, calves are placed in group boxes. Up to 10 weeks they were fed with liquid feed (milk substitute up to 6 L/day), hay and concentrate (up to 2 kg/day), and from eleven weeks with forage (silage, hay, concentrate). Water was available *ad libitum*. Calves old 16 to 18 weeks were moved to farm where they were kept until the end of the fattening period under same accommodation and feeding conditions, and in group boxes with ten animals. Young bulls and heifers are kept in separate fattening facilities. During the completely fattening period, animals were fed with a total mixed ratio (TMR). The average meal consisted of maize silage (~ 32% dry matter, DM), maize of high humidity (~ 35% DM), concentrate (34% crude protein, CP) and straw in mass ratio of 45: 40: 10: 5. The TMR meal was available for the whole day. Transportation of animals to authorized abattoirs, slaughter, processing and cutting of carcasses were carried out according to standard procedure. After the primary processing on the warm carcasses EUROP classification of conformation (E, U, R, O, P) and estimation of the coverage of the carcasses with fat tissue (score from 1 to 5) was made. Warm carcasses are weighed and placed in the cooling chambers (24 h/+ 4ºC) and then weighed again to determine the mass of the cold carcass and the cooling loss. On the rib eye area (between the 10th and 12th ribs), dissection of muscle, bone/cartilage and fatty tissue was performed to estimate their ratio in carcass.

From each individual tissue sample was taken for isolation of DNA under manufacturer's protocol (Sigma-Aldrich, USA). Using the oligonucleotide primers 5'-GCTAACTTCATCGTGGACAAC-3' and 5'-CTATGGCATGATTTTGTTCAG-3', a DNA strand length of 342 base pairs was multiplied. Polymerase Chain Reaction (PCR) was performed according to the manufacturer's protocol (Takara Bio Inc., Otsu, Shiga, Japan) in a total volume of 15 µL including 1.2 µL of genomic DNA, 7.5 µL EmeraldAmp® MAX HS PCR Master Mix, 0.45 µL of each oligonucleotide primers and 5.4 µL of water. Multiplication of the sequence involved activation of the Taq polymerase (98ºC/3 min), 35 cycles for multiplying DNA sequence (98ºC/10 s, 53ºC/30 s, 72ºC/50 s) and its final extension (72ºC/5 min). Determination of *GHR* allele variants was done by restriction with *AluI* enzyme (Promega Corporation, USA). Visualization of DNA sequences after enzymatic restriction was performed on 3% agarose gel with standard of 50 base pairs. Statistical analysis of the results was done using the GLM procedure and SAS statistical program (SAS STAT, V8, 1999).

Results and discussion

Body weight at birth of male and female calves did not differ significantly (33.7 vs. 37.3 kg). Fattening period of young bulls lasted 16.2 months (≈ 495 days) while heifers were fattened up to the age of 15.5 months (≈474 days). Bulls compared to the heifers during fattening period reached higher average daily gains (1176 vs. 901 g/day, p<0.01). During that time bulls were kept 21 days longer than heifers and reached a higher body weight of 103.1 kg (598.8 vs. 495.7 kg). The mass of hot and cold beef carcasses compared to the heifers carcasses was significantly higher (p<0.01) what was expected. Differences in dressing percentage and carcasses chilling loss between young bulls and heifers were not significant. The effect of sex on the EUROP class and the level of body fat coverage was significant (p<0.01; p<0.05). Influence of the sex on bone ratio in the loin eye rib was less pronounced (p<0.05) than the influence on the ratio of muscle and fat tissue (p<0.01). The slaughtering indicators of young bulls and heifers are shown in Table 1.

Table 1. Production and slaughter indicators of young bulls and heifers in this research (X± *SD*)

Category of animals / Carcass traits	Young bulls (n=23)	Heifers (n=17)	Average (n=40)
Birth weight (kg)	*37.70±6.88*	*37.33±7.48*	*37.55±7.03*
Fattening period (days)	*495.0±27.47*	*473.9±28.62*	*486.7±27.89*
Average daily gain (kg)	*1.176±0.324*^{a}	*0.901±0.112*^{b}	*1.07±0.293*
Final live weight (kg)	*598.8±55.58*^{a}	*495.7±38.08*^{b}	*558.1±70.67*
Hot carcasses weight (kg)	*353.9±33.86*^{a}	*290.5±31.13*^{b}	*328.9±45.11*
Carcasses chilling loss (%)	*1.50±0.335*	*1.60±0.337*	*1.54±0.335*
Cold carcasses weight (kg)	*348.6±33.21*^{a}	*285.8±30.69*^{b}	*323.8±44.54*
Dressing percentage (%)	*58.2±1.634*	*57.9±7.348*	*58.1±4.69*
EUROP carcass score	*3.70±0.470*^{a}	*3.27±0.458*^{b}	*3.53±0.506*
EUROP coverage with fat tissue	*2.78±0.422*^{A}	*3.20±0.561*^{B}	*2.95±0.517*
Share of muscle tissue (%)	*67.1±3.067*^{a}	*61.42±4.15*^{b}	*64.9±4.47*
Share of fat tissue (%)	*13.0±2.553*^{a}	*20.92±3.79*^{b}	*16.1±4.95*
Share of bone tissue (%)	*19.9±2.193*^{A}	*17.67±2.60*^{B}	*19.0±2.57*

Different large A-B letters in row indicate p<0.05; different small a-b letters in the row indicate p<0.01

GHR gene polymorphism analysis was performed by restriction of 342 bp long GHR codogene sequence with *AluI* restriction enzyme and product visualization on 3% agarose gel (Figure 1). The homozygous *AA* genotype was identified by three fragments (191 pb, 101 bp and 50 bp), *GG* genotype by two fragments (191 bp and 151 bp), and heterozygous *AG* genotype by four *GHR* gene fragments (191 bp, 151 bp, 101 bp, and 50 bp). In the investigated sample of 40 animals, 19 homozygous and 21 heterozygote individuals were observed (Table 2).

Table 2 Frequency of genotypes and allele variants of the GHR gene, observed and expected heterozygosity in the studied population of fattening cattle

Genotype	No. observed genotype	Genotype frequency	H_O H_E	χ^2	Allele	Frequency of allele	*s.d.*
AA *AG* *GG*	13 21 6	0.325 0.525 0.150	0.525 0.485	0.736	A G	0.588 0.412	± *0.0541*

Ho/He – observed/excepted heterozygosity; χ^2 – Chi square; *s.d.* – standard deviation

Allele variant *A* in relation to *G* allele dominates in the studied population of cattle (0.588: 0.412). *Hadi et al. (2015)* in the Holstein population observe frequency of *A* and *G* allele variants of the *GHR* gene (0.65: 0.35) while *Hradecka et al. (2008)* in the Holstein population notice dominance of *A* versus the *G* allele variant (0.95: 0.05). *Di Stasio et al. (2005)* for the Piedmontese breed suggests a uniform distribution of *A* and *G* allele variants of the *GHR* gene (0.49: 0.51). Higher value for observed than expected heterozigosity can be explained by the disequilibrium because of crossing Simmental and Holstein breeds in the sample.

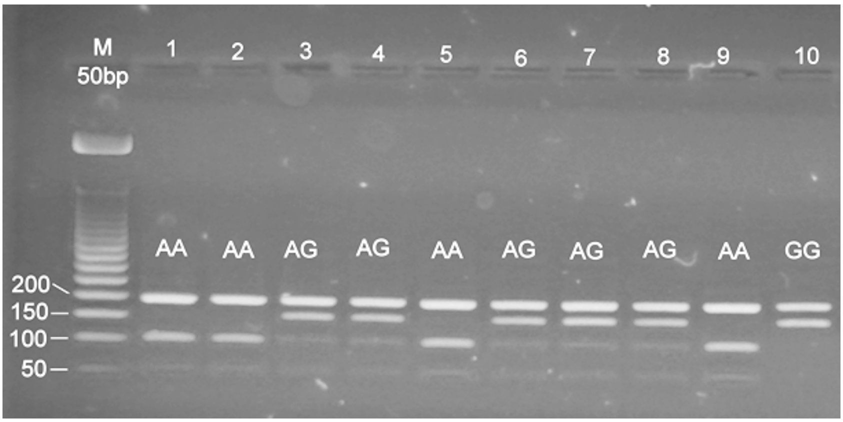


Figure 1 Identification of genotypes of *GHR* gene (M - standard with spacing of 50 base pairs)

Considering the observed genotypes of *GHR* gene and production indicators in the sample, certain interactions can be found. It has been observed that the genotype *AA* has a higher degree of fat cover and a lower ratio of muscle tissue (p<0.05). Association of the *AA* genotype of *GHR* gene with a higher proportion of fatty tissue in the loin eye rib was also observed (p<0.01; Table 3).

Table 3 Indicators of carcass quality and meat of fatty young bulls and heifers considering observed *GHR* genotypes (X± *SD*)

Genotype / Carcass traits	*AA* (n=13)	*GA* (n=21)	*GG* (n=6)
Average daily gain (kg)	1.001±*0*.203	1.106±*0.346*	1.052±*0.234*
Final live weight (kg)	537.09±*57.94*	575.04±*76.16*	537.16±*65.82*
Hot carcasses weight (kg)	315.35±*37.94*	336.63±*47.99*	313.33±*50.23*
Carcasses chilling loss (%)	1.673±*0.329*	1.515±*0.353*	1.530±*0.363*
Cold carcasses weight (kg)	310.06±*37.19*	331.53±*47.38*	308.62±*49.97*
Dressing percentage (%)	59.50±*8.251*	57.58±*1.824*	57.23±*2.704*
EUROP carcass score	3.307±*0.480*	3.667±*0.483*	3.333±*0.516*
EUROP coverage with fat tissue	3.076±*0.640* ^{A}	3.000±*0.316* ^{A}	2.501±*0.548* ^{B}
Share of muscle tissue (%)	62.12±*5.238* ^{A}	65.74±*3.495* ^{B}	66.22±*4.243* ^{B}
Share of fat tissue (%)	19.60±*5.648* ^{a}	14.85±*3.857* ^{b}	14.60±*3.467* ^{b}
Share of bone tissue (%)	18.28±*2.535*	19.41±*2.459*	19.16±*2.780*

Different large A-B letters in order signify p<0.05; different small a-b letters in order signify p<0.01

Comparing data of fattening beef production with regard to the presence of *A* and *G* allele variants of *GHR* gene, a significant influence of *A* allelic variation on a higher proportion of fatty tissue in the loin eye rib was observed (Table 4). Thus, individuals with *AA* genotype with respect to *GA/GG* genotypes have significantly higher proportion of fatty tissue (19.6 vs. 14.8; p<0.01) and a lower proportion of muscle tissue (62.1 vs. 65.8; p<0.05). It was observed that the *GG* genotype with respect to *AA/GA* genotypes has lower body fat coverage (3.03 vs. 2.50; p<0.05).

Table 4 Quality indicators of carcass and beef carcass in view of the observed combinations of GHR genotypes (X± *SD*)

Genotype / Carcass traits	*AA/AG* (n = 34)	*GG* (n = 6)	*AA* (n = 13)	*GG/GA* (n = 27)
Average daily gain (kg)	1,067±*0.306*	1,053±*0.234*	1,001±*0.203*	1,094±*0.322*
Final live weight (kg)	562.0±*71.84*	537.2±*65.82*	537.1±*57.94*	566.6±*74.52*
Hot carcasses weight (kg)	328.5±*45.05*	313.3±*50.23*	315.3±*37.94*	331.4±*48.53*
Carcasses chilling loss (%)	1.576±*0.348*	1.530±*0.363*	1.673±*0.329*	1.519±*0.348*
Cold carcasses weight (kg)	323.3±*44.45*	308.6±*49.97*	310.1±*37.19*	326.4±*47.97*
Dressing percentage (%)	58.24±*4.996*	57.23±*2.704*	59.50±*8.251*	57.51±*1.997*
EUROP carcass score	3.529±*0.506*	3.334±*0.516*	3.308±*0.480*	3.593±*0.501*
EUROP cov. with fat tissue	3.029±0.46^{A}	2.501±0.55^{B}	3.071±*0.641*	2.889±*0.424*
Share of muscle tissue (%)	64.36±*4.537*	66.23±*4.243*	62.12±5.23^{A}	65.85±3.59^{B}
Share of fat tissue (%)	16.66±*5.111*	14.60±*3.467*	19.60±5.65^{a}	14.79±3.71^{b}
Share of bone tissue (%)	18.98±*2.513*	19.17±*2.781*	18.28±*2.536*	19.36±*2.480*

Different large A-B letters in row indicate p<0.05; different small a-b letters in the row indicate p<0.01

There was no correlation of the *GHR* gene polymorphisms with the dynamics of animal growth, but there were significant correlation with proportion of muscle and fat tissue in loin eye rib. *Reardon et al. (2010)* have also determined the association of polymorphism of *GHR* gene with meat quality. In some earlier studies, the correlation of the polymorphism of *GHR* genes with performance traits was observed *(Di Stasio et al., 2005; Sherman et al., 2008; Waters et al., 2011).* However, in this study, such association was not observed. Since the share of fatty tissue in the carcasses and meat is an important factor of the quality (market prices, nutritional and sensory quality of meat, consumer perception), the apparent association of the *GHR* gene allelic variants with the share of fatty tissue indicates the need for further research to ensure the safety of observed interactions and eventual confirmation of the *GHR* as a valuable gene candidate.

Conclusion

In the investigated sample of beef carcass the dominance of *A* allele of the *GHR* gene was observed. Significant association between the *AA* genotype of *GHR* gene and the higher body fat content in the beef carcasses and higher coverage with fat was observed. Results indicate the potential utilization of *GHR* gene variants in cattle selection related to carcass and meat quality of beef carcasses.

Povezanost polimorfizma *GHR* gena sa kvalitetom trupova junadi

Ante Ivanković, Valentino Držaić, Jelena Ramljak, Miljenko Konjačić

Rezime

Odgajivačko-selekcijski rad u govedarstvu zadnjih decenija u sve značajnijoj meri koristi prednosti genomike i neposredne selekcije na poželjne varijante polimorfnih gena. U konkurentnoj proizvodnji goveđeg mesa važna je optimizacija dinamike rasta, proizvodnja kvalitenih trupova i mesa bogatog ukusa. Cilj istraživanja je utvrđivanje alelnih varijanti *GHR* gena u populaciji tovnih goveda te njihove povezanosti s odlikama rasta, kvalitetom trupa i mesa. U istraženoj populaciji goveda utvrđena je dominacija alelne *A* varijante *GHR* gena (0,588). Nije zapažena povezanost alelnih varijanti *GHR* gena s odlikama rasta, klaničnom masom životinja, masom toplih i hladnih polutki i randmanom. Uočena je značajna povezanost *AA* genotipa *GHR* gena s većim udelom masnog tkiva ($p<0,01$) i manjim udelom mišićnog tkiva u trupu ($p<0,05$). Takođe, zapažena je povezanost alelne *A* varijante *GHR* gena sa prekrivenosti trupa masnim tkivom ($p<0,05$). Istraživanje ukazuje na potencijal polimorfizma *GHR* gena u neposrednoj selekciji goveda kao genetskog markera. Protežiranjem alelne *G* varijante *GHR* gena može se umanjiti udeo masnog tkiva u junećim trupovima te povećati udeo mišićnog tkiva. Pri tome je poželjno nastaviti praćenje učinaka alelnih varijanti *GHR* gena na fenotipske odlike goveda te interakciju s drugim selekcijskim kandidat genima.

Ključne reči: polimorfizam, *GHR* gen, kvalitet trupa, kvalitet govedine

References

ALBERTI P., PANEA B., SANUDO C., OLLETA J., RIPOLL G., ERTBJERG P., CHRISTENSEN M., GIGLI S., FAILLA S., CONCETTI S. (2008): Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. Livestock Science, 114: 19-30.

ARDICLI S., DINCEL D., SAMLI H., BALCI F. (2017): Effects of polymorphisms at LEP, CAST, CAPN1, GHR, FABP4 and DGAT1 genes on fattening performance and carcass traits in Simmental bulls. Arch. Anim. Breed., 60: 61-70.

DI STASIO L., DESTEFANIS G., BRUGIAPAGLIA A., ALBERA A., ROLANDO A. (2005): Polymorphism of the GHR gene in cattle and relationships with meat production and quality. Animal Genetics, 36: 138-140.

GE W., DAVIS M.E., HINES H.C., IRVIN K.M. (1999): Two-allelic DGGE polymorphism detected in the promoter region of the bovine GHR gene. Animal Genetics, 30: 161-168.

GE W., DAVIS M.E., HINES H.C., IRVIN K.M. (2000): Rapid communication: single nucleotide polymorphisms detected in exon 10 of the bovine growth hormone receptor gene. Journal of Animal Science, 78: 2229-2230.

HRADECKA E., CITEK J., PANICKE L., REHOUT V., HANUSOVA L. (2008): The relation of GH1, GHR and DGAT1 polymorphisms with estimated breeding values for milk production traits of German Holstein sires. Czech Journal of Animal Science, 53: 238-245.

HADI Z., ATASHI H., DADPASAND M., DERAKHSHANDEH A., GHAHRAMANI SENO M.M. (2015): The relationship between growth hormone polymorphism and growth hormone receptor genes with milk yield and reproductive performance in Holstein dairy cows. Iran Journal of Veterinary Research, 16: 224-248.

KOKNAROGLU H., LOY D., WILSON D., HOFFMAN M., LAWRENCE J. (2005): Factors affecting beef cattle performance and profitability. Prof. Anim. Sci., 21: 286-296.

REARDON W., MULLEN A., SWEENEY T., HAMILL R. (2010): Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. Meat Science, 86: 270-275.

SAS (1999): OnlineDoc® Software Release 8. SAS Institute Inc., Cary, NC, USA.

SHERMAN E.L., NKRUMAH J.D., MURDOCH B.M., LI C., WANG Z., FU A., MOORE S.S. (2008): Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. Journal of Animal Science, 86: 1-16.

WATERS S., MCCABE M., HOWARD D., GIBLIN L., MAGEE D., MACHUGH D., BERRY D. (2011): Associations between newly discovered polymorphisms in the Bos taurus growth hormone receptor gene and performance traits in Holstein–Friesian dairy cattle. Animal Genetics, 42: 39-49.

ANALYSIS OF BETA-LACTOGLOBULIN IN SMALL HERD OF CATTLE BY PCR-RFLP

Violeta Caro Petrović, Milan M. Petrović, Nevena Maksimović, Marina Lazarević, Aleksandar Stanojković, Nenad Micić, Milos Marinković

Institute for Animal Husbandry, 11080 Belgrade, Serbia
Corresponding author: violycaro@yahoo.com
Original scientific paper

Abstract: The Beta-lactoglobulin loci affecting the milk production traits as well as the milk quality and their polymorphisms describe a part of the genetic variance and improve the breeding value estimation of breeding value. The study aim was to identify the Beta-Lactoglubulin genotypes of one small herd of cows Simmental breed. For the preliminary investigation had utilized 10 Simmental cows from a private farm (small herd). The detection of the genetic polymorphism of the bovine Beta-Lactoglobulin was performed by digestion the PCR products of 262 bp with restriction endonuclease *Hae III*. The Beta-Lactoglobulin genotype of all the cows tested showing that they belong to an AA genotype having the lengths of restriction fragments of 153 and 109bp. PCR-RFLP is an efficient method that can be used to determine the dairy cattle genotype and the use of molecular markers is the substitute to the current methods of interest traits in animals.

Keywords: Beta-lactoglobulin, genotyping, PCR-RLFP

Introduction

The milk protein comprises of casein and whey protein. Beta-lactoglobulin gene is situated on bovine chromosome 11 and encodes the main protein of whey (*Karimi et al., 2009).* Beta-lactoglobulin is 1 of 2 major whey proteins found in the mammal's milk, mainly ruminants (*Remus-Alexandru et al., 2010; Alim et al., 2015)* and exists in different allelic forms which are controlled by co-dominant autosomal genes *(Madureira et al., 2007; Meignanalakshmi S. et al., 2013*). Beta-lactoglobulin (b-LG) is a globular protein member of the lipocalin family (large group of small extracellular proteins) and is the main whey protein of ruminant milk consisting around 50% of total whey proteins (*Flower, 1996; Badola 2003; Selvaggi et al., 2014).* Polymorphism of β -lactoglobulin gene was discovered in 1955 and a total of 15 alleles are known (*Threadgill and Womack,1990; Mat et al., 2007*). The common alleles are A, B, C and D, with alleles A and B being the most frequent *(Farrell et al., 2004).* The Beta-Lactoglobulin loci affecting the milk production traits as well as the milk quality and their polymorphisms describe a part of the genetic variance and improve the breeding value estimation of breeding value *(Alim et al., 2015).* Genetic polymorphism of milk protein received considerable research interest in the past years due to the probable associations between milk protein genotypes and economically important traits in dairy cattle **(***Tsiaras et al., 2005*; *Rachagani et al., 2006; Ilie et al., 2010; Selvaggi et al. 2014).*

The study aim was to identify the Beta-Lactoglubulin allele A and allele B and Beta-Lactoglubulin genotypes AA; AB and BB in one small herd of Simmental cows.

Materials and Methods

For the preliminary investigation has utilized 10 Simmental cows from a private farm (small herd). The blood samples are having stored at -20^{0}C in a vacutainer tubes EDTA Lavender top. Isolation of DNA was done using the Quick DNA universal Kit (Epigenetics) from thawed blood samples. The isolated DNA was then placed in a micro centrifuge tube and stored in freeze at -20^{0}C until used for amplification.

Primers sequences used for amplification of beta-Lactoglobulin: 5' GTCCTTGTG CTGGACACCGACTACA-3 (forward) and 5'-CCCAGGACACCGGCTCCCGGTATAT -3' (reverse). The PCR amplification reactions carried out containing 1 µl of DNA, 10 µl mastermix, 1 µl of each primer (forward and reverse) and 7 µl distilled water with a final volume of 20 µl.

The study of Beta-Lactoglobulin genotyping has performed through a plain polymerase chain reaction using of the Qantarus Q Cycle. The following applied steps includes primary denaturation step at 95^{0}C for 5 minutes followed by 40 cycles of which include the denaturation at 95^{0}C at 30 seconds, annealing at 57^{0}C at 30 seconds, elongation at 72^{0}C for 1 minute and 10 minutes extension at 72^{0}C.

The PCR products are having analyzed with electrophoresis of 2% agarose gel and visualizing under UV rays. For the genotyping identification, the PCR products are having digested with the endonuclease *Hae III* and incubated for 3 hours at 37^{0} C.

Results and Discussions

For the evaluation of beta-Lactoglobulin genotypes distribution was used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method with certain designed sequences of interest. A 262bp have acquired after electrophoresis in agarose gel having stained with advance blue (Figure 1).

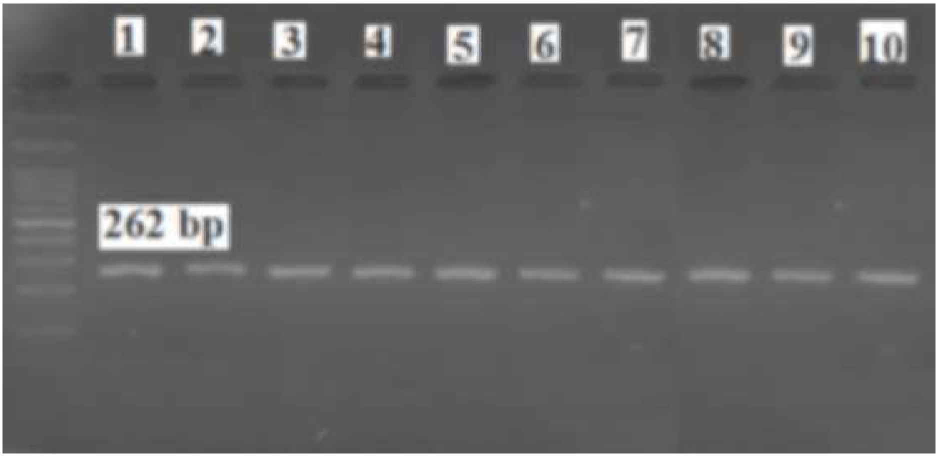


Figure 1. Electrophoresis pattern of amplified bovine genomic DNA with beta- Lactoglobulin specific primers separated on 2% agarose gel stained with advance blue.

The Lane 1 (Figure 1), indicating the molecular size marker. Lane 2-11 were the fragments amplified for Beta-Lactoglobulin gene. (100bp DNA Step Ladder).

Table 1. Milk production for 305 days lactation period of the 10 cows

ID No./Name	Milk yield	% Milk fat	Milk fat (kg)	% Milk Protein	Milk Protein (kg)
7154374170 Lana	6,031	4.10	247.565	3.42	206.370
7165188741 Marija	5,122	4.05	207.594	3.40	174.225
7134770915 Lisa	6,806	4.09	278.340	3.42	232.952
7124770911 Cakana	6,822	4.08	278.315	3.42	233.544
7175602711 Neda	5,910	4.09	241.796	3.41	201.588
7102827406 Vanda	4,683	4.10	192.016	3.38	158.296
7113407791 Lela	4,998	4.08	203.930	3.40	169.941
7175565172 Majda	5,213	4.08	212.620	3.39	176.909
7185601216 Cura	5,705	4.06	231.658	3.41	194.810
7155602712 Mona	5,312	4.05	214.923	3.41	180.920

The detection of the genetic polymorphism of the bovine Beta-Lactoglobulin was performed by digesting the PCR products of 262 bp with restriction endonuclease *Hae III (Medrano and Aguilar-Cordova, 1990*). The lengths of restriction fragments are 153 and 109bp for the AA genotype, 109 and 79 bp for BB genotype. Three fragments of 153, 109 and 79bp for heterozygous AB genotype (combination of the two alleles A and B). The result of the study, revealing that all the cows tested are having a homozygous Beta-Lactoglobulin AA genotype (Figure 2).

Figure 2. Electrophoresis pattern for beta-Lactoglobulin digested with Hae III enzyme. (100bp DNA Step Ladder).

The milk fat and protein percent (Table 1) of the tested cows used in this study precisely connected with the acquired Beta-Lactoglobulin genotype.

A lot of studies were performed to investigate the effect of B-LG genotypes on milk production traits, milk composition and quality (*Gouda et al., 2011; Selvaggi et al. 2014; Dogru 2015).* Some authors informed the association of Beta-Lactoglobulin protein variants A and B in bovine milk. *Remus-Alexandru et al. (2010*) informed that the AA homozygote supply milk with a low percentage of fat but in a larger quantity. On the other hand, *Ali at al. (2015)* stated that the β-LG protein variants A and B are having associated with different amounts of β-LG protein in the bovine milk of which the variant A has a higher β –LG protein concentration than variant B. According to *Medrano and Aguilar-Cordova (1990)* the cows with the AA BLG genotype produced more milk with higher protein as compared to the BB homozygote**.** *Zaglool et al., (2016)* found that the genotype AA produced significantly higher milk yield, protein % in Holstein Friesian cattle breed. The AA genotype cows compared to cows with AB and BB genotypes produced milk with a higher milk fat content and more favorable share of protein in milk with cows AB and BB genotype however the mentioned differences were not significant *(Dokso et al., 2014).* The milk fat and protein percent (Table 1) of the tested cows used in this study precisely connected with the acquired B-Lg genotype.

We do agree with the notes of *Rachagani et al. (2006)* that the use of polymorphic genes as detectable molecular markers is an alternative way to the current methods of trait selection that associated with traits of interest in animals.

Conclusion

The results of Rapid Fragment Length Polymorphism (RFLP) analysis indicating that the cows tested are all homozygous AA genotype for Beta-Lactoglobulin. The Polymerase Chain Reaction- Rapid Fragment Length Polymorphism (PCR-RFLP) is an efficient method that can be used to determine the dairy cattle genotype. Thus, the use of molecular markers is the substitute to the current methods of interest traits in animals.

PCR-RFLP analiza Beta-laktoglobulina malog stada krava

Violeta Caro Petrović, Milan M. Petrović, Nevena Maksimović, Marina Lazarević, Aleksandar Stanojković, Nenad Mićić, Miloš Marinković

Rezime

Beta-laktoglobulinski lokusi koji utiču na osobine proizvodnje mleka, kao i na kvalitet mleka i njihovi polimorfizmi opisuju deo genetičke varijacije i poboljšavaju procenu priplodne vrednosti. Cilj istrazivanja je bio da se identifikuju genotipovi beta-laktoglobulina jednog malog stada krava Simentalske rase goveda. Za preliminarnu analizu korišćen je uzorak od 10 Simentalskih krava sa privatne farme (malog stada). Detekcija genetskog polimorfizma goveđeg beta-laktoglobulina obavljena je digestijom PCR proizvoda od 262 bp sa restrikcionom endonukleazom *Hae III*. Genotip beta-laktoglobulina svih testiranih krava pokazuje da pripadaju genotipu dominantnog homozigota AA koji ima dužinu restrikcionih

fragmenata od 153 i 109 bp. PCR-RFLP je efikasna metoda koja se može koristiti za određivanje genotipa muznih krava i upotreba molekularnih markera je zamena za savremene metode u analizama osobina životinja koje su od interesa.

Ključne reči: Beta-laktoglobulin, genotipizacija, PCR-RLFP

Acknowledgement

This study is part of the project TR 31053 “Modern biotechnology solutions in the breeding and feeding of cattle and sheep and goats for the production of valuable and safety food” supported by the Ministry of Education, Science and Technological Development, Republic of Serbia.

References

ALIM M.A., SUN D., ZHANG Y., ZHANG Y., ZHANG Q., LIU L. (2015): DNA Polymorphisms in the *β-lactoglobulin* and *κ–casein* Genes Associated with Milk Production Traits in Dairy Cattle Bioresearch Communications, 1 (2): 82-86.

BADOLA S., BHATTACHARYA T.K., BISWAS T.K., SHIVAKUMAR B.M., KUMAR P., MISTRA S.S., SHARMA A. (2003): Bubaline beta-lactoglobulin gene polymorphism: A contrast from its bovine counterpart. Buffalo J., 19 (4): 291-298.

DOGRU U. (2015): B-Lactoglobulin Genetic Variants In Brown-Swiss Dairy Cattle And Their Association With Milk Yield And Quality Traits. The Journal of Animal & Plant Sciences, 25(2): 595-598.

DOKSO *A*., IVANKOVIĆ A., BRKA M., ZEČEVIĆ E., IVKIĆ Z. (2014): Utjecaj genetskih varijanti β-laktoglobulina, κ-kazeina i αs1-kazeina na količinu i kvalitetu mlijeka holstein, simentalske i smeđe pasmine goveda u Hrvatskoj. Mljekarstvo 64 (1), 49-56.

FARRELL JR H.M., JIMENEZ-FLORES R., BLECK G.T., BROWN E.M., BUTLER J.E., CREAMER L.K., HICKS C.L., HOLLAR C.M., NG-KWAI-HANG K.F. & SWAISGOOD H.E. (2004): Nomenclature of the proteins of cows' milk-- sixth revision. Journal of Dairy Science 87, 1641-74.

FLOWER D.R. (1996): The lipocalin protein family: structure and function. Biochem J.**,** 15; 318(Pt 1): 1–14.

GOUDA E.M. , GALAL M.K., WASFY M.A.,, ABDELAZIZ S.A. (2011): Phenotypes, Genotypes and Allele Frequencies of B-lactoglobulin in Egyptian Cattle and Buffalo. Journal of Agricultural Science, 3(4):203-210.

ILIE D.E., SĂLĂJEANU A., MAGDIN A., NEAMŢ R., VINTILA I. (2010): Early Determination of Animals with Favorable Genes in Milk Production for Profitable Private Farms. Animal Science and Biotechnologies, 43 (1): 279-282.

KARIMI K., BEIGI NASIRI M.T., FAYYAZI J., MIRZADEH K.H., ROUSHANFEKR H. (2009): Allele and genotype frequencies of _-lactoglobulin gene in Iranian Najdi cattle and buffalo populations using PCR-RFLP. African Journal of Biotechnology 8 (15): 3654-3657.

MADUREIRA A.R., PEREIRA C.I., GOMES A.M.P., PINTADO M.E., MALCATA F.X. (2007): Bovine whey proteins—Overview on the main biological properties, *Food Research International*, 40, 1197–1211**.**

MEDRANO J.F., AGUILAR E. (1990). Genotyping of bovine kappa casein loci following DNA sequence amplification. Biotechnology, 8, 144-146.

MEIGNANALAKSHMI S., GOPI H. AND MAHALAINGA NAINAR A. (2013): Effect of Genotypes of Beta Lacto Globulin on Milk yield and Milk composition during four Lactation Periods in Red Sindhi cows Research Journal of Animal, Veterinary and Fishery Sciences, 1(11): 1-4.

RACHAGANI S., GUPTA I.D., GUPTA N., GUPTA S.C. (2006). Genotyping of beta-lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. BMC Genet. 7: 31-34

REMUS-ALEXANDRU V.B., GEORGESCU S. E., MANEA M. A., DINISCHIOTU A., COSTACHE M. (2010): University of Bucharest, Faculty of Biology, Molecular Biology Center, Bucharest. https://www.ibna.ro/arhiva/AZ%2010/AZ%2010_14%20Vatasescu.pdf

SELVAGGI M., LAUDADIO V., DARIO C., TUFARELLI V. (2014): Investigating the genetic polymorphism of sheep milk proteins: a useful tool for dairy production. Journal of the Science of Food and Agriculture, 94 (15): 3090–3099**.** doi: 10.1002/jsfa.6750.

THREADGILL D.W., WOMACK J.E. (1990): Genomic analysis of the major bovine milk protein genes. Nucleic Acids Research 18 (23): 6935-6942.

TSIARAS A.M., BARGOULI G.G., BANOS G., BOSCOS C.M. (2005): Effect of kappaCasein and betaLactoglobulin loci on milk production traits and reproductive performance. J Dairy Sci., 88: 324-334.

ZAGLOOL A.W., AWAD A., EL SAYED EL ARABY I., EL-BAYOMI K.M. (2016): Association of β –Lactoglobulin Gene Polymorphism with Milk Yield, Fat and Protein in Holstein-Friesian Cattle. World Vet J, 6(3): 117-122.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE INFLUENCE OF KAOLIN E559 ON MILK PRODUCTION AND SOMATIC CELLS COUNT IN MILK DURING THE INDOOR AND OUTDOOR PERIODS

Vigilijus Jukna[1], Česlovas Jukna[2], Vaidas Prusevičius[3], Edita Meškinytė-Kaušilienė[4], Žydrūnė Vyšniauskaitė[5]

[1] Institute of Energy and Biotechnology Engineering, Aleksandras Stulginskis University
Studentų str. 11, Akademija, Kaunas distr., Lithuania
[2] Veterinary Academy, Lithuanian University of Health Sciences
Tilžės str. 18, Kaunas, Lithuania
[3]The Ministry of Agriculture of the Republic of Lithuania
Gedimino str. 19, Vilnius, Lithuania
[4]Center of Animal Husbandry Selections, Breeding Values and Dissemination,
Aleksandras Stulginskis University
Studentų str. 11, Akademija, Kaunas distr., Lithuania
[5]Center of Animal Husbandry Selections, Breeding Values and Dissemination,
Aleksandras Stulginskis University
Studentų str. 11, Akademija, Kaunas distr., Lithuania
Corresponding author: zydrune.vysniauskaite@asu.lt
Original scientific paper

Abstract: The effect of kaolin E559 was studied on dairy cattle during the indoor and outdoor periods. Cows were divided into the control and treatment groups each including 40-45 cows. Cows were selected on the basis of their age, the phase of lactation, productivity, and parents. During the study, cows were fed a ration of indoor. Cows in both groups received the same concentrated feeding with respect to produced milk, 350 grams per one kilogram. Cattle in the treatment group received 1.5 kg of kaolin powder. The somatic cells count was lower in milk produced by cows fed the clay supplement as compared to the control group ($p<0.001$). During the indoor period, cattle fed mix of combined food kaolin E559 produced 10.3% more milk, the level of fat content was 0.09 % higher, and the level of protein was 0.28% higher as compared to the control group. At the end of the study, the level of leukocytes was significally higher in the blood of cows from the control group. The blood of cows from the treatment group was higher in hemoglobin by 9.7 g/l ($p<0.005$) and in globulin by 5 g/l ($p<0.001$). The clay supplements did not significantly affect cows housed outside ($p<0.05$).

Key words: kaolin E 559, milk, production, somatic cells

Introduction

The dairy cattle breeding is one of the most challenging branches of agriculture. Grass feeding is very beneficial to cattle, but bad weather conditions and technological failures can cause the growth of fungi in feed which can be toxic to cattle. As a result, fungi reduce animals productivity, weakens the immune system, and cause sexual dysfunctions. Eventually, a wide range of reproduction, food consumption, and productivity problems occur (*Bakutis, 2007; Zainukov et al., 2008*). Moldy feed and toxins affect the developing fetus. Mold toxins also accumulate in milk and pose a potential risk to a human health. Moreover, feeding calves moldy milk causes severe diarrhea which leads to death (*Ani et al., 2014*).

The growing interest in organic food production encourages farmers to use natural resources in livestock farming systems. Nowadays, one can find information that bentonite clays can be used not only as a supplement, but as absorbents as well. *Morozo et al., (2009)* claimed that feed supplemented with crushed grains and bentonite had a positive effect on milk yield and milk fat content. *Trckova et al., (2006)* and *Demirel et al. (2011)* stated that 1.5 % of zeolite on a dry matter had increased milk production. *Andrejkovičová et al. (2016)* stated that 1.5 % of zeolite on a dry matter improved the digestibility of 12 to 18 months old bulls by 4.7-9.1 %.

Aluminosilicates (e.g., zeolite, bentonite, kaolin, etc.) can absorb toxins, toxic metals, radionuclides (U, Ra, Cs, I), ammonia, carbon dioxide, methane, and other organic pollutants *(Adamovič et al., 2011; Liang et al., 2013)*. Kaolin is a soft, plastic clay mainly composed of the mineral kaolinite which is a hydrated aluminum silicate $Al_2\ O_{32}\ SO_{22}\ H_2O$ *(Murray, 2002)*. It was identified that kaolin and other silicate clays have a positive effect on calves *(Lee et al., 2010; Sidorova, 2009; Kardišauskas et al., 2015)*, pigs *(Trckova et al., 2004)*, and poultry *(Owen et al., 2012; Ani et al., 2014)*.

Bentonite clays contain all the basic macro- and microelements necessary for the normal development of an organism. Bentonite clays absorb bodily toxins, bacteria, poisons. Bentonite also gently covers the mucous membrane of the stomach and helps reduce inflammations *(Huwig et al., 2001; Trskova et al., 2004; Liang et al., 2013)*. The effectiveness of clay depends on the amount of clay added to feed which is different for each animal *(Katouli et al., 2001)*.

The aim of this study was to investigate the influence of kaolin E559 on milk production and somatic cells count in milk during the indoor and outdoor periods.

Material and methods

While analyzing the influence of kaolin E559 on dairy cattle during the indoor period, cows were divided into the control and treatment groups each including 40-45 cows. Cows were selected on the basis of their age, the phase of lactation, productivity, and parents. During the study, cows were fed a ration of indoor. The study was carried out at Agriculture Company in Griškabūdis. Cattle in the control and treatment groups were fed and treated equally during the indoor and outdoor periods. Cows in both groups received the same concentrated feeding with respect to produced milk, 350 grams per one kilogram. Cattle in the treatment group received 1.5 kg of kaolin powder. The chemical composition of kaolin E559 used in the study was as follows: the dry matter, 95,14; Ca, 0,304; Mg, 0,141; Fe, 0,962; P, 0,086; Mn, 0,007; Na, 0,097; K, 1,382; Cu, 0,024; Se, 0,00011; C, about 30; aluminum silicates, 67,00; $Al_{203,}$ 29,70 (g/100g). During the indoor period, cows were tied up and during the outdoor period cows were grazed outside. Cattle in the treatment group received 1.5 kg of kaolin E559 powder per 100 kg of the body weight. During the indoor period, the concentrate feed was distributed manually according to the individual productivity of each cow, while during the outdoor period, feed was distributed during milking.

The energy value and composition of feed (Table 2) were determined while using “AgroSoft” analyzer. The concentrate for dairy cows was K-7340410-64.

During the indoor period, cows received the following feed (Table 1).

Table 1. The type and amount of feed cows were fed during the indoor period

Feed	Amount per day
Grass silage	12 kg (4.65 kg of DM*)
Corn silage	17 kg (6.22 kg of DM)
Wheat straw	0.7 kg (0.595 kg of DM)
Saladin	4 kg (2.0 kg of DM)
Compound feed	8 kg (7.15 kg of DM)

*Amount of the dry matter

Table 2. Feed composition

Parameters	Values
Dry matter kg	21.625
Net energy for lactation (NEL)	139.40
Crude protein g	2689
Crude fat g	648
Sugars g	2260
Starch g	3082
NDF g	1320
ADF g	965
Ca g	140
P g	92
Mg g	50
K g	143
Na g	34
S g	49
Fe mg	1240
Cu mg	253
J mg	16
Vitamin D3, thousand IU	100
Vitamin A, thousand IU	18
Vitamin E mg	710

Cows were milked twice a day. The milk production was recorded while using a standard milk recording method – A4. The control milking was done by control assistants. The composition and quality of milk were evaluated by the State Enterprise “Pieno tyrimai”. Cows’ productivity and fat, protein, glucose, and *urea* content were calculated according to the data obtained by “Pieno tyrimai”.

In order to analyze the influence of kaolin E559 on cows grazing outdoors, cows were divided into two groups, namely, the control and treatment groups, each including 20 cows. The selection was based on the similar level of productivity and the phase of lactation. Both groups were kept together. Concentrates of 100 kg were mixed with 1.5 kg of kaolin E559 powder. Cows received concentrated feed during milking. Cows were milked twice a day. Milk production was recorded while using a standard milk recording method, A4. The composition and quality of milk were evaluated by the State Enterprise “Pieno tyrimai”. The study was carried out in July, August, and September.

In order to evaluate the health status of cows, blood samples from cows of similar characteristics were taken at the end of the study. Biochemical and morphological indices of blood had been studied. Blood samples were analyzed while using a blood analyzer. Moreover, the health status of cows was observed throughout the entire study period. Data was processed while using the “R Stats Package”.

Results and discussion

The findings of the study revealed that kaolin E559 has a positive effect on the productivity of dairy cattle and the quality of milk during the indoor period. The findings also showed that kaolin helps reduce the number of somatic cells (SC) (Table 3).

Table 3. Milk production during the indoor period

Parameters		Control group				Treatment group			
		Months				Months			
		In the beginning	I	II	III	In the beginning	I	II	III
Milk production, kg	x	11,42	11,55	12,05	11,85	11,48	12,70	13,40	13,01
	Sx	0,22	0,20	0,29	0,32	0,15	0,21	0,25	0,29
	lim	7,5-16,3	7,0-14,5	6,7-17,6	7,6-19,2	7,8-15,9	8,0-18,5	7,0-19,5	5,2-19,8
Milk proteins, %	x	3,61	3,37	3,42	3,37	3,54	3,71	3,60	3,72
	Sx	0,062	0,036	0,030	0,028	0,024	0,020	0,041	0,051
	lim	2,89-5,17	2,81-4,15	3,08-4,19	2,98-4,02	3,13-5,08	2,98-4,71	2,90-4,41	3,21-5,79
Milk fat, %	x	4,17	4,31	4,39	4,36	4,11	4,38	4,47	4,62
	Sx	0,066	0,046	0,046	0,075	0,054	0,060	0,042	0,065
	lim	3,13-5,59	3,38-5,10	3,54-5,24	3,19-5,96	3,48-5,49	3,35-6,35	3,75-5,87	3,55-6,81
Milk proteins, kg	x	0,41	0,40	0,41	0,40	0,41	0,47	0,48	0,48
	Sx	0,017	0,014	0,014	0,015	0,016	0,117	0,008	0,018
	lim	0,21-0,84	0,20-0,59	0,21-0,73	0,23-0,77	0,24-0,81	0,24-0,87	0,20-0,61	0,17-1,15
Milk fat, kg	x	0,48	0,50	0,53	0,52	0,47	0,56	0,59	0,60
	Sx	0,075	0,013	0,015	0,009	0,162	0,018	0,018	0,023
	lim	0,36-0,64	0,39-0,59	0,43-0,63	0,38-0,71	0,27-0,87	0,27-1,17	0,26-1,14	0,18-1,34
Somatic cell count/ ml	x	526	542	471	406	875	535	367	183
	Sx	45,9	40,7	15,1	402	33,0	38,4	36,4	24,8
	lim	54-1848	40-1547	53-615	29-1515	23-1674	31-1956	31-1852	43-1284
The amount of lactose, %	x	4,22	4,31	4,31	4,35	4,30	4,46	4,37	4,36
	Sx	0,024	0,022	0,031	0,017	0,028	0,022	0,016	0,021
	lim	3,60-4,49	3,77-4,58	3,46-4,62	3,94-4,59	3,50-4,75	3,77-4,58	3,69-4,49	3,41-4,48
The amount of lactose kg	x	0,48	0,50	0,52	0,51	0,49	0,58	0,58	0,57
	Sx	0,012	0,011	0,016	0,015	0,009	0,011	0,016	0,014
	lim	0,27-0,73	0,26-0,66	0,23-0,81	0,30-0,88	0,27-0,75	0,30-0,84	0,26-0,87	0,18-0,88
Urea, mg %	x	44,50	27,10	26,80	23,61	45,9	24,3	24,5	19,97
	Sx	0,59	0,31	0,29	0,27	0,52	0,48	0,50	0,42
	lim	29-52	19,38	19,37	17,33	32-58	16-40	16-41	15-36

The data presented in the table above shows that 1.5 kg of kaolin E559 powder added to 100 kg of concentrated feed affected milk production during the first month of the study. The treatment group cows produced more milk that the control group cows; milk production increased by 1.22 kg (10.6%) and 0.13 kg (1.0%), respectively. During the second month of the study, the treatment group cows increased milk production by 0.70 kg (5.5%). It is 1.35 kg (1.7%) more as compared to the control group. During the third month of the study, milk production for the treatment group cows decreased by 0.39 kg (2.9%) and for the control group cows decreased by 0.20 kg (1.7%) as compared to the second month. During the whole study period, the average amount of milk produced by the control group was larger by 11.82 kg and the amount of milk produced by the treatment group was larger by 13.04 (10.32%). Throughout the study period, the average amount of milk produced by each cow from the treatment group was 1081.53 kg, whereas, from the control group it was 1193 kg; that is 111.63 kg (10.3%) more of the amount.

The addition of clay to food affected milk protein composition. Although in the beginning of the study milk protein level was higher by 0.07% ($p>0.05$) in milk from the control group cows, it has increased when food included clay supplements. During the first month of the study, milk protein level in milk produced by cows from the treatment group was higher by 0.34%, during the second month, by 0.24%, and during the third month, by 0.23%, as compared to milk produced by the control group cows. Clays also had a positive effect on fat content of milk. During all months of the study, cows fed with kaolin E559 produced milk with a higher fat content as compared to the control group. During the first month, the difference was 0.07%, during the second month, 0.08%, and during the last month, 0.26%.

The increase in milk production and improved milk composition resulted in increased fat and protein levels. During the study, cows from the treatment group produced 43.61 kg of protein, whereas cows from the control group produced 37.21 kg. The difference was 6.4 kg (17.2%). The total amount of protein in milk produced by one cow from the treatment group was 53.34 kg, whereas in the control group it was 47.27 kg. The difference was 6.07 kg (12.84%).

Cows from the treatment group produced milk having a higher level of lactose ($p>0.05$). Clay supplements affected the somatic cell count (SCC); it was lower. Even though, milk produced by cows from the treatment group had 349 000 more somatic cells before the study, one month later the somatic cell count was a bit lower as compared to the control group. During the last month of the study, the somatic cell count in milk produced by cows from the treatment group was significantly lower as compared to the count of the control group. The difference was 223 000 (54.93%) ($p<0.001$).

The findings of urea in milk have shown that the ration for cows was not balanced correctly before the study. When the ration was balanced, the level of urea in milk produced by cows of both groups normalized as well. However, the analysis of urea levels of an individual cow revealed that the ration of cows was not balanced correctly according to the protein levels. Thus, proteins were not properly used. Other authors, (for example, *Zainukov and Mironova, 2008; Morozov et al., 2009*) have also found out that bentonite clay has a positive effect on milk production. According to their studies, bentonite clay supplements increased milk production from 8.5% to 10.2% and fat content increased from 0.10% to 0.22%.

Koba (2008) have found out that natural zeolite-based additives in cattle feeding have significantly reduced the amount of heavy metals stored in their bodies.

The health status of cows was determined by visual assessments and blood samples which presented morphological and biochemical parameters. Visual observations of cows revealed that the hair coat of cows fed with kaolin E559 was shinier as compared to the control group. What is more, cows were more active and udder diseases rarely occurred. The morphological data of blood is presented in the table below (Table 4).

Table 4. The morphological data of the cattle blood

Parameters	**Treatment group**		**Control group**		**Norm**
	$\bar{x} \pm Sx$	lim	$\bar{x} \pm Sx$	lim	
Leukocytes (WBC), x 10^9 /L	12,72 ± 2,78	9,0-22,9	23,56 ± 5,04	11,9-55,60	5,0-16,0
Lymphocytes (LYMPH), x 10^9 /L	5,36 ± 1,84	2,6-14,2	15,42 ± 4,40	1,0-44,5	1,5-9,0
Monocytes (MON), x 10^9 /L	0,88 ± 0,10	0,6-1,3	1,08 ± 0,16	0,3-1,8	0,3-1,6
Granulocytes (GRAN), x 10^9 /L	6,48 ± 0,66	4,9-8,2	7,06 ± 1,50	2,3-9,8	2,3-9,1
Lymphocytes (LYMPH), %	37,52 ± 7,22	25,8-61,9	49,34 ± 10,16	29,1-79,9	20,0-60,3
Monocytes (MON), %	7,36 ± 0,62	5,9-9,0	6,24 ± 1,16	3,3-9,1	4,0-12,1
Granulocytes (GRAN), %	57,54 ± 6,94	32,2-66,9	44,42 ± 11,01	16,8-63,0	30-65,0
Erythrocytes (RBC), x 10^{12} /L	6,94 ± 0,38	5,30-7,2	6,63 ± 0,34	5,6-7,3	5,0-10,1
Hemoglobin (HGB), g/L	107,3 ± 3,10	93-109	97,6 ± 3,40	98-117	90-139

As presented in the Table 4, cows from the control group had an increased number of white blood cells ($p<0.05$). The analysis of leukocytes revealed that several cows from the control group were experiencing severe inflammatory processes. These findings explain why the somatic cells count was significantly higher in milk produced by cows from the control group. The fact that the number of somatic cells in milk produced by cows from the treatment group was significantly higher before the study than in milk produced by the control group must be consider as well. This confirms the assumption that bentonite clay helps reduce inflammation, neutralizes toxins and heavy metals, improves live functions, and increases productivity. The blood of cows from the treatment group was higher in hemoglobin as compared to the blood of cows from the control group. It contained 9.7 g/l (p<0.05). This suggests that metabolic processes were more intense for cows from the treatment group.

The biochemical data of blood is presented in the table below (Table 5). It shows that parameters of cows from both groups were within normal limits. However, there were some differences between both groups. The level of globulin in blood of cows from the treatment group was 5 g/l higher that in blood of cows from the control group (p<0.001). It suggests that their resistance is higher.

Table 5. The biochemical data of the cattle blood

Parameters	Treatment group		Control group		Norm
	$\bar{x} \pm Sx$	lim	$\bar{x} \pm Sx$	lim	
Glucose (GLU), mmol/L	10,3 ± 3,01	0,60-18,21	6,5 ± 0,53	4,6-9,9	0-34
Cholesterols (CHL), mmol/L	7,73 ± 0,35	6,0-8,09	7,32 ± 0,36	5,66-9,26	1,16-5,17
Hematocrits (HCT), %	29,07 ± 1,06	27,9-1,06	32,20 ± 0,80	29,0-35,6	28-46
Mean platelet volume (MPV), t/L	50,40 ± 1,20	46,0-54,3	48,8 ± 1,44	42,9-53,3	38-53
Cell hemoglobin mass (MCH), pg	16,50 ± 0,48	15,0-17,0	16,40 ± 0,70	14,2-17,5	13-19
Mean corpuscular hemoglobin concentration (MCHC), g/L	328,2 ± 3,20	324,0-340,0	331,2 ± 1,03	327-337	300-370
Red blood cell distribution width (RDW), %	16,1 ± 0,22	15,1-16,7	16,1 ± 0,34	15-16,7	14,0-19,0
Platelets (PLT), 10^9 /L	200,4 ± 7,60	121-309	172,8 ± 2,80	119-233	120-600
Mean platelet volume (MPV), t/L	6,60 ±0,26	5,9-7,1	6,40 ± 0,10	6,5-6,6	-
Thrombus volume (PCI)	0,130 ±0,021	0,076-0182	0,110 ± 0,015	0,074-0,151	-
Globulin (SLOB), g/L	48,0 ± 0,83	39,10-43,25	413,0 ± 0,75	45,0-48,90	30-49
Besoin protein (TP), g/L	77,0 ± 1,05	70,6-77,3	75,0 ± 0,85	74,3-79,0	62-80
Liver enzyme (ALKP), u/l	64,1 ± 2,10	58,0-67,5	51,8 ± 1,10	49,0-54,3	28-233

Cows from the control group had a lower platelet count as compared to the cows from the treatment group (p<0.05). The platelet count decreases because of liver, blood, and oncological diseases. Increased levels of platelet count in blood of cows from the treatment group shows that physiological processes in livers were more favorable to cows from the treatment group, even though the parameters of platelet from both groups were within normal limits.

The data of clay usage for cows housed outdoors is presented in the table below (Table 6).

Table 6. The data of kaolin E559 usage for cows housed outdoors.

Parameters	Month	Control group		Treatment group	
		$\bar{X}$	Sx	$\bar{X}$	Sx
Milk production, kg	I	12,52	0,61	12,99	0,51
	II	12,29	0,67	12,96	0,49
	III	10,76	0,56	12,02	0,63
Milk fat, %	I	4,19	0,18	4,27	0,16
	II	3,95	0,22	4,09	0,18
	III	4,17	0,42	4,16	0,09
Milk protein, %	I	3,39	0,08	3,45	0,04
	II	3,36	0,07	3,33	0,07
	III	3,84	0,14	3,80	0,08
Milk fat, kg	I	5,24	-	5,54	-
	II	4,85	-	5,30	-
	III	4,49	-	5,00	-
Milk protein, kg	I	4,24	-	4,48	-
	II	4,13	-	4,31	-
	III	4,49	-	4,56	-
Milk protein and fat, kg	I	9,48	-	10,02	-
	II	8,98	-	9,61	-
	III	8,98	-	9,56	-
Lactose, %	I	4,31	0,03	4,37	0,03
	II	4,35	0,02	4,28	0,05
	III	4,26	0,02	4,25	0,04
Lactose, kg	I	5,40	-	5,67	-
	II	5,35	-	5,54	-
	III	4,58	-	5,11	-
Urea, %	I	13,60	0,89	18,30	0,84
	II	24,60	0,70	22,20	0,45
	III	18,86	0,70	21,05	0,75
Somatic cells, thousand/ml.	I	213	42,51	207	31,12
	II	235	40,80	229	21,15
	III	207	30,00	201	22,36

According to the data presented in the table above, the individual differences between the control and treatment group are small and statistically unreliable. It can be suggested that cows eating natural grass increase their level of micronutrients. The fresh grass usually does not contain toxins which may appear in silos or haylage, thus, the usage of clay had no significant benefits.

Conclusions

During the indoor period, cattle fed 1 kg of combined food and 1.5 kg of kaolin E559 produced 10.3% more milk, the level of fat content was 0.09 % higher, and the level of protein was 0.28% higher as compared to the control group. The level of protein in milk produced by cows from the treatment group was 6.07 kg (12.8%) higher than in milk produced by the control group.

The somatic cells count was lower in milk produced by cows fed the clay supplement as compared to the control group ($p<0.001$).

At the end of the study, the level of leukocytes was significally higher in the blood of cows from the control group. This suggests that due to inflammatory processes, the SCC had increased as well. The blood of cows from the treatment group was higher in hemoglobin by 9.7 g/l ($p<0.005$) and in globulin by 5 g/l ($p<0.001$). Cows from the control group had a lower platelet count suggesting that physiological processes in livers were less favorable to the control group cows.

The clay supplements did not significantly affect cows housed outside ($p<0.05$).

Uticaj kaolina E559 na proizvodnju mleka i broj somatskih ćelija tokom perioda boravka mlečnih goveda u zatvorenom i otvorenom prostoru

Vigilijus Jukna, Česlovas Jukna, Vaidas Prusevičius, Edita Meškinytė-Kaušilienė, Žydrūnė Vyšniauskaitė

Rezime

Efekat kaolina E559 je proučavan na mlečnim govedima u toku perioda njihovog boravka u objektu, znači zatvorenom prosotru, i na otovorenom. Krave su podeljene u grupe za kontrolu i tretmane, od kojih svaka sadrži 40-45 krava. Krave su odabrane na osnovu starosti, faze laktacije, produktivnosti i roditelja. Tokom ispitivanja, krave su hranjene obrocima u zatvorenom prostoru.

Krave u obe grupe su hranjene istim koncentrovanim obrokom, prema proizvodnji mleka, 350 grama po kilogramu. Goveda u oglednoj grupi su dobijala 1,5 kg kaolinskog praha. Broj somatskih ćelija u mleku bio je niži kod krava koje su u obroku dobijale dopunu u obliku gline u odnosu na kontrolnu grupu ($p<0,001$).

Tokom perioda boravka u zatvorenom prostoru, krave koje su dobijale obrok – smešu u kombinaciji sa proizvodom kaolin E559, proizvele su 10,3% više mleka, nivo sadržaja masti je bio veći za 0,09%, a nivo proteina je bio viši za 0,28% u odnosu na kontrolnu grupu.

Na kraju ispitivanja, nivo leukocita je značajno bio veći u krvi krava iz kontrolne grupe. Krv krava iz ogledne grupe je imala veću vrednost za hemoglobin za 9,7 g/l (p<0,005) i globulin za 5 g/l (p<0,001). Dodatak gline nije značajno uticao na krave smeštene na otvorenom (p<0,05).

Ključne reči: kaolin E 559, mleko, proizvodnja, somatske ćelije

References

ADAMOVIĆ M., STOJANOVIĆ M., GRUBIŠIĆ M., ILEŠ D., MILOJKOVIĆ J. (2011): Importance of aluminosili cate minerals in safe food production. Macedonian Journal of Animal Science. Vol. 1. No. 1. P. 175–180.

AKAHOSHI, A., KOBA, K., ICHINOSE, F., KANEKO, M., SHIMODA, A., NONAKA, K., IWATA, T., YAMAUCHI, Y., TSUTSUMI, K., AND SUGANO, M. (2004): Dietary protein modulates the effect of CLA on lipid metabolism in rats. Lipids 39, 25-30.

ANDREJKOVIČOVÁ, S., SUDAGAR, A., ROCHA, J., PATINHA, C., HAJJAJI, W., FERREIRA DA SILVA, E., VELOSA, A., ROCHA, F., (2016): Theeffect of natural zeolite on microstructure, mechanical and heavy metals adsorption properties of metakaolin based geopolymers. Appl. Clay Sci. 126, 141–152.

ANI A. O., OGBU C. C., ILOH E. A. (2014): Response of broiler chicks to diets containing graded levels of clay. Journal of Animal and Plant Sciences. Vol. 24. No. 1. P. 30–34.

BAKUTIS B. Gyvulių auginimo sąlygų įtaka produktyvumui. Monografija. (2007): P. 126.

DEMIREL R., YOKUS B., SENTURK D., KETANI M., BARAN S. (2011): Effects of dietary zeolite on serum contents and feeding performance in rats. International Journal of Agriculture & Biology. P. 345–355.

HUWIG A., FREIMUND S., KAPPELI O., DUTLER H. (2001): Mycotoxin detoxication of animal feed by different adsorbents. Toxicology Letters. Vol. 122. P. 179–188.

KARDIŠAUSKAS A., KREGŽDYTĖ K. Pašarinio papildo kaolino E559 poveikis telyčių auginimui, vystymuisi ir reprodukcijai. Žemės ūkio mokslai. (2015): T. 22. Nr. P. 93 – 98.

KATOULI M., SAFAEI JAFARIAHANGARI Y., BAHARLOUEI A. (2011): An evaluation on the effects of dietary kaolin and zeolite on broilers blood parameters, T4, TSH and growth hormones. Pakistan Journal of Nutrition. Vol. 10. No. 3. P. 233–237.

LEE S., KIM Y., KWAK W. (2010): Effects of dietary addition of bentonite on manure gas emission, health, production and meat characteristics of Hanwoo (Bos Taurus Coreanae) steers. AsianAustralasian Journal of Animal Sciences. Vol. 23. No. 12. P. 1594–1600.

LIANG X., GAN Q., LIN J., QI B., LIU Q., DU Y., KE F., ZHENG S. (2013): Effects of bentonite on rat growth and organ microstructure. Journal of Animal and Veterinary Advances. Vol. 12. No. 6. P. 664–670.

MOROZOV, V., A. MOROZOV, AND S. LAGAYE. (2009): Endogenous JSRV like proviruses in domestic cattle: analysis of sequences and transcripts. Virology 367:59-70.

MURRAY H. (2002): Industrial clays case study. Mining, Minerals and Sustainable Development. Vol. 64. P. 1–9.

SIDOROVA O. J., NODU M. B., DIKE U. A., IDEOZU H. M. (2012): The effects of dietary kaolin (clay) as feed additive on the growth performance of broiler chickens. Greener Journal of Agricultural Sciences Vol. 2. No. 6. P. 233–236.

TRCKOVA M., MATLOVA L., DVORSKA L., PAVLIK I. (2004): Kaolin, bentonite and zeolites as feed supplements for animals: health advantages and risks. Veterinary Medicine – Czech. Vol. 49. No. 10. P. 389–399.

WAFAR R. J., GABDO I. I., BILHAM D. Y., ONALEYE K. J., OJINNAKA E., BILHAM R. Y. (2014): Effects of kaolin supplementation on growth performance, carcass characteristics and hematological indices of weaner rabbits. International Journal of Applied Sciences and Engineering Research. Vol. 3. No. 1. P. 91–98.

ZAINUKOV R.S., MIRONOVA I.V. (2008): Milk productivity and milk quality of bestuzhev firstcalf heifers fed rations supplemented with natural alumosilicate glauconite // Известия Оренбургского государственного аграрного университета. Т. 1. Nr. 22-2. С. 98-101.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

VARIABILITY OF THE BODY DEVELOPMENT TRAITS OF SIMMMENTAL COWS IN SERBIA

Dragan Nikšić[1], Vlada Pantelić[1], Dušica Ostojić-Andrić[1], Predrag Perišić[2], Marina Lazarević[1], Nenad Mićić[1], Maja Petričević[1]

[1] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding autor:draganniksic84@gmail.com
Original scientific paper

Abstract: Assessment of cows' exterior is important because of the assessment of the capability of animals to guarantee, through their overall exterior appearance as well as the appearance of certain parts of the body, not only their good health but also long production life (longevity). The main goal of measuring of domestic animals is to determine the body dimensions, to compare animals of the same or different species, to understand the numerous physiological and biochemical processes that occur in the animal organism. Only by measuring, the accurate and reliable data on the general physical development of the animal and the harmony of its structure are obtained. In the present study, most of the physical measures were taken on total of 954 animals. The average height of the cow of the Simmental breed was 141.95 cm, the length of the pelvis was 51.44 cm, the width of the pelvis was 50.45 cm and the body depth was 78.84 cm. By analyzing the impact of the unified factor of the rearing system and origin, its very significant influence ($p < 0.001$) was determined on all four properties of body development.

Key words: Simmental breed, body development, rearing system, origin

Introduction

The Simmental breed of cattle is most common in the Republic of Serbia. At the beginning of 2013, preliminary results of the Agricultural census carried out in the Republic of Serbia in 2012 were announced. According to the results of the census, Serbia has a total of 908,990 cattle, of which about 450,000 cows and heifers. In the breed structure of cattle in Serbia, it is estimated that the Simental breed makes about 85%, or about 360,000 cows and heifers (*Perišic et al., 2009 and Petrović et al., 2013*). Simental cattle are reared increasingly in intensive

conditions of housing, care and nutrition, i.e., the number of farms in Serbia with more than 50 females is increasing (*Bogdanović et al., 2012*).
In some areas of Serbia, semi-intensive rearing in the herds of several cows is still present. The genetic improvement of this breed is mainly performed by breeding and selection in pure breed (*Petrović et al., 2009*). The improvement of the genetic basis of the Simmental cattle population in our conditions is mainly done through quality bulls (Germany, Austria, etc.) and in recent years, the number of genetically high quality heifers imported from the best European populations of this breed has not been negligible.

The experience of the breeders shows that health, resistance, constitution, fertility, length of exploitation and productive characteristics largely depend on the development of the body, its structure and the individual parts of the body (*Perišić et al., 2008).* Deficiencies in the type characteristics lead to poorer production, poor health status and premature culling of cows from the herd (*Pantelić et al., 2007*).
Romčević (1999) in his monograph "Simental Cattle in Serbia" presents the morphometric measurements of bull dams of the Simental breed for 1995 and 1996: the height to withers for both years was 136 cm, the chest circumference (girth) 199 and 202 cm, and the body weight 697 and 692 kg, respectively.

The Institute for the Application of Science in Agriculture (1999), in the report on the conducted livestock breeding measures in Serbia for 1999, provides information on the exterior measures of 610 selected bull dams of Simmental breed: height to withers 136 cm, chest depth 72 cm, body length 163 cm, chest circumference (girth) 197 cm and body weight 697 kg.

According to the Report on the implementation and results of the Breeding program in 2014, the Institute of Animal Husbandry (2015) the values of body measures taken on bull dams were as follows: 143 cm (137-150 cm) height to rump, 81 cm (71-92 cm) for body depth, 59 cm (43-77 cm) length of the pelvis, 55 cm (41-62 cm) width of the pelvis and 201 cm (188-218 cm) for chest circumference (girth). The average weight of bull dams was 689 kg (551-822 kg).
Examining the morphometric properties and the incidence of foot deformities in cows in tied system of rearing, *Stojanović (2012)* states the following data on the average body dimensions of Simmental cows in Kolubara district: height to withers 134.3 cm, height to rump 137.1 cm, body length 157.1 cm, chest width 47.8 cm, chest depth 86 cm, chest circumference (girth) 200.5 cm, pelvis width 47.5 cm and tibia circumference of 20.6 cm.

In the breeding programs of European countries for Simmental breed, different traits are given and different values for them. Thus, in Croatia, the breeding objective for height to withers is 138 cm to 148 cm, and the body weight of an adult cow 650 kg to 750 kg; in Germany: height to withers 136-142 cm, height to rump 138-145 cm, body weight 550-650 kg, etc.

Material and methods

Basic data on morphometric properties, as well as data on the origin of all examined cows, were collected in cooperation with the dairy farm "Lazar" Blace, which housed certain number of animals included in this research. For animals reared on individual farms, data on these characteristics were collected in cooperation with the breeding organizations, which carry out all activities in the implementation of the breeding program in the area of Toplica district.

The total animals (n = 954) included in the study of morphometric traits were divided into four groups based on origin and rearing system, in the following way:

Group 1: animals of domestic origin reared by individual agricultural producers (n = 436);
Group 2: imported animals reared by individual agricultural producers (n = 68);
Group 3: domestic animals reared on farms (n = 282);
Group 4: imported animals reared on farms (n = 168).
The following traits of body development were taken after the first calving: height to rump, pelvis length, and body depth and pelvis width.

The analysis of the collected data consisted of determining the parameters of descriptive statistics (average, minimum, maximum, standard deviation, standard error of average and 95% confidence interval), while the analysis of variance, using the single factorial analysis model, examined the influence of the unified factor of the rearing system and the origin of the animals on their body development:

The model with a fixed unified influence of the rearing system and origin (NP):

$Yij = \mu + NPi + eij$

- Yij: examined trait,
- μ: population average for a given trait,
- NPi: a fixed unified influence of the rearing system and origin (i = 1,2,3,4),

E_{ij}: a random error

Subsequent to the analysis of the variance and determination of the basic parameters of descriptive statistics by the least significant difference test (LSD), the differences were determined by groups individually for all body development traits.

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

Results and Discussion

On the basis of the obtained results shown in Table 1, it can be concluded that the average height of the Simmental cows was 141.95 cm, the length of the pelvis was 51.44 cm, the pelvis width was 50.45 cm, and the body depth was 78.84 cm.

The highest height to rump was observed in imported cows reared on the farm (143.56 cm), and the lowest in domestic cows reared by individual agricultural producers (140.76 cm). The results obtained are lower than the results from the Report on the implementation of the Breeding program in Serbia, and higher than the results reported by *Stojanović (2012).* The results presented agree with the breeding goal in Germany for the Simmental cows. The difference between the groups was statistically very significant ($p \leq 0.001$) in the comparison of groups 1 and 2, 1 and 3, 1 and 4, 2 and 4, statistically significant ($p \leq 0.05$) between groups 3 and 4, while between groups 2 and 3, 2 and 4, no statistically significant ($p > 0.05$) difference was found (Table 2).

The value for the length of the pelvis was the highest among the cows of domestic origin reared on the farm (53.72 cm), and the lowest among the imported cows from reared by individual producers (51.11 cm). Comparing the obtained values according to the Report on the implementation of the Breeding program in Serbia in 2014 of the *Institute for Animal Husbandry (2015)*, it can be concluded that the first heifers from the import reared by individual agricultural producers had the length of the pelvis as bull dams in Serbia, and other three groups, individually, had lower values for pelvic length. Statistically very significant ($p \leq 0.001$) difference between all observed groups was established by the LSD test, except for groups 1 and 3 where the presence of statistically significant ($p > 0.05$) difference in pelvic length (Table 2) was not established.

Table 1. Mean value and variability of body development traits by groups of first calving heifers

Trait	Group	No. of calvings	$\bar{X}$	SD	SE	95% confidence interval		Min.	Max.
						LB	UB		
Rump height (cm)	1	436	140.76	3.163	0.151	140.46	141.06	134	151
	2	68	142.72	3.570	0.433	141.86	143.58	136	152
	3	282	142.65	4.229	0.252	142.16	143.15	134	151
	4	168	143.56	3.562	0.275	143.02	144.10	135	155
Total		**954**	**141.95**	**3.775**	**0.122**	**141.71**	**142.19**	**134**	**155**
		F=31.554***			**p=0.000**				
Pelvis length (cm)	1	436	51.14	1.681	0.081	50.99	51.30	42	57
	2	68	53.72	2.072	0.251	53.22	54.22	46	57
	3	282	51.11	2.401	0.143	50.83	51.39	44	59
	4	168	51.83	2.094	0.162	51.51	52.15	46	57
Total		**954**	**51.44**	**2.128**	**0.069**	**51.30**	**51.57**	**42**	**59**
		F=36.579***			**p=0.000**				
Pelvis width (cm)	1	436	50.05	1.820	0.087	49.88	50.22	39	54
	2	68	52.69	2.160	0.262	52.17	53.21	45	56
	3	282	50.02	2.675	0.159	49.70	50.33	41	61
	4	168	51.30	2.490	0.192	50.92	51.68	43	58
Total		**954**	**50.45**	**2.377**	**0.077**	**50.30**	**50.60**	**39**	**61**
		F=38.568***			**p=0.000**				
Body depth (cm)	1	436	77.83	3.401	0.163	77.51	78.15	66	87
	2	68	83.72	3.709	0.450	82.82	84.62	75	90
	3	282	78.84	4.169	0.248	78.35	79.33	69	85
	4	168	79.51	2.624	0.202	79.11	79.91	75	90
Total		**954**	**78.84**	**3.850**	**0.125**	**78.60**	**79.09**	**66**	**90**
		F=56.552***			**p=0.000**				

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

As well as the length, both the width of the pelvis was the highest among the cows of domestic origin reared on the farm (52.69 cm), and the lowest among the imported cows reared by the individual producers (50.02 cm). Based on the obtained results, it can be concluded that all four groups had significantly lower values than the values for the pelvis width of bull dams in Serbia, as stated in the Report on the implementation of the Breeding program in Serbia in 2014 by the *Institute for Animal Husbandry (2015*), significantly higher than the values stated by *Stojanovic (2012)* for cows in the Kolubara district. A statistically significant (p≤0.001) difference between all observed groups was established by the LSD test, except between groups 1 and 3 where the presence of statistically significant (p> 0.05) difference was not established, Table 2.

The greatest depth of the body was recorded for imported first calvers reared by individual producers (83.72 cm), and the lowest for domestic cows reared by individual producers (77.83 cm). If the obtained results are compared with the results stated in the Report and the results of the implementation of the breeding program in 2014 of the *Institute for Animal Husbandry (2015)*, only imported cows reared by individual agricultural producers have greater body depth than bull dams, while all four groups have much lower values for body depth than stated by *Stojanović (2012).* The least significant difference test (LSD) established statistically very significant (p≤0.001) difference between all observed groups in regard to the trait of body depth, Table 2.

The statistically very significant impact (p <0.001) on all observed properties was determined in the analysis of the influence of the unified factor (the rearing system, the origin) on the variability of the body development traits, which can be seen in Table 1.

Table 2. Differences of average for observed traits by groups of the first calving heifers (LSD test)

Rump height				**Pelvis length**			
group	2	3	4	group	2	3	4
1	-1.959***	-1.891***	-2.798***	1	-2.576***	0.031 nz	-0.683***
2		-0.068 nz	-0.839 nz	2		2.607***	1.893***
3			-0.907*	3			-0.714***
Pelvis width				**Body depth**			
group	2	3	4	group	2	3	4
1	-2.641***	0.033 nz	-1.247***	1	-5.893***	-1.012***	-1.678***
2		2.673***	1.394***	2		4.880***	4.215***
3			-1.280***	3			-0.666***

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

Conclusion

On the basis of the obtained results, it can be concluded that all the observed traits of body development were the most pronounced in imported cows reared by individual producers, with the exception of the height to rump which was the highest in the nimported first calvers reared on the farm.

By analyzing the effect of the unified factor (rearing system x origin) on the variability of the body development properties, a statistically very significant influence ($p < 0.001$) was established for all observed traits.

The least significant difference test (LSD) established statistically very significant ($p \leq 0.001$) difference to absence of difference ($p > 0.05$) among all observed groups.

Assessment of cows' exterior is important because of the assessment of the capability of animals to guarantee, through their overall exterior appearance as well as the appearance of certain parts of the body, not only their good health but also long production life (longevity). The main goal of measuring of domestic animals is to determine the body dimensions, to compare animals of the same or different species, to understand the numerous physiological and biochemical processes that occur in the animal organism. Only by measuring, the accurate and reliable data on the general physical development of the animal and the harmony of its structure are obtained.

Varijabilnost osobina telesne razvijenosti krava simentalske rase u srbiji

Dragan Nikšić, Vlada Pantelić, Dušica Ostojić-Andrić, Predrag Perišić, Marina Lazarević, Nenad Mićić, Maja Petričević

Rezime

Procena eksterijera krava je važna zbog ocene sposobnosti grla da svojim ukupnim spoljašnjim izgledom kao i izgledom pojedinih delovima tela garantuju pored dobrog zdravlja i trajnu proizvodnju (dugovečnost). Osnovni cilj merenja domaćih životinja jeste utvrđivanje telesnih dimenzija, međusobno poređenje životinja iste ili različitih vrsta, razumevanje brojnih fizioloških i biohemijskih procesa koji se dešavaju u životinjskom organizmu. Jedino se merenjem dobijaju tačni i sigurni podaci o opštoj telesnoj razvijenosti grla i harmoničnosti njegove građe. U radu je većina telesnih mera utvrđena na ukupno 954 prvotelki. Prosečna visina krsta krava simentalske rase iznosila 141,95 cm, dužina karlice 51,44 cm, širina karlice 50,45 cm i dubina tela 78,84 cm. Analizom uticaja objedinjenog faktora načina držanja i porekla, utvrđen je njegov vrlo visoko značajan uticaj ($p<0,001$) na sve četiri osobine telesne razvijenosti.

Ključne reči: simentalska rasa, telesna razvijenost, način držanja, poreklo

Acknowledgment

Research was financed by the Ministry of Education and Science, Republic of Serbia, project TR- 31053.

References

BOGDANOVIĆ V., ĐEDOVIĆ R., PERIŠIĆ P., STANOJEVIĆ D., PETROVIĆ M.D., TRIVUNOVIĆ S., KUČEVIĆ D., PETROVIĆ M.M. (2012): An assessment of dairy farm structure and characteristics of dairy production systems in Serbia. Biotechnology in Animal Husbandry, 28(4):689-696.

INSTITUT ZA PRIMENU NAUKE U POLJOPRIVREDI (1999): Selekcija stoke u Srbiji za 1999. godinu, Beograd.

INSTITUT ZA STOČARSTVO (2015): Stručni izveštaj i rezultati o sprovođenju odgajivačkog programa u 2014. godini, Beograd-Zemun.

PANTELIĆ V., SKALICKI Z., PETROVIĆ M.M., ALEKSIĆ S., MIŠČEVIĆ B., OSTOJIĆ-ANDRIĆ D. (2007): Phenotypic correlations of productive and reproductive traits of Simmental cows. Biotechnology in Animal Husbandry, 23(3-4):11-17.

PERIŠIĆ P., SKALICKI Z., PETROVIĆ M.M., BOGDANOVIĆ V., RUŽIČIĆ-MUSLIĆ D. (2009) : Simmental cattle breed in different production system, Biotehnologija u stočarstvu 25 (5-6), str. 315-326, Institut za Stočarstvo, Beograd-Zemun.

PERIŠIĆ P., SKALICKI Z., PETROVIĆ M. M., BOGDANOVIĆ V., TRIFUNOVIĆ G. (2008): Simentalska rasa u kombinovanoj i specijalizovanoj proizvodnji, Biotehnologija u stočarstvu, izdanje 25, posebno izdanje str. 25-37., Institut za stočarstvo Beograd-Zemun.

PETROVIĆ M.M., ALEKSIĆ S., PETROVIĆ M.P., PETROVIĆ M., PANTELIĆ V., NOVAKOVIĆ Ž., RUŽIĆ-MUSLIĆ D. (2013): Potentials of Serbian livestock production: Outlook and future Biotechnology in Animal Husbandry, 29(1):1-17

PETROVIĆ M.M., SRETENOVIĆ LJ., ALEKSIĆ S., PANTELIĆ V., NOVAKOVIĆ Ž., PERIŠIĆ P., PETROVIĆ M.D. (2009): Investigation of the heritability of phenotypes of fertility and milk performance of Simmental cattle breed in Serbia. Biotechnology in Animal Husbandry, 25(5-6-1):285-292.

ROMČEVIĆ, LJ. (1999): Simentalska goveda u Srbiji. Beograd.

STOJANOVIĆ M. (2012): Morfometrijske karakteristike i učestalost pojave deformacija nogu krava simentalske rase u vezanom sistemu držanja. Specijalistički rad. Poljoprivredni fakultet, Zemun. Univerzitet u Beogradu.

THE MODERN STATE OF SHEEP BREEDING IN RUSSIA

Yusup A. Yuldashbaev[1], Maria I. Dongak[1], Ksenia A. Kulikova, Elena V. Pakhomova[1], Zhaziraim M. Abenova[1], Salbak O. Chylbak-ool[1], Milan P. Petrovic[2]

[1] Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Faculty of Animal Science and Biology, Moscow, Russia
[2]Institute for Animal Husbandry, Belgrade, Serbia
Corresponding author: Yusup. A. Yuldashbaev, zoo@rgau-msha.ru
Review paper

Abstract: Sheep farming in Russia is developing dynamically in farms, but it is unable to compensate for the decline in production in the public sector. In years 2010 - 2016 import of mutton to the country has increased by 3.3 times, reaching 11 thousand tons (comparable to the volume of mutton production in agricultural organizations of the country). Compared with the pre-reform period the number of sheep and goats in the country in 2010 declined by 63% (36.4 million head). The negative trend of relief stock, which lasted until 2000, is now overcome by 2010 was able to increase the number of animals of 7 million head. Now the number of sheep and goats in farms of all categories totals 24 million heads. Giving new impetus to the development of sheep breeding is an important national economic task, in the course of solving which it is necessary to perform not so much a restoration as an update of the industry on the basis of the necessary structural, pedigree, technological changes. An important condition for its implementation is the activation of innovation. Scientists of academic, sectoral and university science make certain efforts in this area.

Key words: sheep breeding, farming, production, wool, meat, innovative activity

Introduction

Russia in the priority the national project "Development of the agro-industrial complex", the State program "The development of agriculture and the regulation of markets for agricultural products, raw materials and foodstuffs" emphasizes that it is possible to solve the accumulated problems in agriculture, to restore the disturbed reproduction processes, to overcome the gap from developed industrial countries to reorient the industry to an innovative path of development, which will increase production efficiency, reduce the country's dependence on food imports, to ensure the competitiveness and sustainability of domestic agriculture.

Sheep breeding requires special approaches to solving the tasks. This branch is traditional for the agriculture of Russia, especially the regions of the North Caucasus (*Aboneev et al., 2013)* and Kalmykia. For many years sheep breeding has been working extensively with the use of inefficient technologies. In this regard, there is a need to deal with issues related to the validity of the priority directions of the further development of the industry, the use of more efficient organizational and economic mechanism in the manufacturing and sales of products. An essential condition for their solution is the activation of innovation.

Problems of activization of innovative activity in modern sheep breeding have studied by *Yuldashbaev and Leshcheva (2011).* Management of industry in the small farms of ownership was studied by *Kiryanov (2013).* Problem production of sheep meat were investigated by *Trukhachev et al. (2012), Kubatbekov and Mamaev (2013).* Activization of the main problems of innovative activity in modern sheep farming was also reflected in the researches (*Yuldabashev, Lescheva, 2013).* Foreign experience in efficient production of sheep products was studied in the works of *Scherbakova (2006), Petrovic et al. (2013).*

Sheep breeds and their productivity depended by considerable variety of climatic, social and economic conditions of various Russian regions. Special attention should be given to sheep breeding development not only because of valuable products (mutton, milk, wool, etc.) but also as a factor ensuring the employment in regions not suitable for breeding other farm animals because of unfavourable climatic conditions (*Erohin et al.,1985;2001; 2013).*

The study aim was to analyze the modern state of sheep breeding in Russia and provide scientific views on the further development of this important sector of animal husbandry.

Status and prospects of sheep breeding

Analyzing the changes that have occurred in the domestic sheep industry in recent years, in the first place, it should be noted a significant reduction in the number of livestock and the restructuring of the sheep breeding complex.

Compared with the pre-reform period the number of sheep and goats in the country in 2010 declined by 63% (36.4 million head). The negative trend of relief stock, which lasted until 2000, is now overcome by 2010 was able to increase the number of animals of 7 million head. Now the number of sheep and goats in farms of all categories totals 24 million heads.

The distribution of livestock by categories of farms has changed significantly. If in 1990 70.6% of sheep were kept in agricultural organizations and 29.4% in personal part-time farms of the population, at present the share of sheep and goats in agricultural enterprises had only 20%, in households was 52%, 28 % in peasant (farm) households. Currently, the population's economy produces 54% of wool and 72% of lamb, the share of K (F) X is 26% and 19% respectively, of agricultural organizations - only 20 and 9%.

Agricultural organizations have lost a leading role in the production of wool and sheep meat. Many of the breeding farms lost their significance, a significant part of the genetically most valuable livestock was sold as commodity animals.

Agricultural organizations have completely reduced the number of sheep in Ascania, Vyatka; Gorky; Kuchugurov breeds. On the verge of extinction are Kuibyshev, Russian long-haired breeds. The Salsk breed of sheep has 2.1 thousand heads, and the Lincoln is the Kuban type with only 800 heads. The small number of these breeds of sheep can be in the coming years lead to their complete disappearance from the territory of the Russian Federation and will cause damage to the gene pool of sheep country.

More than half of the sheep are concentrated in private farms of the population with very limited possibilities for breeding work, the intensification of production, the use of science. Reproduction in them is carried out on an extensive basis and does not provide acceptable rates of growth and production volumes.

On average, over the past 10 years, the average annual growth rate of wool production was 3.3%; Mutton - 2.7%.

Sheep farming is developing dynamically in farms, but it is unable to compensate for the decline in production in the public sector. In 2010 - 2016 years, import of mutton to the country has increased by 3.3 times, reaching 11 thousand tons (comparable to the volume of mutton production in agricultural organizations of the country).

With 80.4 million hectares of natural hayfields, pastures and fallow land, Russia imports not only wool, lamb, 95% of which comes in frozen form, but also by-products. The production of sheep products for commodity producers is unprofitable, the organizational, economic, technological and technical backwardness of the industry takes place. The negative consequences of this are manifested not only in economic, but also social aspects, lead to incomplete use of pasture lands, and in some cases, loss of control over the territories. The current situation does not meet national interests.

Scientific aspects for improvement of sheep breeding

Giving new impetus to the development of sheep breeding is an important national economic task, in the course of solving which it is necessary to perform not so much a restoration as an update of the industry on the basis of the necessary structural, pedigree, technological changes. An important condition for its implementation is the activation of innovation.

Scientists of academic, sectoral and university science make certain efforts in this area.

In recent years, researchers of scientific institutions have created fourteen fundamentally new types and breeds of sheep with increased productivity and consumer properties of the products: southern meat; Buubei; Aginskaya; Kulunda; Tashlin breeds; Type solar (tsigay breed); Mountain type (Tuva short-tailed rock); Steppe type (Tuva short-tailed rock); Aksaray type (Soviet meat and wool breed); Udmurt type (Soviet meat and wool breed); Dogoy type (Transbaikalian rock); Argument type (Transbaikalian rock); Kadutun type (Mountain Altai breed).

New resource-saving technologies have been created, recommendations have been developed for the modernization of existing methods of production of sheep breeding, which significantly improve the profitability of the industry, namely: innovative technologies for the production of broiler lamb; System of feeding young sheep with intensive cultivation and fattening; Low-cost technology of pasture-stables maintenance of sheep; A system for assessing the hereditary qualities of breeding animals using genetic markers and DNA technologies. Methodical provisions have been developed for obtaining, staining, and creating a cryobank of epididymal seed of rare, endangered species of argali, snow sheep, Siberian ibex, Edilbaev, Hissar and Romanov sheep breeds; Method of integrated assessment of sheep-producers on the quality of offspring; A method for estimating and predicting meat productivity at an early age on the basis of blood groups of DNA markers of sheep; Biotechnological methods for assessing the productivity of sheep, methodological and methodological recommendations for the creation of sheep maintenance and feeding systems, biotechnological approaches to product control and selection assessment using computer programs, cryopreservation of sperm of rare and endangered sheep and goat breeds, mobile chip systems, improved machine systems and Equipment, etc.

However, the efforts of Russian scientists are minimized by an ineffective system for managing the innovative development of the industry. Branch government bodies, scientific, educational institutions, agricultural producers are a poorly coordinated community. The lack of a centralized directing influence from the sectoral research institute and the necessary infrastructure hampers the introduction of innovations in practical sheep breeding, the rate of promotion of new products and processes in sheep breeding is very low compared with the potential one.

In these conditions, it is required to form a certain system-structured education of an organizational and information character in the management of various aspects of innovation activity, as well as the use of mechanisms and tools to expand innovation that provide the opportunity for practical implementation of this task based on the integrated use of information technology.

The National Union of Sheep-breeders was created, the program "Development of sheep breeding for 2010-2020 was developed. And the plan for the breed placement of sheep for the federal districts, the organizational and economic assessment of standard models of industrial-type sheep farms, farms and households is given, organizational and economic normative indicators of effective sheep breeding are determined, the information-analytical system "Selex-sheep" is prepared for implementation; The software is developed and the base of selection-genetic data is formed. To strengthen the capacity of the industry in the country's universities, the Master's program is being prepared according to the program "Intensive technologies in sheep breeding".

However, the gap between the scientific provision of sheep breeding and the practical implementation of innovations has not been overcome. The main problem of implementing the innovative strategy for the development of the industry is that, even with new breeds and technologies in the industry, channels for their introduction into practical sheep breeding are not developed and there are no qualified personnel for their development. The level of financial, personnel, logistical and information support of the industry is extremely low. This determines the low susceptibility of rural commodity producers to innovations. Of great importance in this regard is the organizational and economic support for the development of innovations in the mass production of sheep products by improving the organization of the innovation process and the economic stimulation of innovation.

Innovative activity by country is as follows: USA - 50%; Netherlands - 62%; Austria - 67%; Germany - 69%; Denmark - 71%; Ireland - 74%; Russia - 5-7%.

It should be borne in mind, however, that successful innovation activity is far from always associated with own extraction and development of new knowledge. In the agro-industrial complex, the financing of new developments is limited by the shortage of financial resources, the lack of the necessary institutional infrastructure for the development of innovative institutions, and a weak market infrastructure, so innovations, as such, are not of a mass nature, their share in the cost of domestic agri-food products is very small. In this regard, the most relevant for the industry is the use of a broader approach, according to which, by innovation, we mean not only investing in the creation of something new, but also investing in the acquisition of innovations, including the practical implementation of the world knowledge base.

The main directions of activization of innovative activity in modern domestic sheep breeding are:

• Improvement of the organization of the industry as a whole, since until the general issues are resolved, the introduction of innovations will not be effective;

• Development and implementation of integrated scientific and technical programs for the development of sheep breeding;

• improving the organizational forms of innovation; Development of management and increasing the manageability of the innovation process.

• economic stimulation of innovation activity, consisting in the formation and effective functioning of the market of scientific and technical products; Strengthening the work on the commercialization of scientific and technical developments, increasing the interest of research institutes and scientists in this; Support of innovative activity on the part of the state; Granting benefits to business entities for the introduction of innovations.

Scientific developments should be more focused on the actual state and requirements of the industry. To increase the susceptibility of agricultural producers to innovations, it is required to understand that the main sphere of realization of innovation activity in sheep breeding is now the rural farmstead. In view of this, it is necessary to implement measures of an organizational and economic nature that ensure the activation of innovative activity, in particular, at a qualitatively new level, organize the work of the tribal service, and more fully implement the principles of cooperation and integration in the forms of management, which make it possible to accelerate the advancement of innovations in the business environment.

Conclusion

Analyzing the situation and development opportunities of sheep breeding in Russia, we can conclude the following.

Our country has enormous natural and human resources for the development of sheep production. New trends of economic development have caused a new approach to the organization and management of sheep production. After the fall of the number of sheep in the nineties, the trend is turning positive, especially in the last few years. There are new and modern private farmers. Many of our agricultural universities and institutes have the knowledge, scientific experience and capacity to introduce modern technological solutions open up new prospects for development of sheep breeding Russia, as one of the World's leading countries in this field of sheep production.

Moderno ovčarstvo u Rusiji

Yusup A.Yuldashbaev, Maria I. Dongak, Ksenia A. Kulikova, Elena V. Pakhomova, Zhaziraim M. Abenova, Salbak O. Chylbak-ool, Milan P. Petrovic

Rezime

Ovčarstvo u Rusiji se dinamički razvija na farmama, ali nije moguće nadoknaditi pad proizvodnje u javnom sektoru. U periodu od 2010. – 2016. godine, uvoz ovčijeg mesa u zemlju porastao je za 3,3 puta i dostigao 11 hiljada tona (u poredjenju sa obimom proizvodnje ovčetine u poljoprivrednim organizacijama zemlje). U poređenju sa periodom pre reforme, broj ovaca i koza u zemlji u 2010. smanjen je za 63% (36,4 miliona grla). Negativni trendovi u stočarstvu, koji su trajali do 2000. godine, sada su prevaziđeni, do 2010. godine broj životinja je povećan za 7 miliona. Sada je broj ovaca i koza na farmama svih kategorija ukupno 24 miliona grla. Davanje novih podsticaja razvoju ovčarstva je važan nacionalni ekonomski zadatak, u toku rešavanja kojih je neophodno izvršiti ne toliko restauraciju, koliko ažuriranje industrije na osnovu potrebnih strukturalnih, kao i tehnoloških promena. Važan uslov za njegovu implementaciju je aktiviranje inovacija. Naučnici akademskih, sektorskih i univerzitetskih nauka vrše određene napore u ovoj oblasti.

Ključne reči: uzgoj ovaca, poljoprivreda, proizvodnja, vuna, meso, inovativna delatnost

References

AMERKHANOV KH.A. (2010): Comparative characteristics of productive qualities of different types of Tuvan short-tailed sheep // Kh.A. Amerkhanov, S.I. Biltuev, L.D. Shimit, B.B. Mongush, Yu.A. Yuldashbaev Sheep, goats, woolen business, №2, 16-17c.

ЕРОХИН А.И., ЕРОХИН С.А. (2001): Овцеводство / под ред. А.и.ерохина. – М. Изд-во мгуп, 480 с.

EROHIN A.I, RIBIN G.I, YULDASHBAEV YU.A., LEŠCEVA M.G. (2013): Development of meat sheep breeding in central Russia. Sheep goat and wool, 1, 2-9.

KUBATBEKOV TS, MAMAEV S.SH. (2013): Slaughter indicators of the sheep of the Kirghiz fine-fleeced breed of different ages // Sheep, goats, woolen business, No.3, 30-31.

On the current situation in the agro-industrial complex of the Russian Federation in October 2016 [Electronic resource]. - Access mode: http://www.mcx.ru. - The title from the screen. - (Date of circulation: 14.11.2016). 8. Sheep population in different countries [Electronic resource] - Access mode: FAOSTAT database.

PETROVA, T. A. (2012): Повышение эффективности функционирования отрасли овцеводства на основе формирования инвестиционной привлекательности, Ph.D. dissertation, Saratov State Agrarian University n.a. N.I. Vavilov, Saratov, Russia.

PETROVIC, M. P., PETROVIC, V. C., ILIC, Z. G., MILOSEVIC, B., MILENKOVIC, M., SPASIC, Z.,STOIKOVIC, J. (2013): Состояние и перспективы овцеводства в Сербии, Sheep, goats and wool production, vol. 1, pp. 13-15.

TRUKHACHEV VI, LESHCHEVA MG, YULDASHBAEV YU.A. (2012): Meat market of Russia: analysis of the state and prospects of development // Achievements of science and technology of agroindustrial complex, №11, 3-9.

Yearbook (2016): Breeding work in sheep breeding and goat breeding in Russian farms / VNIIPLEM Publishing House, p.42; p. 71.

YULDASHBAEV YU. A., LESHCHEVA M.G. (2011): Problems of activization of innovative activity in modern sheep breeding // Achievements of a science and engineering of agrarian and industrial complex, No. 11, 6-8.

YULDASHBAEV YU.A., ARILOV A.N., NEZOVANA V.F., BACHAEV B.T. (2010): Kurdjuchevoe sheep breeding - the factor of increase of meat resources of Kalmykia // Zootechnics, № 5, 12-13.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

SHEEP BREEDING ECONOMIC, PLUSES AND MINUSES

Marina I. Selionova, Galina T. Bobryshova

FSBSI -All-Russian Research Institute for Sheep and Goat Breeding, Stavropol, Russia
Corresponding author: Marina I. Selionova, m_selin@mail.ru
Review paper

Abstract: In the article the comparative analysis of economic and industrial parameters in sheep breeding of the Stavropol Territory in the period from 1976 to 1990 and now is resulted. The role of state support in the stabilization of sheep breeding and its further development is considered. Over the years of economic transformations, the number of sheep in the Stavropol Territory has declined by more than 7.1 times since 1990, and in agricultural organizations it has amounted to 11.8 times. Wool and mutton volumes of output have decreased by 10.5 and 6.7 times, respectively. The number of sheep for this period was reduced in 3 times as a whole across Russia. If in 1990 the sown area of cereal crops in the Stavropol Territory was 1792.2 thousand hectares, then in 2010 it was 2138.9 thousand hectares. The area of fodder crops for this time has decreased in 6 times and has made in 2010 only 216.5 thousand hectares. In addition to reducing the number of sheep, the ownership structure has changed and profit is the main driver of the development of this business. Today it is quite clear that sheep breeding in Russia cannot become a profitable branch without balanced comprehensive state support. The package of measures should include the regulation of pricing processes, the elimination of prices disparity for agricultural and industrial products, subsidies in amount covering production and sales costs, preferential taxation and lending, and protection of domestic market from foreign commodity producers.

Key words: sheep breeding, sheep breeding products, wool, mutton, proceeds, production costs, selling price, profitability, state support.

Introduction

"Sheep is the most profitable animal A man only can have"
Fitzherbert

A large number of factors of genetic and external nature affect the development of sheep farming *Petrovic et al., (2013), Selionova et al.,(2015), Vershinin (2015).*

The state and the prospects of sheep breeding depend in many respects on the economic conditions of branch conduct, the conjuncture of domestic and foreign markets for sheep breeding products. The general law of economic efficiency that is products realization at the price exceeding the cost of its production, to the full relates to sheep breeding. At the same time, it is well known that not all branches and sub-sectors of the AIC are profitable and favorable from the economic point of view. There are a number of objective reasons, primarily such as protection of domestic market, employment of the population and others, on which the state subsidizes the production of agricultural products, in order to bring it to a profitable level. Analysis of schemas and mechanisms of state support and their effectiveness in the stabilization and development of sheep breeding in Russia over the past period has determined the relevance of this article.

Economic indicators of sheep breeding

One of the economic advantages that make sheep breeding attractive to other types of farm animals is the small amount of initial financial investments (Table 1).

Table 1. Charges on one animal in a year, rubles
(According to data of agricultural organizations of the Stavropol Territory)

Species of animal	Years 2013		2014		2015	
	In total	For 1kg of live weight	In total	For 1kg of live weight	In total	For 1kg of live weight
Horned cattle (dairy direction)	94221	57.0	107284	178.8	116050	193.4
Horned cattle (meat direction)	19184	38.4	20703	41.4	19251	38.5
Pigs	9458	47.3	11103	55.5	11998	60.0
Sheep	1831	36.6	1980	39.6	1950	39.0

The ability of sheep to use all year round pasture forage almost free of charge, transforming it to wool, meat and milk, reduces expenses of production to a minimum. For the keeping of sheep, you can use any cattle barns, and depending on the weather conditions you can manage without them at all. The sheep uses those areas of agricultural land, where plowing of land by natural topographical conditions is impossible and grazing is the only possible means for its use.

Recently, the important factor, to which the increasing importance is attached, is the ecological purity of the resulting cattle-breeding products. And in this sense, meat and milk of sheep have certain advantages, since sheep are kept almost everywhere on natural pastures, and in their feeding hormones and other biological active substances of anabolic character are not used. Milk of sheep is an excellent and sometimes irreplaceable raw material for the production of elite cheese grades and other kinds of dairy products. In its nutrition, sheep's milk is far superior to cow's milk, contains a large number of substances and minor elements useful for the human body, and at the same time can be assimilated by more than 90.0 percent. Among other things, sheep feel perfectly near to other farm animals.

Nevertheless, all these advantages are not reflected in the price of sheep products, and up to now this branch is unprofitable as a whole (Table 2).

Table 2. The main economic indicators of sheep breeding in the Stavropol Territory (according to data of agricultural organizations of the Stavropol Territory)

Indicators	Years		
	2013	2014	2015
Expenses for production of products, thousand rubles	760666.0	766333.0	853564.0
Gain from sales of production, thousand rubles	438269.0	395782.0	518046.0
Profit from sales of production, thousand rubles	-205645.0	-273304.0	-251507.0
Profitability, %	-32.0	-41.0	-33.0

In the current economic conditions, agricultural organizations continue to decline in the number of sheep and falling-off of production, since with the existing ratio of costs and incomes agricultural producers cannot carry out extended reproduction.

Over the years of economic transformations, the number of sheep in the Stavropol Territory has declined by more than 7.1 times since 1990, and in agricultural organizations it has amounted to 11.8 times. Wool and mutton volumes of output have decreased by 10.5 and 6.7 times, respectively. The number of sheep for this period was reduced in 3 times as a whole across Russia.

Sheep breeding recession is also promoted by reduction of the fodder areas as a result of plowing of hayfields and pastures for growing grain crops, which profitability unlike sheep breeding is high (Table 3).

Table 3. Profitability of grain and industrial crops production in the Stavropol Territory (according to the data of the Territorial Body of the Federal State Statistics Service for the Stavropol Territory)

Appellation	Years				
	1990	1995	2005	2010	2015
Grain	292.0	115.0	18.1	19.9	47.0
Sunflower	208.0	181.1	47.6	95.6	83.0
Sugar beet	89.3	43.2	18.3	48.7	56.0

If in 1990 the sown area of cereal crops in the Stavropol Territory was 1792.2 thousand hectares, then in 2010 it was 2138.9 thousand hectares. The area of fodder crops for this time has decreased in 6 times and has made in 2010 only 216.5 thousand hectares.

The situation that develops in the sheep breeding of the Stavropol Territory repeats the events of 100 years ago. In 1902, there were 3.5 million sheep in the Stavropol province, and in 1911 there were 1.9 million sheep left. In 1912, Mordvin L.N., the senior animal husbandry specialist of the Stavropol province wrote:”... with the development of agriculture and communication lines, sheep breeding becomes an unprofitable occupation, and the number of sheep begins to decline faster”, “... at best, the sheep gives the gross income from 3 to 4 rubles per tithe; the value of the pure rent for plowing in most of our regions is about 15-18 rubles for corn, about 25 rubles for sunflower sowing, and 40 rubles for beet sowing”. “ My visit to the sheep farms of the Stavropol province, undertaken in this year (1912), revealed that these farms are in the period of gradual elimination of sheep breeding and the transition to grain crops; sheep breeders, who had tens of thousands of heads have left now 1500-2000 sheep at themselves, and then they want to stop sheep breeding. Fine-fleeced sheep breeding in the Stavropol province remains only on unsuitable lands or as a by-product branch at a large grain economy for the purpose of using the waste of this economy” (*Mordvin, 1912*).

Speaking about the period of the greatest development in sheep breeding, scientists and practitioners often turn to the “golden” 70-80 years of the last century, when the number of sheep in the Stavropol Territory reached a maximum, and it was profitable to engage with sheep breeding. In order to understand what factors contributed to this, we will analyze the situation that has developed in the sheep breeding of the Stavropol Territory since 1976.

In those years, the number of sheep in the Stavropol region exceeded 6.5 million heads. More than 3.0 million heads of sheep were kept only in the east of the region. All sheep inventory were kept in agricultural organizations, and only 10.0 percent, or about 800.0 thousand heads, were kept at the farmstead of the population. Annually more than 30.0 thousand tons of wool, which was the main product of sheep breeding at that time, was produced in physical weight.

Table 4. Economic indicators of wool production in the Stavropol Territory (according to the data of the Territorial Body of the Federal State Statistics Service for the Stavropol Territory)

Indicators	Years								
	1976	1980	1985	1990	1995	2000	2005	2010	2015
Profitability level, %	-24.4	-8.3	11.3	35.6	-65.7		-69.0	-72.5	-61.0
Cost of 1 centner production, rubles	609	742	862	995	1351000		9227	11977	22290
Selling price of 1centner, rubles	509.6	672.0	951.8	1325.0	410145.3		2810.4	4254.8	8741.0
Number of sheep, thousand heads	5858,3	6192.5	6433.7	6337.6	3408.2	1352.9	1500.0	2212.9	2276.9
Wool production, thousand tons	27.7	28.3	30.7	33.3	14.0	6.2	6.0	7.0	6.8
Wool clip from 1 sheep in physical weight, kg	4.3	4.3	4.4	4.5	3.6	3.8	3.8	3.7	3.9
Lambs dropped per 100 ewes, heads	73	86	82	84	64	77	77	82	82

Table 5. Economic indicators of mutton production in the Stavropol Territory (according to the data of the Territorial Body of the Federal State Statistics Service for the Stavropol Territory)

Indicators	Years								
	1976	1980	1985	1990	1995	2000	2005	2010	2015
Profitability level, %	-0.8	-31.0	-30.4	26.8	-40.3		-6.3	-4.9	-10.0
Cost of 1 centner production, rubles	109.0	152.0	187.0	226.0	339547.0		3245	4382	9452.0
Selling price of 1 centner, rubles	72.8	97.2	161.9	338.3	122784.4		2313.1	4077.6	7023.2
Mutton production in live weight, thousand tons					39.7		25.7	35.6	

The data in Tables 4 and 5 show that in the best of their years, as in today, the situation in the economy of the Stavropol Territory was ambiguous. The production of both wool and mutton was and still is unprofitable.

The situation in other sheep-breeding regions of the country is similar. In the Republic of Dagestan (according to the data of the agricultural organizations) in 2015, the production cost of 1kg of wool was 29.7 rubles, and the selling price was 24.1 rubles, as a result, the loss ratio amounted to minus 18.9 percent. The profitability level of mutton production has made 4.8 percent at the cost price of 54.4 rubles per 1kg and the selling price of 57.0 rubles. In 13 districts of Dagestan mutton production was unprofitable.

The Republic of Kalmykia, the second for the number of sheep and production of sheep-breeding products region, also has ambiguous indicators on the sheep breeding economy. In 1995, the profitability of wool production has made minus 67.0 percent, and in 1999 it reached almost 0.13 percent. The profitability of mutton fluctuated within 45.0-50.0 percent (Taunova, 2000).

As a whole, the sheep breeding in Kalmykia continues to be profitable, but a certain proportion of sheep farms has a negative economy or is unprofitable. In 2006, the profitability of sheep breeding in this republic has made 9.6 percent, according to the result of 2015 it was X percent.

In various years, analysis of the sheep breeding economy in the Trans-Baikal Territory shows significant fluctuations. Thus, in the early nineties of the last century, the profitability indicators of the branch in agricultural enterprises were very high; the profitability of wool production has made 27.8-139.8%, while for mutton production it was 53.0-93.5%. However, since the md-nineties the situation has changed drastically and the profitability of the wool has dropped to minus 85.6%, while the profitability of mutton decreased to minus 66.7%; in 2013-2014, it has made minus 47.3-48.1%, respectively, and for mutton it is minus 0.17-9.1%. Over the period from 1990 to 2000, the sheep population in the province decreased by almost 5 million or 80 percent (*Vershinin, 2015*).

Thus, the analysis of the sheep breeding economy shows that the best period for the development of the branch falls at 1985-1990. In this regard, it is important to determine, what economic conditions were created by the state, how the sheep breeding yielded a high income under various climatic conditions and other factors in different regions of the country, and sheep farms were millionaires.

Measures of the Government

One of such factors in the pre-reform period was the regulation of the sheep breeding profitability in agricultural organizations by surcharges to purchase prices. Thus, in the Resolution No. 1032 of the Council of Ministers of the USSR from November, 14th, 1980 “On Improving Planning and Economic Incentives for the Production and Procurements of Agricultural Products” it is stated: “To establish that in 1981-1985 collective farms, state farms, other agricultural enterprises and associations are paid a surcharge in a size of 50 percent of purchase prices for sale of wool to the state over the middle level achieved in the tenth five-years period".

In addition, the procurement prices included all operating differentiated extra charges, surcharges for sale to the state of products above the average level achieved in the previous five-year plan, and so on. (Resolution of the Council of Ministers of the USSR No. 792 from August, 9th, 1990 "On the Introduction of New State Procurement Prices for Agricultural Products")

In 1993, the Government of the Russian Federation adopted Resolution No. 629 "On Measures of State Support for Sheep Breeding in the Russian Federation", which provided for the establishment from July, 1st, 1993 of a subsidy for wool sold to the state resources in the amount of 600 rubles per 1 kg in terms of pure fiber (300 rubles per 1 kg in physical weight). At the same time, the cost of 1 kg of wool has made 4933.1 rubles in physical weight, and the selling price was 4559.7 rubles. However, the amount of state support did not cover the difference between the cost price and the sale price and did not allow the production of wool to be profitable.

The same resolution stipulated that from centralized credit resources for 1993, a preferential loan at a rate of 25 milliard rubles should be allocated to the enterprises of primary processing of wool for the purchase of a haircut of 1992 and 50 milliard rubles for the purchase of haircut of 1993. And with the purpose of ensuring the protection of the Russian wool market, starting from July, 1st, 1993, the rates of import customs duties on wool import should be increased meaning an establishment of the price for imported wool for 10-15 percent higher than on wool of domestic production.

However, these measures also did not have a stimulating effect on the stabilization of sheep breeding. In 1993-1995, the sheep population in the Stavropol Territory decreased by 2.0 million heads, including 1.9 million heads in agricultural organizations, where an array of the best fine-fleeced sheep was concentrated. The volume of wool production decreased by 6.2 thousand tons or by 30.0 percent. As a whole, in Russia, the reduction in the number of sheep since the year has made 48.0 percent, and in production of wool it was 40.0 percent.

The new Resolution No. 1092 of the Government of the Russian Federation "On Measures of the State Support for Sheep Breeding" was adopted in 1995. The resolution provided for the following: "To pay agricultural producers differentiated on subjects of the Russian Federation subsidies for the wool sold in 1995 to state resources at the expenses of the funds provided for in the federal budget for 1995 for these purposes, as well as for providing state support to the agrarian industrial complex. It was also made provision for the allocation to the enterprises of primary wool processing on a return basis 100 milliard rubles for the wool purchase.

Unfortunately, this decision also did not have that effect which the state counted on; the sheep population in Russia continued its reduction to 13.2 million heads by the year 2000. In the current economic conditions, the enterprises could not overcome the trend of a decline in production, since with the existing ratio of expenses and incomes agricultural commodity producers could not carry out extended reproduction.

And only the implementation of the national project “Development of the AIC”, and then the State program for the development of agriculture and regulation of markets for agricultural products, raw materials and food, providing subsidies on charges for ewes’ stock keeping, has stopped the catastrophic decline in the number of sheep. In the period from 1990 to 2015, the number increased by xx million sheep, and currently there are almost 25 million in Russia, including 2.2 million in the Stavropol region.

At the same time, it should be noted that in this period there have been significant changes in the breed structure of sheep breeding in Russia. Specific weight of fine-fleeced sheep decreased from 90% in 1990 to 56.0% in 2015, respectively, the specific weight of sheep with coarse-woolen direction of productivity and crossbreds number increased from 3.5% to 38.4% or more than 10 times. A significant reduction in the number of fine-woolen sheep, a decrease in their productivity against the background of widespread deterioration of selection and breeding work and a general culture of sheep breeding conducting led to destabilization of primary processing enterprises and the textile industry of the country. In addition, the volatility of the Russian ruble in the past two years has contributed to an even greater competitiveness of Russian merino wool in the world market, and the domestic wool processing industry has practically remained without high-quality wool raw materials. Considering this circumstance, the state introduced a new type of support in 2015, it was subsidizing of fine and half-fine wool production. In particular, the Stavropol sheep breeders have received on 37.0 rubles for each kilogram of certified wool with a thinness of up to 23 micrometers realized for domestic processing. However, this support has not yet made the production of wool cost-effective. Perhaps, the envisaged increase in the size of subsidy will allow sheep breeders to reach profitable production and will serve as an incentive for increasing the number of Merino sheep in agricultural organizations and peasant farms.

However, leading economists note that direct subsidization of products in the agricultural sector cannot be an endless process. It is necessary to search for other incentive measures, at which consumer prices for agricultural products cover the costs of their production (Vershinin, 2015, Amerkhanov et.al., 2015).

One approach, on our opinion, can be the state control of prices for animal husbandry products, in particular, wool and mutton. In most developed countries, indirect subsidy measures prevail, including price intervention in the food market through supporting domestic prices for agricultural products, setting quotas, tariffs, taxes on exports and imports of food. We are talking about the so-called price corridor, when the minimum price for products brings profitability of production to zero or minimum level, and the maximum price does not allow us to receive super-profit. If the minimum price falls below the zero mark of profitability, then there are subsidies for products that allow agricultural producers to conduct breakeven production.

Conclusion

The foregoing allows us to conclude that the state always and to date to a greater or lesser extent supported sheep breeding, searched for most optimal and effective measures for stabilizing and developing the branch, regulating the production volumes of wool and mutton. Today it is quite clear that sheep breeding in Russia cannot become a profitable branch without balanced comprehensive state support. The package of measures should include the regulation of pricing processes, the elimination of prices disparity for agricultural and industrial products, subsidies in amount covering production and sales costs, preferential taxation and lending, and protection of domestic market from foreign commodity producers.

This is relevant today and was relevant one hundred and two hundred years ago. P.N. Kuleshov wrote in 1907: “One cannot expect, of course, that the peasant himself will pay serious attention to the improvement of wool and meat qualities of simple sheep, but it is regrettably that for the last decade neither the government nor the agricultural societies have done anything either for the past century”.

Ekonomičnost ovčarske proizvodnje, prednosti i nedostaci

Marina I. Selionova, Galina T. Bobryshova

Rezime

U radu je prikazana uporedna analiza ekonomskih i industrijskih parametara u ovčarstvu na teritoriji Stavropola, u periodu od 1976. do 1990. godine i sada. Razmatra se uloga državne podrške u stabilizaciji uzgoja ovaca i njenom daljnjem razvoju. Tokom godina ekonomskih preobražaja, broj ovaca na teritoriji Stavropola je opao za više od 7,1 puta od 1990. godine, a u poljoprivrednim organizacijama 11,8 puta. Količine proizvedene vune i ovčijeg mesa su smanjene za 10,5 i 6,7 puta, respektivno. Broj ovaca za ovaj period smanjen je 3 puta u cijeloj Rusiji. Ako je 1990. godine posejana površina žitarica na teritoriji Stavropola iznosila 1792,2 hiljada hektara, onda je u 2010. godini iznosila 2138,9 hiljada hektara. Površina pod krmnim biljkama za ovo vreme smanjena je za 6 puta, e u 2010. godini posejano je samo 216,5 hiljada hektara. Pored smanjenja broja ovaca, vlasnička struktura se promenila i profit je glavni pokretač razvoja ovog posla. Danas je sasvim jasno da uzgajanje ovaca u Rusiji ne može postati profitabilna grana bez uravnotežene sveobuhvatne državne podrške. Paket mera bi trebalo da uključi i regulaciju procesa utvrđivanja cena, eliminaciju dispariteta cena za poljoprivredne i industrijske proizvode, subvencije u iznosu koji pokriva troškove proizvodnje i prodaje, preferencijalno oporezivanje i pozajmljivanje i zaštitu domaćeg tržišta od stranih robnih proizvođača.

Ključne reči: uzgoj ovaca, proizvodi ovčarstva, vuna, ovčije meso, prihodi, troškovi proizvodnje, prodajna cena, profitabilnost, državna podrška.

References

VERSHININ A.S. (2015): Scientific and technical and selective aspects of improving the efficiency in sheep breeding in the Trans-Baikal Territory. Thesis Doctor of Agricultural Sciences. – Chita, 343 p.

MORDVIN L.N. A short outline of the present situation of sheep breeding in the Stavropol province and the reasons for its decay. Materials of the All Russian congress on sheep breeding. – 1912. – P.95-101.

AMERKHANOV H.A., EGOROV M.V., SERDEYUKOV V.N. Sheep and goat breeding of the Russian Federation in figures. National Union of Sheep Breeders. – Stavropol. – 2015. – 111 p.

The main indicators of the development of the state complex in the Stavropol Territory. Issue 8. Information and Computing Center of the Agro-Industry Committee of the Stavropol Territory. – Stavropol. – 1987. – 476 p.

Agriculture of the Stavropol Territory for the years 1976-1997. (Statistical Digest). State Statistics Committee of Russia. Stavropol Committee of State Statistics. – Stavropol. – 1998. – 29 p.

Agriculture in the Stavropol Territory for 2005-2010. Federal Service of State Statistics. Territorial body for the Stavropol Territory. – Stavropol. – 2011. – 144 p.

TAUNOVA A.I. (2000): Development of sheep breeding in the system of the AIC in the Republic of Kalmykia. – Elista: APP “Dzhangar”.

PETROVIC P.M, ILIC Z., CARO PETROVIC V. (2013): Sheep and goat breeding (Biology and technics of small ruminants breeding). Institute for animal Husbandry, Belgrade, 365pp.

SELIONOVA, M.I. (2015): Effective scientific support of production domestic sheep and goat breeding - a worthy response to global challenges modernity. Sheep, goats, woolen business. -2015. - №1. - P. 2-5.

SHEEP AND GOAT FLOCK BOOK AND HERD BOOK RECORD KEEPING IN CENTRAL SERBIA

Nevena Maksimović[1], Milan P. Petrović[1], Dragana Ružić-Muslić[1], Violeta Caro Petrović[1], Bogdan Cekić[1], Marina Lazarević[1], Rossen Stefanov[2]

[1]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[2]Institute of Biology and Immunology of reproduction, Bulgarian Academy of Science, 1113 Sofia, Bulgaria
Corresponding author: Nevena Maksimović, nevena_maksimovic@yahoo.com
Review paper

Abstract: Traditionally, Serbia has always been directed toward sheep and goat raising and the use of products of these animals. Geographically, the area of Central Serbia, as the hilly-mountainous area, is rich in natural pastures, and therefore the most suitable area for small ruminant production. There are varieties of sheep and goat breeds raised in Serbia, both domestic and imported. In Central Serbia, both flock and herd books are kept for sheep and goats raised on this territory. Quality breeding animals are registered at Central flock and herd book and are under the control of productive and reproductive traits. According to the Institute for animal husbandry's annual report for Central Serbia in 2016, there were 76,877 heads of sheep and 4,040 heads of goats under performance recording. This makes 5.25% of total sheep and 2.35% of total goats raised in this area. All sheep and goat breeds that are of national interest for breeding in Central Serbia are included in breeding-selection programs which are implemented through collaboration of farmers and breeding organizations. Presently, there are 146 (of which 112 are active) breeding organizations for sheep and goats registered on the territory of Central Serbia. In order to improve small ruminant production, government is funding breeders for implementation of breeding programs and production of high quality breeding animals.

Key words: sheep, goats, record keeping, Central Serbia

Introduction

In the past, Serbia was traditionally directed toward sheep and goat breeding and the use of products of these animals. This is primarily due to the natural environment that is particularly favourable for sheep and goat production, but also to the fact that these animals can produce highly valued products even in the humblest conditions. Geographically, the area of Central Serbia, as the hilly-mountainous area, is rich in natural pastures, and therefore the most suitable area for small ruminant production. Also, small individual land holdings/farms were not adequate for the organization of economically efficient cattle production in which farmers had to provide high quantity of quality food. In former Yugoslavia, 50% of agricultural population lived in hilly-mountainous area and raised 60% to 80% of total sheep in the country *(Mitić, 1984)*. However, in recent decades, along with rapid industrialization, colonization of cities and emptying of villages, as well as unfavourable political and economic situation in the country, sheep and goat production was significantly neglected, even abandoned, especially in mountainous regions.

Mountains as traditional centres for breeding of sheep and goats have been abandoned, and the small ruminants were moved to lowland areas and raised in expensive and non-organic way *(Petrović, 2015)*. In addition, the Law on the prohibition of goat keeping, which was adopted in 1954 *(Anonymous, 1954)*, led to destruction of about 80% of the goats. It was not until the last decade of twentieth century, that the lack of goat products on the market and a new understanding and knowledge about the quality of the food for people, led to tacit abolition of the controversial Law of 1954 *(Krajinović and Pihler, 2014)*.

Unfortunately, at about the same time, the disintegration of Yugoslavia was in process, and large state agricultural conglomerates which had large farms of sheep and other domestic species, were closed.

All of this negatively contributed to the small ruminant production in the country, and centres of sheep and goat breeding, once very much active and operational, were completely devastated. In recent years, the government has been trying to revitalize and prevent further deterioration of this type of production, mostly by funding breeders for production of quality breeding animals.

Sheep and goat breeds in Central Serbia

All sheep breeds raised in Central Serbia can be divided into two mayor groups, of which one constitutes of domestic sheep breeds and the other group includes imported breeds.

All domestic sheep breeds in Serbia are in fact different strains of one larger group which is called Pramenka. These strains were formed in different environmental and nutrition conditions and are different in terms of morphology and production. Due to significant differences among individual strains raised in different conditions, strains acquired the status of the breed *(Ružić-Muslić et al., 2015)*. These strains/breeds were named after the regions or towns were they were originally formed. Most of these breeds are still raised in their original form, while some of them were somewhat modified using different imported breeds. However, originally they were all characterized by triple combined production ability (for meat, milk and wool), they are low productive, but well adapted and biologically resistant *(Mitić, 1984; Maksimović et al., 2011; Savić et al., 2014)*. Presently, there are seven domestic sheep breeds/strains raised in Central Serbia: Sjenica strain (Sjenička pramenka) which is the most numerous strain of Pramenka, Svrljig strain (Svrljiška pramenka), Lipe strain (Lipska pramenka), Pirot strain (Pirotska pramenka) and Pirot improved sheep, Krivovir strain (Krivovirska pramenka), Karakachan strain (Karakačanska pramenka) and Bardoka (White Metohian sheep). There is also one population of sheep called Mis sheep (Meat, Institute, Sheep), a meat type of sheep of good fertility *(Maksimović et al., 2015a)*, which was formed as a result of crossing of three different sheep breeds (Wurttemberg, Ile de France and Pirot pramenka), but it is still in experimental stage *(Maksimović et al., 2016)*.

As for imported sheep, there are several different breeds, including: Wurttemberg, Ile de France, Bergamo, Suffolk, Texel, Charollais and Romanov sheep. The most popular of all imported breeds, and the largest in number, is Wurttemberg breed, which started to be imported to Serbia after the Second World War. Thanks to its exceptional ability for adaptation in unfavourable conditions and very good wool quality, Wurttemberg sheep was used to improve domestic sheep breeds in hilly-mountainous regions *(Mitić, 1984)*. However, it soon became popular amongst sheep breeders in Serbia who began raising it as purebred sheep. Nowadays, it is the most numerous imported breed raised in Central Serbia. All of the other imported sheep breeds are much less in number and are raised in small flocks.

There is much less variety in goat breeds raised in Central Serbia compared to sheep. There are two domestic and three imported goat breeds.

Balkan goat and Serbian white goat are domestic, low productive breeds with combined production ability for milk and meat, usually raised in high lands. Balkan goat is indigenous breed, well adapted to modest conditions of care, housing and nutrition *(Žujović et al., 2011)*. It is included in the program of protection and preservation of animal genetic resources on the territory of Serbia. Serbian white goat was created as a result of crossing Balkan goat does with Saanen bucks. This improved the milk yield, as well as fertility and body weight of domestic goats *(Memiši and Bauman, 2003)*. However, this crossing was usually random and not planned, which resulted in pronounced variability of the breed's productive traits.

Imported goats are of dairy breeds, such as Alpine, Saanen and Bunte Deutsche Edelziege goat. Alpine goat is the most dominant and preferred by breeders in Central Serbia. Alpine goats can be found all over Central Serbia, in different regions, from lowlands to hilly-mountainous areas *(Maksimović et al., 2015b)*. It is well adapted to both off-grazing production system and pasture. Other two breeds are represented in small number and are practically very rare in the territory of Central Serbia.

Flock and herd book record keeping

In Central Serbia, both flock and herd books are kept for sheep and goats raised on this territory. Quality breeding animals are registered in the Central flock and herd book and are under the control of productive and reproductive traits. However, only small number of animals are registered. Census of agriculture, which was conducted in 2012, showed that there are total of 1,464,666 heads of sheep and 171,774 heads of goats raised in Central Serbia (RZS, Statistical Office of the Republic of Serbia). According to the Institute for Animal Husbandry's annual report for Central Serbia in 2016, there were 76,877 heads of sheep and 4,040 heads of goats under performance recording. This makes 5.25% of total sheep and 2.35% of total goats raised in this area. In Republic of Croatia, for example, in 2015 there were 600,000 heads of breeding sheep and 65,000 heads of breeding goats, of which 39,883 sheep (around 7%) and 6,277 goats (approximately 10%) were under productivity control (HPA, Annual report for sheep, goats and small animals, 2015). In 2016 Republic of Slovenia had approximately 14% (15,600 out of 109,406 heads) of sheep and almost 17% (4,500 out of 26,959 heads) of goats under control (Statistical office RS). Although this number of registered animals is not very high, there was a positive trend in recent years indicating a certain increase (Table 1). In five year period, percentage of registered sheep increased from 1.59% to 5.25%, while in goats that increase was from 0.68% to 2.35%.

Table 1. Number of sheep and goats under performance recording in Central Serbia for the period of five years

	Years				
	2012	**2013**	**2014**	**2015**	**2016**
Heads of sheep	23,378	27,157	36,683	52,724	76,877
Heads of goats	1,179	1,228	1,817	3,050	4,040

Activities report and results of the control of realization of breeding programs in 2016.

Within breed structure, the most dominant amongst registered sheep is Sjenica strain, followed by Wurttemberg breed. In 2016 there were 36 582 heads of

Sjenica strain and 25 341 heads of Wurttemberg sheep, and these two breeds accounted for 80% of all registered sheep.

Table 2. Breed structure of registered sheep in Central Serbia in 2016

Genotype	**No of heads in 2016**
Sjenica strain	36,582
Wurttemberg	25,341
Svrljig strain	8,866
Ile de France	3,750
Lipa strain	591
Pirot improved sheep	511
Krivovir strain	476
MIS sheep population	336
Karakachan strain	132
Pirot strain	89
Bardoka	86
Texel	47
Bergamo	46
Romanov	12
Charollais	12

Activities report and results of the control of realization of breeding programs in 2016.

Of the total number of goats registered in Central heard book on the territory of Central Serbia in 2016, the Alpine breed is the most dominant with 92%, local breeds Balkan goat and Serbian white goat are represented with 3% and 4%, while the least is Saanen breed with only 1%. This breed structure has been more or less similar throughout the years (Table 3), with Alpine goat remaining the most dominant breed amongst registered animals.

Table 3. Breed structure of registered goats in Central Serbia for the period of five years

Genotype/year	**2012**	**2013**	**2014**	**2015**	**2016**
Alpine (in heads)	839	967	1583	2733	3712
Serbian White goat (in heads)	150	144	155	150	116
Balkan goat (in heads)	152	100	37	129	154
Saanen (in heads)	38	17	42	38	58

Activities report and results of the control of realization of breeding programs in 2016.

Selection programs and breeding organizations

Programmed selection is one of the prerequisites for successful farm animal production, especially on national level. Therefore, for the purpose of unique methodology of productivity control, testing and recording data and the scientific principle of assessment of animals' breeding value on the basis of objective measurement of their productivity, there is one Main breeding program for sheep and goats in Central Serbia.

The purpose of the Main breeding program is the implementation of organized breeding and selection practices, with a goal of achieving the selection progress for sheep and goats raised in Serbia. It defines breeding objectives, population size, breeding methods and selection programs, all for the purpose of increasing the efficiency of program implementation and successful breeding of sheep and goats. In this way, it provides genetic improvement of animals and the improvement of the quality of their products, in accordance with the zootechnical standards *(Ružić-Muslić, 2015)*.

In the implementation of breeding-selection program, central place take breeding organizations which are formed in hierarchical organizational structure. Main breeding organization, which is the official holder of Main breeding program, coordinates the functioning of regional breeding organizations for different districts and breeding organizations which act directly with farmers. At the moment, there are 146 (of which 112 are active) breeding organizations for sheep and goats registered on the territory of Central Serbia. They represent a link between farmers, scientific organizations and government in the field of sheep and goat breeding. The main role of these breeding organizations is to directly implement basic breeding programs, which must be in accordance with the Main breeding program. They participate in the selection of quality breeding animals, they are engaged in animal productivity control and manage record keeping.

All sheep and goat breeds that are of national interest for breeding in Central Serbia are included in these breeding-selection programs. In order to revitalize and improve small ruminant production, government is funding breeders for implementation of breeding programs and production of high quality breeding animals. These funding propose lower limit of 10 breeding heads of sheep and 5 breeding heads of goats that are under productivity control. In 2017, the funding for production of male breeding animals (rams and bucks) was implemented as a new stimulating measure. The importance of using only highly fertile, healthy rams in breeding programs cannot be over emphasized, which is particularly important in the case of small producers who use only one ram in the flock *(Maksimović, 2014)*. This also applies for bucks in goat herds.

Conclusion

Sheep and goat production in Central Serbia went through some difficult period in last few decades, which resulted in decrease in number of these animals and consequently led to lack of their products on the market. Luckily, in recent years that negative trend has stopped and some stimulating measures were taken in order to revitalize small ruminant production. As a result, number of quality breeding animals raised, and breeders are more interested in this type of domestic animal production. However, there is still a need for improvement. Average flocks and herds are small, genetic potential of animals is not fully utilized and the market for their products is still uncertain. In this process, goat production is in far more need for help and improvement, since it was neglected for a very long time. Present status of goat breeding in Central Serbia is not favourable, despite the recent rise in the number of quality breeding goats. Government funding of breeders is very important, but it is only the beginning as the improvement of wider economic and social environment is as necessary

Matična evidencija u ovčarstvu i kozarstvu u Centralnoj Srbiji

Nevena Maksimović, Milan P. Petrović, Dragana Ružić-Muslić, Violeta Caro Petrović, Bogdan Cekić, Marina Lazarević, Rossen Stefanov

Rezime

U prošlosti, Srbija je imala izraženu tradiciju u gajenju ovaca i koza i korišćenju proizvoda od ovih životinja. Geografski posmatrano, područje Centralne Srbije, kao brdsko-planinsko područje, bogato je prirodnim pašnjacima, te najviše pogoduje upravo ovoj vrsti proizvodnje. Na teritoriji centralne Srbije gaji se veći broj različitih rasa ovaca i koza, kako autohtonih tako i uveženih, a za sve ove rase ovaca i koza vodi se matična evidencija. Kvalitetne priplodne životinje registrovane su u glavnoj matičnoj evidenciji i nalaze se pod kontrolom proizvodnih svojstava. Prema stručnom izveštaju Instituta za stočarstvo i rezultatima sprovođenja odgajivačkog programa u 2016. godini na teritoriji centralne Srbije, ukupno je bilo 76.877 kvalitetnih priplodnih grla ovaca i 4.040 kvalitetnih priplodnih grla koza u matičnom zapatu. To čini 5,25% od ukupnog broja ovaca i 2,35% od ukupnog broja koza koje se gaje na ovoj teritoriji Sve rase ovaca i koza koje su od nacionalnog interesa za gajenje na teritoriji centralne Srbije uključene su u odgajivačko-selekcijske programe koji se implementuju kroz saradnju farmera i odgajivačkih organizacija. Trenutno, na teritoriji centralne Srbije postoji 146 registrovanih odgajivačkih organizacija, od kojih 112 aktivno učestvuje u sprovođenju odgajivačko-selekcijskih programa u ovčarstvu i kozarstvu. U ciju unapređenja proizvodnje malih preživara, država učestvuje davajući određena finansijska sredstva odgajivačima za implementaciju odgajivačkih programa i proizvodnju kvalitetnih priplodnih grla.

Ključne reči: ovce, koze, matična evidencija, centralna Srbija

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 3105.

References

ACTIVITIES REPORT AND RESULTS OF THE CONTROL OF REALIZATION OF BREEDING PROGRAMS IN 2016. Institute for animal husbandry, 109-133 135-156

ANONYMOUS (1954): Zakon o zabrani držanja koza. *Službeni glasnik Narodne Republike Srbije 37*, 269.

HPA. Annual report for sheep, goats and small animals, 2015. http://www.hpa.hr/wp-content/uploads/2014/06/04-Ov%C4%8Darstvo-kozarstvo-i-male-%C5%BEivotinje.pdf

KRAJINOVIĆ M., PIHLER I (2014): Tehnologija kozarske proizvodnje. Univerzitet u Novom Sadu, Poljoprivredni fakultet, pp 237.

MAKSIMOVIĆ N. (2014): Variability of testosterone concentration evaluation and relationship to the primary and secondary sexual characteristics and behaviour of rams. Ph.D. Dissertation, University of Belgrade, Faculty of agriculture.

MAKSIMOVIĆ N., DELIĆ N., STANKOVIĆ B., HRISTOV S., PETROVIĆ P.M., CARO-PETROVIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N. (2015a): Oestrus synchronization efficiency and fertility in ewes of MIS sheep population during anoestrous and breeding season. Invited Paper. Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, October 7 – 9, Belgrade, Serbia, 104-112

MAKSIMOVIĆ N., BAUMAN F., PETROVIĆ P.M., CARO PETROVIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N., MILOŠEVIĆ-STANKOVIĆ I. (2015b): Productive characteristics and body measurements of Alpine goats raised under smallholder production systems in central Serbia. Biotechnology in animal husbandry 31, 2, 245-253.

MAKSIMOVIĆ N., HRISTOV S., STANKOVIĆ B., PETROVIĆ P.M., MEKIĆ C., RUZIĆ-MUSLIĆ D., CARO-PETROVIĆ V. (2016): Investigation of serum testosterone level, scrotal circumference, body mass, semen characteristics, and their correlations in developing MIS lambs. Turkish Journal of Veterinary and Animal Sciences, 40, 53-59.

MAKSIMOVIĆ N., ŽUJOVIĆ M., PETROVIĆ P.M., HRISTOV S., STANKOVIĆ B., RUŽIĆ-MUSLIĆ D., STANIŠIĆ N., (2011): Reproductive indicators and development of lambs of Sjenica pramenka breed from birth to weaning: The effect of non-genetic factors. 2nd Conference of the Balkan Network for the Biotechnology in Animal reproduction, Sofia, Bulgaria, Proceeding, 23-26.

MEMIŠI N., BAUMAN F. (2003): Domaće rase koza. Poljoprivredne aktuelnosti, 5-6, 41-56.

MITIĆ N. (1984): Ovčarstvo. Zavod za udžbenike i nastavna sredstva, Beograd.

PETROVIĆ P.M. (2015): Glavni odgajivački program u ovčarstvu. Institut za stočarstvo, Beograd, 77-119.

RUŽIĆ-MUSLIĆ D. (2015): Glavni odgajivački program u kozarstvu. Institut za stočarstvo, Beograd, 121-148.

RUŽIĆ-MUSLIĆ D., BIJELIĆ Z., CARO-PETROVIĆ V., ŠKRBIĆ Z., CIVIDINI A., BOJKOVSKI D., SIMČIČ M., KOMPAN D. (2015): Conservation of autochthonous sheep breeds in Serbia and Slovenia. Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, October 7 – 9, Belgrade, Serbia, 83-103.

RZS (2012): Statistički godišnjak Republike Srbije.

SAVIĆ M., BALTIĆ M., BECSKEI Z., DIMITRIJEVIĆ B., DIMITRIJEVIĆ V., SAVIĆ Đ., VEGARA M. (2014): Evaluation of Zackel lamb meat quality with the aim of increasing the conservation value of the breed. Acta veterinaria 64 (4), 438-446.

ŽUJOVIĆ M., MEMIŠI N., IVANOVIĆ S. (2011): Present status, possibilities and perspective of development of goat production in republic of Serbia. Biotechnology in Animal Husbandry 27, 3, 431-443.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

PERSPECTIVES OF PIG BREEDING SECTOR IN EUROPE

Vesna Gantner[1], Pero Mijić[1], Maja Gregić[1], Vladan Bogdanović[2], Tina Bobić[1], Dragan Solić[3], Krešimir Kuterovac[4]

[1]Josip Juraj Strossmayer in Osijek, Faculty of Agriculture in Osijek, Vladimira Preloga 1, Osijek, Croatia, e-mail: vgantner@pfos.hr
[2]University of Belgrade, Faculty of Agriculture, Institute of Zootechnics, Nemanjina 6, 11080 Belgrade – Zemun, Serbia
[3]HPA, Ilica 101, 10000 Zagreb, Croatia
[4]Inagra Ltd, Biljska cesta 27, 31000 Osijek, Croatia
Corresponding author: vgantner@pfos.hr
Review paper

Abstract: Agriculture worldwide in future period will face with a number of major challenges: rapid population growth, climate change, an increasing demand for energy, resource shortages, accelerated urbanisation, dietary changes, ageing populations in rural areas in developed countries, increased competition on world markets, and lack of access to credit developing countries. Agriculture in the European Union is characterised by the gradually declining of the area of land available for agriculture due to increased forestry, biogas production and urbanisation. Furthermore, it is characterised by a long-term decline in the number of holdings with a corresponding increase in the area per holding. Currently, European Union is self-sufficient in cereals, total meat production and milk and dairy products. Forecasts indicate similar level of self-sufficiency in future (till year 2025). Analysis and forecasts of pig meat market development in Europe in year 2025 indicate increase of net production (amount of increase will vary regarding the state), slightly increase of consumption in Europe, significant increase of export (mainly in China) while import remains unchanged.

Croatia and Serbia together breed about 3% of total pig production in Europe. Can we do better? Let’s review the advantages: natural resources (agricultural land; cereal production; water), educated stakeholders, and possibility for investments (CAP – II. Pilar, SAPARD, IPARD). Lastly, for sustainable development of pig breeding it is necessary to ensure: government support (simplification and price cuts of administration; simplification and price cuts of cost of capital as well as institutional support (scientific community, advisory service). The question is, will we organize?

Key words: pig breeding, challenges, perspectives, Europe

Introduction

Agriculture – State of the Art

In accordance to the forecasts, ***agriculture worldwide*** in future period will face with a number of major challenges: rapid population growth, dietary changes, accelerated urbanisation, ageing populations in rural areas in developed countries, an increasing demand for energy, resource shortages, increased competition on world markets, lack of access to credit developing countries as well as ***climate change*** (EPRS, 2016).

EU agriculture is characterised by the ***gradually declining of the area of land available for agriculture*** due to increased forestry and urbanisation (EPRS, 2016). Currently, the total utilised agricultural area is 174 million hectares (ha), which comprises 40% of the EU land area. During the period between 2005 and 2013 the agricultural land area fell by 0.7% (Figure 1). Due to this trend, in order to maintain or increase output, the productivity must be increased.

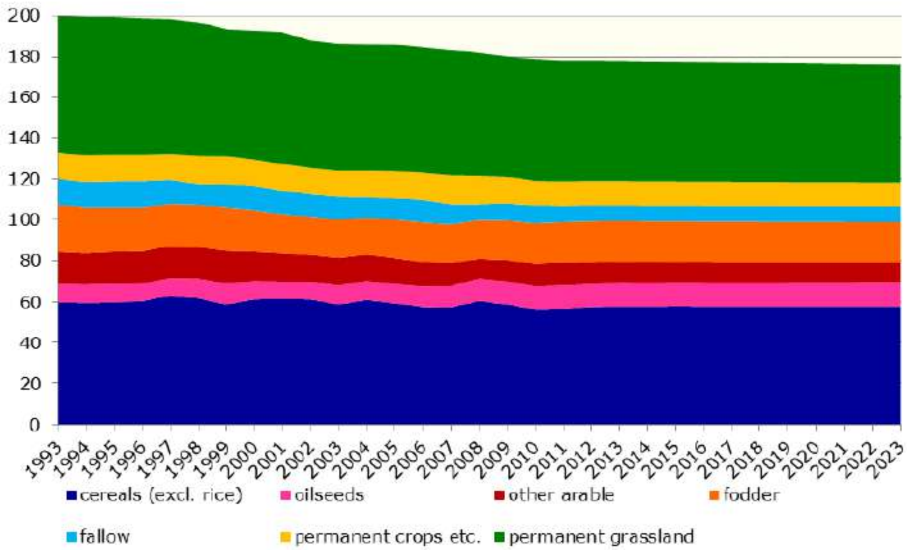


Figure 1. Agricultural land-use developments in the EU in million ha (EC, 2013)

Other important characteristics of EU agriculture is a ***long-term decline in the number of holdings*** with a corresponding increase in the area per holding. In the period between 2005 and 2013, the average rate of decline was 3.7% per year, resulting in the number of holdings reducing by 1.2 million and average holding area rising from 14.4 to 16.1 ha (EPRS, 2016). In year 2013 there were 10.8

million farm holdings (farms) in the EU employing about 22.2 million people (excluding seasonal workers).

Currently, ***European Union is self-sufficient*** in cereals, total meat production and milk and dairy products. Forecasts indicate similar situation in future (till year 2025, EC, 2015).

Challenges of European agriculture

Climate change

In the last decades we have witnessed increasingly pronounced climate change worldwide. These changes are transforming the environment in different regions by making them not convenient for living and agricultural production. FAO experts (FAO, 2013) stated that aiming to hold the increase in global temperature below 2^{o}C and by that to avoid dangerous climate change, global GHG emissions need to be significantly decreased worldwide.

Currently, agriculture plays an important role in global environmental issues, such as climate change, land degradation, water pollution and biodiversity loss. Therefore, future production growth in must be accommodated within the growing insufficiency of natural resources, including land, water and nutrients. Furthermore, waste and greenhouse gasses (GHG) emissions must be significantly reduced. Possible impacts of climate change on EU agriculture in presented of Figure 2.

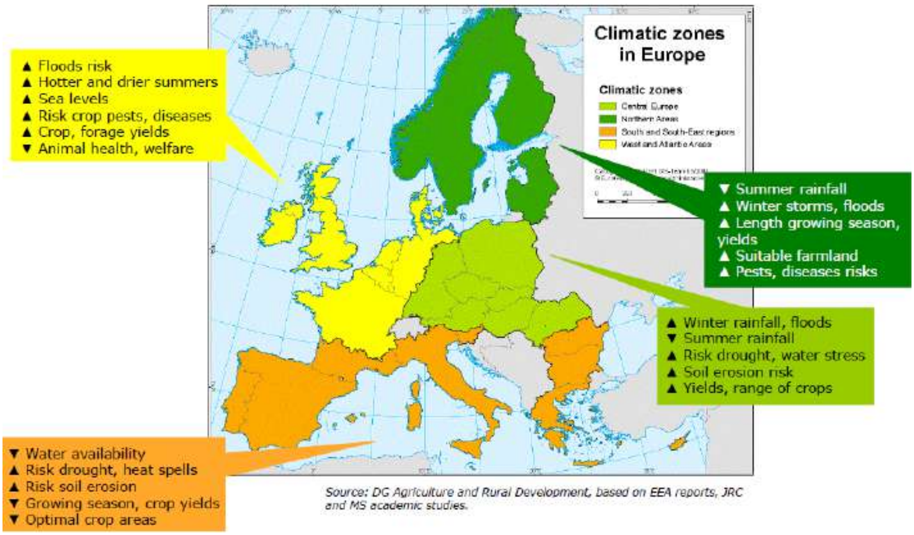
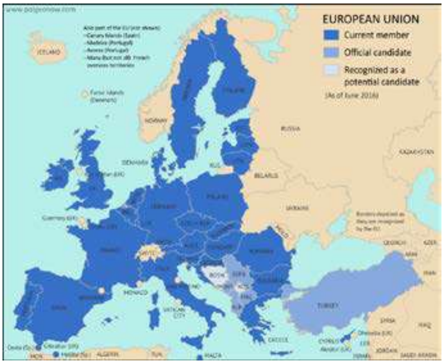


Figure 2. Possible impacts of climate change on European regions (EC, 2013)

Political changes

Recent political movements (USA - election of Donald Trump, Britain's Brexit, current elections in different EU states, China - President Xi - who has moved toward nationalistic policies) clearly indicate that the ***idea of globalization becomes questionable.*** It is obvious that the world is changing.



The Common Agricultural Policy (CAP)

European Union has Common Agricultural Policy (CAP) that applies for all member states and amounts more than 40% of the EU budget (DG, 2013). CAP addresses following challenges:

- economic,
- environmental (climate change),
- territorial.

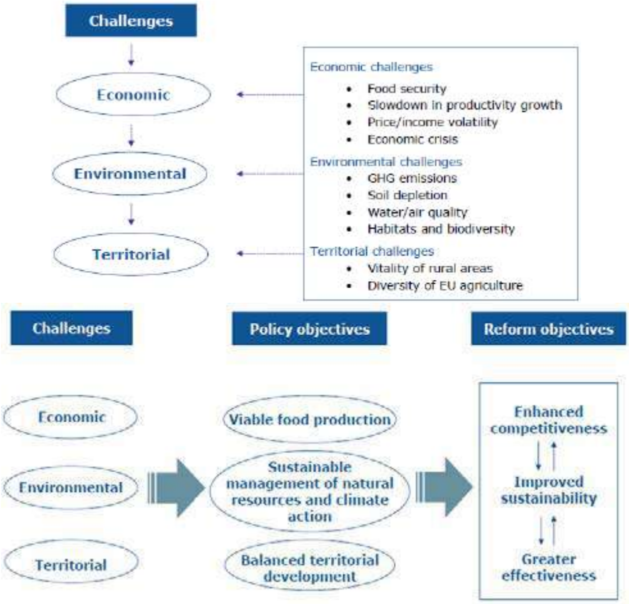


Figure 3. How to address challenges (DG, 2013)

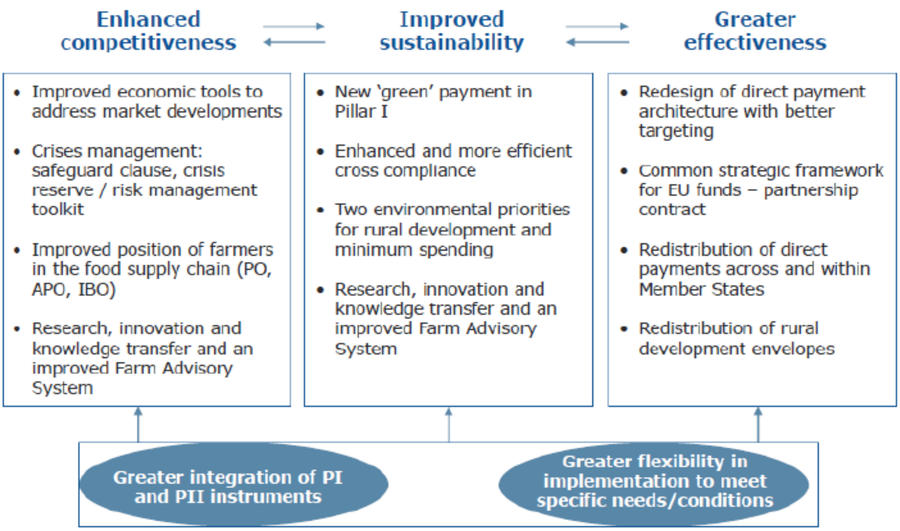


Figure 4. CAP instruments (DG, 2013)

Main statistics

Population and meat production – Europe

Table 1. Livestock population, 2015 (million head, Eurostat, 2017)

	Bovine animals	Pigs	Sheep	Goats
EU-28	**89.2**	**148.7**	**85.5**	**12.5**
Belgium	2.5	6.4	:	:
Bulgaria	0.6	0.6	1.3	0.3
Czech Republic	1.4	1.6	:	:
Denmark	1.6	12.7	:	:
Germany	12.6	27.7	1.6	0.1
Estonia	0.3	0.3		:
Ireland	6.4	1.5	3.3	:
Greece	0.6	0.9	8.9	4.0
Spain	6.2	28.4	16.5	3.0
France	19.4	13.3	7.1	1.2
Croatia	0.5	1.2	0.6	0.1
Italy	6.2	8.7	7.1	1.0
Cyprus	0.1	0.3	0.3	0.2
Latvia	0.4	0.3	0.1	0.0
Lithuania	0.7	0.7	0.1	0.0
Luxembourg	0.2	0.1		:
Hungary	0.8	3.1	1.2	0.1
Malta	0.0	0.0	0.0	0.0
Netherlands	4.3	12.5	1.0	0.5
Austria	2.0	2.8	0.4	0.1
Poland	5.8	10.6	:	:
Portugal	1.6	2.2	2.0	0.4
Romania	2.1	4.9	9.8	1.4
Slovenia	0.5	0.3		:
Slovakia	0.5	0.6	0.4	0.0
Finland	0.9	1.2	:	:
Sweden	1.4	1.4	0.6	:
United Kingdom	9.8	4.4	23.1	0.1
Iceland	0.1	0.0	:	:
Montenegro	0.1	0.0	0.2	0.0
Serbia	0.9	3.3	1.8	0.2
Turkey	14.1	:	31.5	10.4
FYR of Macedonia	0.3	0.2	0.7	0.1

Note: The EU aggregate for sheep and goats corresponds to the sum of the Member States for which data are available. This includes all Member States with a significant number of animals.

Table 2. Production of meat, by type of animal, 2015 (1 000 tonnes of carcass weight, Eurostat, 2017)

	Bovine animals	Pigs	Sheep	Goats	Poultry
EU-28 (*)	7 590.4	22 957.8	725.0	44.8	13 720.0
Belgium	267.9	1 124.3	2.5	0.2	452.9
Bulgaria	5.3	60.7	:	:	101.3
Czech Republic	68.3	227.7	0.2	0.0	151.4
Denmark	120.6	1 598.7	1.8	0.0	134.4
Germany	1 124.0	5 562.0	21.0	0.0	1 511.0
Estonia	9.6	42.4	0.1	:	:
Ireland	564.1	276.4	58.4	0.0	128.0
Greece	41.9	90.0	54.9	21.9	189.6
Spain	633.8	3 895.9	116.5	9.2	1 443.3
France	1 451.0	1 967.6	80.7	6.2	1 718.0
Croatia	42.3	73.0	1.0		63.4
Italy	788.3	1 485.8	33.6	1.8	1 295.0
Cyprus	5.0	43.4	3.4	2.0	23.8
Latvia	17.4	29.3	0.3	0.0	29.5
Lithuania	44.1	66.2	0.1	:	95.8
Luxembourg	9.1	12.3	0.0	0.0	0.0
Hungary	26.4	409.3	0.4	0.0	478.7
Malta	1.0	5.6	0.1	0.0	3.9
Netherlands	382.5	1 456.2	13.0	1.5	:
Austria	228.8	527.8	7.0	0.8	:
Poland	471.0	1 906.1	0.6	:	2 011.0
Portugal	88.6	377.5	10.5	0.8	308.7
Romania	44.5	330.5	9.2	0.1	374.8
Slovenia	33.6	20.2	0.1	0.0	58.8
Slovakia	8.4	45.2	0.5	0.0	:
Finland	85.8	191.9	1.2	0.0	117.3
Sweden	144.0	233.5	5.1	0.0	145.8
United Kingdom	883.2	898.3	300.3	0.3	1 688.8
Iceland	3.5	6.8	10.1	0.0	8.4
Switzerland	141.8	240.1	4.4	0.7	85.0
Montenegro	4.1	0.4	0.9	0.0	0.6
Albania	8.7	8.3	2.0	1.3	4.8
Serbia	40.0	167.2	1.3	0.0	58.6
Turkey	97.8	0.0	58.6	3.7	1 962.1
Bosnia and Herzegovina	22.9	8.5	1.4	0.0	48.7

(:) not available
(*) EU-28 totals include confidential data. They are rounded to keep safe the national confidential values.

Total livestock population in Europe in year 2015 is shown in the Table 1. Total number of pigs in 2015 amounted 148.7 million head. Less than 1% of pig population was breed in Croatia (1.2 million), while on pig farms in Serbia slightly more than 2% was breed.

Perspectives in Europe in period 2025 – 2015

The future trends in Europe regarding the consumption of meat, production and export of pig meat as well as pig market development are shown in following Figures.

Consumption

The forecasted changes in the meat consumption in EU in year 2025 in regard to 2015 indicate increase of total meat consumption in amount of 0.1% per year with different patterns regarding the meat product (EC, 2015). The highest increase in consumption is expected in poultry products (Figure 5).

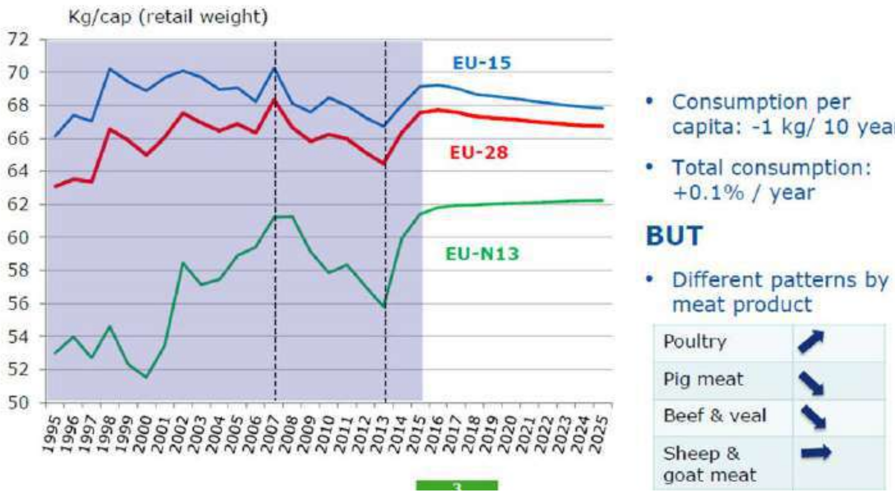


Figure 5. EU changes in consumption (000 t) 2025 vs. 2015 (EC, 2015)

Production and export

In EU in year 2025 expected increase in pig production amount more than 4000000 t that is 1.7% more than in 2015 (Figure 6). Regarding the export of pig meat even higher increase in amount more than 550000 t (26.3% more than in 2015) is forecasted (EC, 2015).

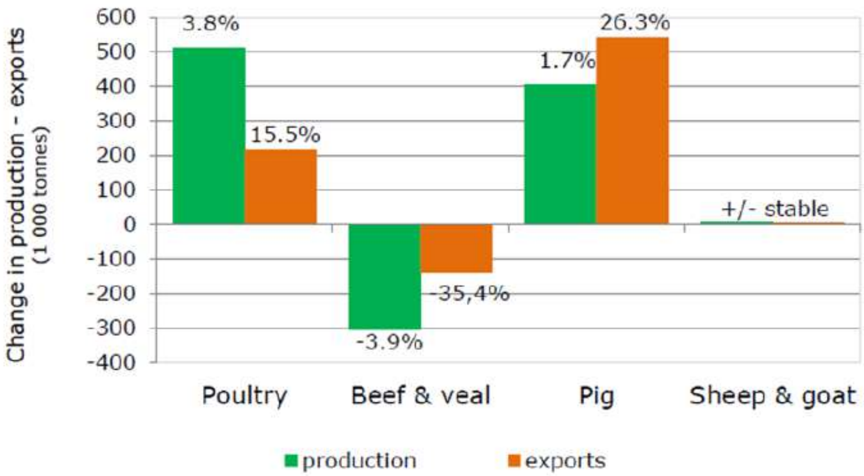


Figure 6. EU changes in production and exports (000 t) 2025 vs. 2015 (EC, 2015)

Pig meat market development

Forecasts indicate in year 2025 (Figure 7)

– increase of net production (amount of increase will vary regarding the state)
– slightly increase of consumption in Europe
– increase of export (mainly in China)
– import remains unchanged (EC, 2015).

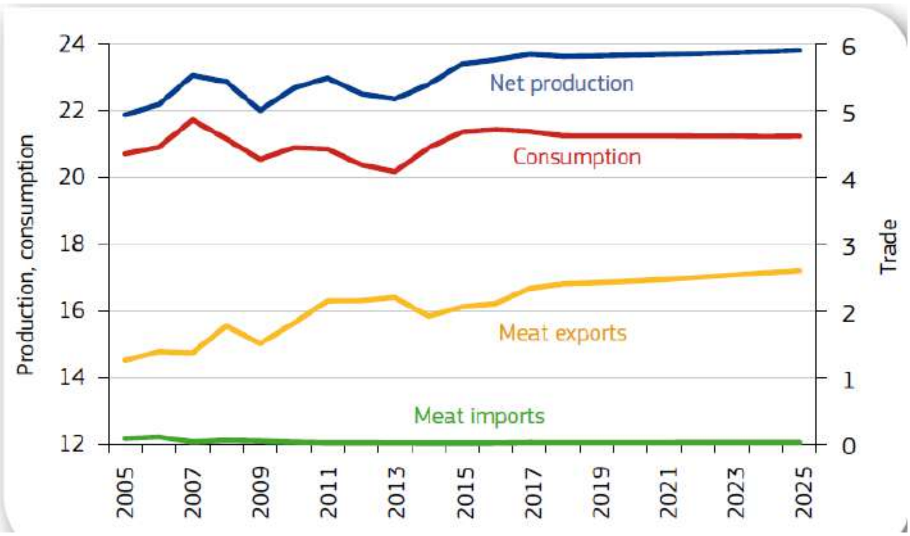


Figure 7. EU pig meat market developments (million t) 2025 vs. 2015 (EC, 2015)

Conclusion

The Perspectives of pig breeding sector in Europe? Currently, European Union is self-sufficient in cereals, total meat production and milk and dairy products. Forecasts indicate similar situation in future (till year 2025). Also, forecasts indicate significant increase of production and export. Croatia and Serbia together breed about 3% of total pig production in Europe. **Can we do better?** Let's review the advantages: **natural resources** (agricultural land; cereal production; water), **educated stakeholders**, and **possibility for investments** (CAP – II. Pilar, SAPARD, IPARD). Lastly, for sustainable development of pig breeding it is necessary to ensure: **government support** (simplification and price cuts of administration; simplification and price cuts of cost of capital as well as institutional support (scientific community, advisory service).

The question is, will we organize?

Perspektive u sektoru uzgoja svinja u Evropi

Vesna Gantner, Pero Mijić, Maja Gregić, Vladan Bogdanović, Tina Bobić, Dragan Solić, Krešimir Kuterovac

Rezime

Poljoprivreda u svetu u budućem periodu će se suočiti s brojnim velikim izazovima: brz rast populacije, klimatske promene, sve veća potražnja za energijom, nedostatak resursa, ubrzana urbanizacija, promene u ishrani, starenje populacije u ruralnim područjima u razvijenim zemljama, povećana konkurencija na svetskim tržištima, i nedostatak pristupa kreditima za zemlje u razvoju. Poljoprivredu u Evropskoj uniji karakteriše postepeno opadanje zemljišnih površina raspoloživih za poljoprivredu zbog povećanog šumarstva, proizvodnje biogasa i urbanizacije. Nadalje, karakteriše ga dugoročno smanjenje broja gazdinstava sa odgovarajućim povećanjem površine po gazdinstvu. Trenutno, Evropska unija je samodovoljna u žitaricama, ukupnoj proizvodnji mesa i mleku i mlečnim proizvodima. Prognoze ukazuju na sličan nivo zadovoljavanja sopstvenih potreba u budućnosti (do 2025. godine).

Analize i prognoze razvoja tržišta svinjskog mesa u Evropi do 2025. godine ukazuju na povećanje neto proizvodnje (količina povećanja će se razlikovati od države do države), blago povećanje potrošnje u Evropi, značajan porast izvoza (uglavnom u Kinu), dok uvoz ostaje nepromenjen.

Hrvatska i Srbija zajedno čine oko 3% ukupne proizvodnje svinja u Evropi. Možemo li bolje? Razmotrimo prednosti: prirodni resursi (poljoprivredno zemljište, proizvodnja žitarica, voda), obrazovani akteri i mogućnost ulaganja (CAP - II. Pilar, SAPARD, IPARD). Na kraju, za održivi razvoj uzgoja svinja potrebno je osigurati: podršku vlade (pojednostavljenje i smanjenje cena administracije, pojednostavljenje i smanjenje troškova kapitala, kao i institucionalna podrška (naučna zajednica, savetodavna služba). Pitanje je da li ćemo uspeti da se organizujemo?

Ključne reči: uzgoj svinja, izazovi, perspektive, Evropa

References

DG Agriculture and Rural Development, European Commission (2013): The CAP towards 2020 Political agreement.
EC – European Commission (2013): Prospect of Agricultural Markets and Income in the EU 2013 – 2023.
EC – European Commission (2015): EU Agricultural Outlook, Prospects for EU agricultural markets and income 2015 – 2025.
EPRS – European Parliament Research Service (2016): Precision agriculture and the future of farming in Europe. Scientific Foresight Study.
EUROSTAT (2017). http://appsso.eurostat.ec.europa.eu/
Food and Agriculture Organization of the United Nations. FAO. (2013): Greenhouse gas emissions from pig and chicken supply chains – A global life cycle assessment, by MacLeod, M., Gerber, P., Mottet, A., Tempio, G., Falcucci, A., Opio, C., Vellinga, T., Henderson, B., Steinfeld, H. Rome.

KRŠKOPOLJE PIG IN TREASURE PROJECT: FROM REARING TO PRODUCT

Marjeta Čandek-Potokar[1,2], Urška Tomažin[1], Martin Škrlep[1], Nina Batorek Lukač[1], Maja Prevolnik Povše[1,2], Peter Dovč[3], Zein Kallas[4], José M. Gil[4]

[1]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana
[2]University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče
[3]University of Ljubljana, Biotechnical faculty, Department of Animal Science, Groblje 3, SI-1230 Domžale
[4]Centre for Agro-food Economy & Development, CREDA-UPC-IRTA, Castelldefels 08860, Spain
Corresponding author: Marjeta Čandek-Potokar, meta.candek-potokar@kis.si
Original scientific paper

Abstract: Krškopolje pig, a Slovenian autochthonous breed, is poorly studied and exploited, as is the case in many autochthonous (local) pig breeds. More knowledge about various aspects of breed as characterisation, performances, product quality and socio-economic relevance is needed for the development of sustainable pork value chains. To answer these challenges, the studies are conducted within H2020 project TREASURE spanning from rearing to product evaluation. In this work, an overview of the first partial and preliminary results are presented for Krškopolje pig which concern the evaluation of growth performance in different rearing systems, effect of the incidence of *RYR1* gene mutation and sensory quality and consumer acceptability of new traditional product (dry-cured sausage – salami type) from Krškopolje pigs.

Keywords: autochthonous breed, Krškopolje pig; growth performance, *RYR1* mutation, sausages, consumer test

Introduction

Slovenia has only one preserved autochthonous (local) pig breed, the Krškopolje pig which has a black coat colour with a white belt across shoulders and forelegs. In the seventies of the last century, the breed was gradually abandoned and became endangered. In the early nineties of the 20th century *in situ* gene bank and herd book were created. An important milestone was also the year 2003 when individual marking of all newborn piglets was introduced (*Kastelic and Čandek-Potokar, 2013)*. The interest for Krškopolje breed has been revived in the last years, esp. on organic farms and on family farms with direct product sales. The public aid for the conservation of this breed contributes to its wider use. Presently, Krškopolje breed is not at risk of extinction, however the breed is far from being safe. In order to develop a sustainable use of Krškopolje pig and thus its preservation, it would be necessary to develop economically sustainable exploitation through valorisation of its products. As is the case for other local pig breeds, Krškopolje pig is reared in very diverse conditions adapted to the specific local environment. Typically, farmers are rearing these pigs on a small scale basis in rather extensive conditions, often using a combination of indoor and outdoor system. The breed is reputed for excellent meat quality and used for traditional pork products appreciated by consumers. However in terms of the scientific substantiation, there is practically no information about nutritional requirements of this breed, the performances, productive traits and quality of products are practically untapped and studies dealing with this breed are lacking. Studies are thus needed to acquire more knowledge as the basis and essential for the development of sustainable pork value chains. In light of this, experiments and studies on Krškopolje breed are conducted within H2020 project TREASURE from rearing to product development and an overview of the first partial or preliminary results are presented in this paper.

Materials and Methods

Growth performance of piglets in lactation phase

Piglets (n=156; 68 females and 88 castrated males) from 7 organic and 11 conventional farms were weighed at weaning (4-6 weeks). Daily gains were calculated based on weaning weight and average birth weight (1.2 kg). Details of the study (experimental design and statistical analysis) are described in *Tomažin et al. (2016).*

Growth performance in the growing phase

Three piglets (castrates) per litter were selected from 12 farms/litters at the average age of 55 days and average weight of 14 kg, and assigned within litter to three groups. One group (two pens of 6 pigs) received organic (ECO) feed mixture (12.8 MJ ME/kg, 17.8% crude protein (CP), 0.8% lysine and 11.9 MJ ME/kg, 15% CP, 0.6% for growing and fattening diets, respectively) while the other group (four pens of 6 pigs) received conventional (CON) feed mixture (13.6 MJ/kg, 16.8% crude protein, 1.0 lysine and 11.6 MJ ME, 15.3% CP, 0.9% lysine for growing and fattening diets, respectively). The pigs were given an *ad libitum* access to feed and were weighed every 2 weeks until the average age of 155 days and daily gains were calculated.

Fattening trial in conventional and organic system

Pigs were assigned within litter to three treatment groups; ECO (n=12), CON (n=12) and CON+L (n=12). Two barley based diets were prepared. For ECO pigs (n=12), the diet (12.4 MJ ME/kg, 12.9% CP, 0.7 % Lys) was based on organically produced crops whereas for CON and CON+L pigs, the diet (13.2 MJ ME/kg, 13.6% CP, 1.2 % Lys) was based on the same ingredients but from conventional crop production. ECO pigs were supplemented with lucerne hay, CON+L pigs were supplemented with pelleted lucerne and received 10% less feed mixture than CON and ECO. Pigs in CON and CON+L were housed indoors, whereas ECO pigs were reared respecting the rules for organic production. Details of the study design are described in *Batorek et al*. (2016).

Fattening performance of Krškopolje pigs in extensive and intensive system

The growing and fattening of pigs was conducted on two farms. Twelve pigs from twelve farms/litters were delivered to both farms at similar age (85.9±2.5 days) and live weight (26.3±2.1 kg). On the extensive (EXT) farm, the pigs (n=6) were housed in a pen (15 m^2) with access to outdoor area (50 m^2) and fed cooked potatoes and root crops supplemented with mixture of cereals, whereas on the intensive farm, the pigs (n=6) were housed indoors (in a pen of 7.5 m^2) and fed complete feed mixture. Pigs were monitored for 138 days and were weighed 4 times (at 86, 129, 195, 224 days) with 4 growth stages (25-45, 45-75, 75-90 and 90-120 kg) considered (for more information see *Tomažin et al., 2017a).*

Effect of the RYR1 mutation in Krškopolje pigs

Unrelated Krškopolje castrates reared in equivalent conditions (at the same farm and fed complete feed mixtures adapted to the stage of growth) were genotyped for recessive mutation c. C1843T (p. Arg615Cys) at *RYR1* locus (recessive allele further denoted as “n”; “N” stands for wild type allele). Carcass traits and meat quality were compared between N/n (n=15) and N/N pigs (n=20) (more information in *Tomažin et al*., *2017b).*

The quality of salami from Krškopolje pigs as compared to standard product

The effect of raw material (meat and back fat) from Krškopolje pigs on quality parameters of non-smoked dry-cured sausage (salami) was assessed. Salami was produced by the same producer with the same processing procedure (additives, temperature and relative humidity regime, dry-curing and ripening duration) using either standard raw material (meat, subcutaneous back fat in proportion 80:20) or using meat and subcutaneous back fat (in proportion 80:20) from Krškopolje pigs.

Consumer acceptance for salami from Krškopolje pigs as compared to standard product

Consumer sensory test were performed in order to determine the consumer acceptability and preferences for non-smoked dry-cured sausage (salami) made from Krškopolje pig as compared to standard product (salami made of conventional modern pig meat). For that purpose products (salami) were made using the same processing method but different raw material (meat and fat of Krškopolje vs. meat and fat of standard pig). Following the Expectation Disconfirmation Theory (EDT) (*Olson and Dover*, *1979*; *Oliver 1997)*, the sensory test included three phases, a blind sensory test with no information; an evaluation of the expected liking based on product description; a sensory test with complete information on the samples. The sensory evaluation was carried out in controlled environment with different sessions of 15 consumers by session. Participants evaluated the overall acceptability using a nine-point category scale from 1 'dislike extremely'; to 9 'like extremely'.

Results and Discussion

Growth rate of piglets in the lactation

Weight at weaning and growth rates of Krškopolje piglets raised on organic farms were approximately 20% lower than of piglets raised on conventional ones, however, the differences were not significant when taking into account the random effect of farm (Figure 1). Such results are expected in Slovenian pig farming conditions as Krškopolje pigs are most often reared on unspecialised, small extensive farms, there are often no big differences between conventionally and organically raised pigs, explaining the small differences between growth rates in lactation period between the two farming systems. The lactation periods practiced are generally longer (4-6 weeks; 38 days in average in the present study) and in such system piglets need to be supplemented with complete feed mixtures to grow well. Diets in organic pig production are often deficient in essential amino acids due to the legislation on organic farming (*Council Regulation (EC) No 834/2007)* which does not allow the use of synthetic amino acids, genetically modified organisms and feed materials processed with the aid of chemically synthesised solvents in organic diets (the latter two are particularly referring to soybean meal, which is one of the main protein sources used in conventional feed for pigs).

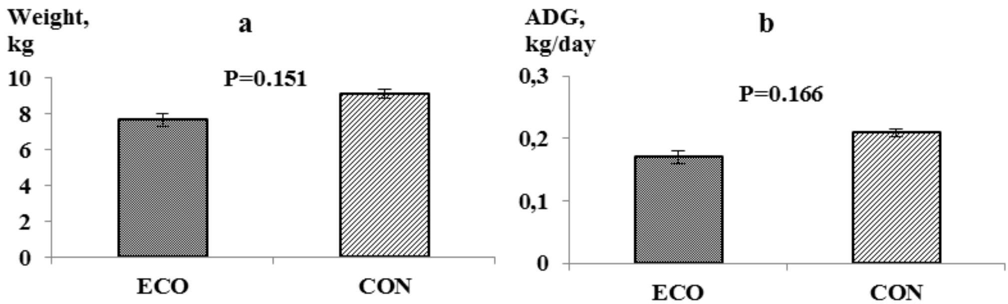


Figure 1: Weaning weight (a) and average daily gain in lactation (b) of conventionally and organically raised Krškopolje piglets

Growth performance in the growing phase (from weaning until 70 kg)

There was no major difference in weight of pigs (Figure 2a) at the beginning of the study whereas after 100 days of fattening, the pigs receiving CON diet were slightly heavier (their growth rate being 10% greater) but the difference was not significant (P>0.05). Daily gain steadily increased with growth, except in a period between 83 and 97 days which could be due to high ambient temperatures (Figure 2b). Average daily gain of pigs in the period from 55 to 155 days was slightly (but not significantly) lower in ECO than CON pigs. These results suggest that no major differences in growth rates of Krškopolje pigs fed organic or conventional diet are to be expected in the case of similar nutritional value of the diet and similar rearing conditions (as was the case in the present study).

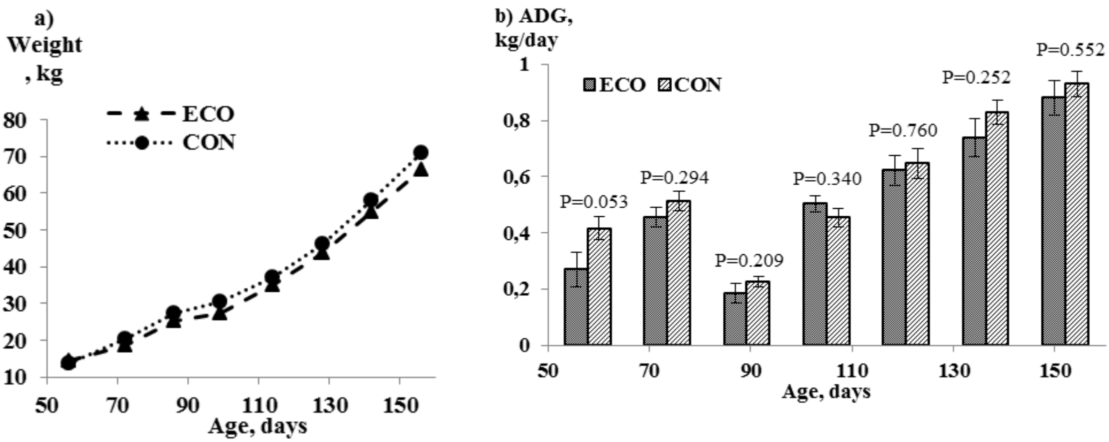


Figure 2: Weight (a) and average daily gain (ADG) (b) of pigs fed conventional (CON) or organic (ECO) feed mixtures

Fattening trial under conventional and organic system

At the beginning of the experiment there were no differences between experimental groups of pigs in body weight (P=0.325, Figure 3), but pigs in group ECO had thinner back fat than pigs in group CON (P=0.019, Figure 3). After 73 days of fattening, no differences among groups in body weight or backfat thickness (Figure 3) were observed. ECO pigs had 13% higher daily gain than pigs in group CON. This could be ascribed to high consumption of lucerne hay and possibly less feed dissipation. Pigs in group CON+L did not compensate slight dietary restriction with consumption of lucerne pellets, consequently leading to lower daily gains (11%) compared to CON pigs. No differences in backfat thickness (Figure 3) at the end of the trial and carcass traits (Figure 4) between treatment groups were noted. There were also no differences in carcass traits between the experimental groups.

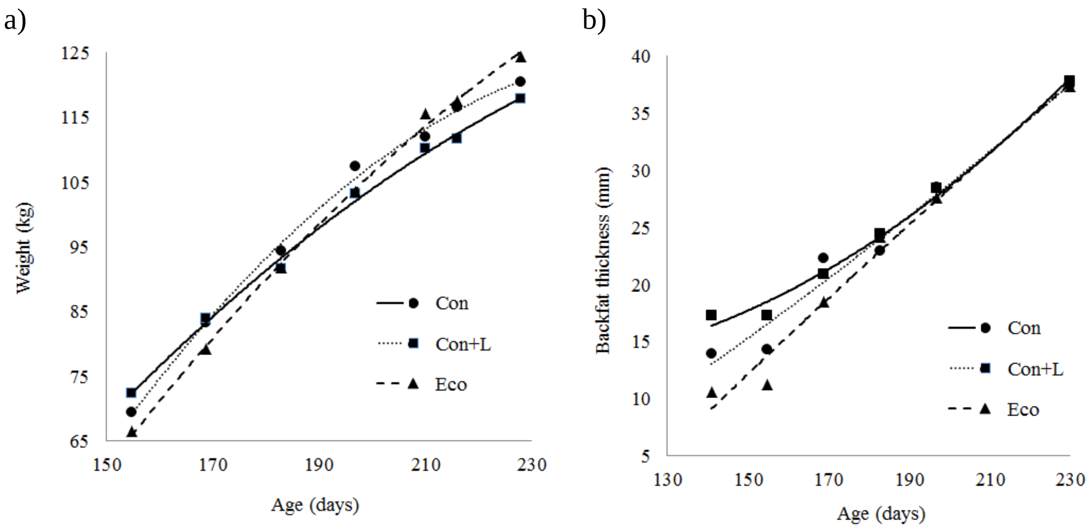


Figure 3: Gain of body weight (a) and backfat (b) in Krškoplje pigs during the experiment

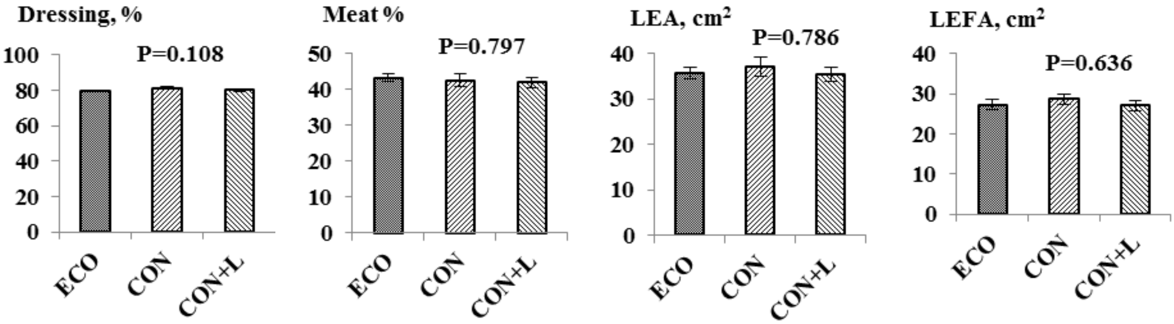


Figure 4: Dressing, meat %, loin eye area (LEA) and loin eye fat area (LEFA) of experimental Krškopolje pigs

Performance of Krškopolje pigs in extensive and intensive system

Pigs fed complete feed mixture achieved in total 49% higher daily gain and were heavier at slaughter than pigs fed traditional meal with root crops and cereals (88 vs. 120 kg, P=0.000). In the first observational period (25-45 kg) similar daily gain was observed for both groups, whereas pigs fed traditional meal with root crops and cereals grew slower than pigs fed complete feed mixture in the periods from 45 to 90 kg. In the last period (90-120 kg) only the pigs fed complete feed mixture were monitored, and they exhibited lower growth rate than in the previous two periods (Figure 5a). Slower growth of pigs fed traditional meal with root crops and cereals can be explained with lysine deficiency of their meal (data not shown; details about nutritional value of both diets are described in *Tomažin et al., 2017a)* As a result, owing to a greater live weight (BW) at slaughter, pigs fed complete feed mixture had thicker backfat than pigs fed traditional meal with root crops and cereals, however at equal BW (88 kg), no differences were observed. With regard to meat quality the results are indicative of more oxidative muscle metabolism of slower growing pigs fed a meal deficient in lysine (they exhibited lower CIE L* and higher CIE a* colour parameters of LD (i.e. darker, redder LD colour).

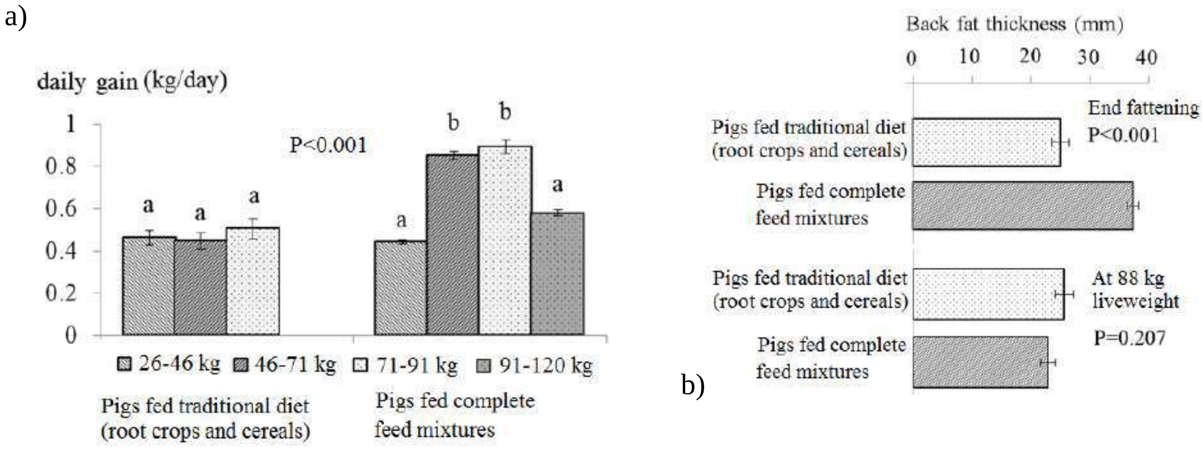
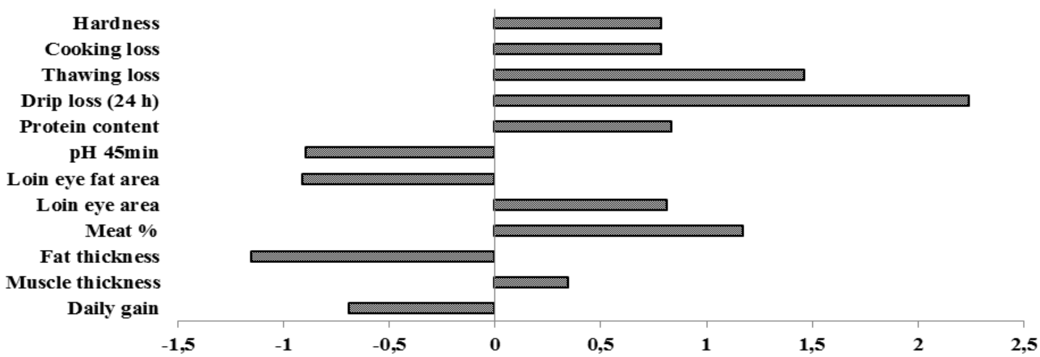


Figure 5: Daily gain (a) and backfat thickness (b) of pigs according to rearing system

Effect of the RYR1 mutation in Krškopolje pigs

Pigs of N/n and N/N genotype at *RYR1* locus reared under the same conditions were compared. Pigs that carry mutated "n" allele exhibited slower growth rate, were more muscular, leaner and less fat and had reduced water holding capacity and increased hardness than N/N pigs (Figure 6) which agrees with the impact of the mutated "n" allele on muscularity and meat quality traits reported for modern white breeds (*Monin et al., 1999; Fisher et al., 2000; Salmi et al., 2010).*



For variables presented in the Figure 6, the effect of "n" allele was significant (P<0.05).

Figure 6: Effect size (of N/n vs. N/N) for carcass and meat quality traits

The quality of salami from Krškopolje pigs as compared to standard product

Using the same processing method, dry-cured sausages, made from meat and back fat of Krškopolje pigs differed from the sausages using usual raw material (conventional pig). Sausages from Krškopolje pigs had higher a_w than standard sausages (0.892 vs. 0.830, P<0.001) despite similar moisture content (28.7 vs. 29.6%, P=0.090). Value of pH was slightly lower in the sausages of Krškopolje pigs than the standard ones (6.00 vs. 6.09, P=0.109). The sausages of Krškopolje pigs had less protein (23.1 vs. 29.9 %, P<0.001), more fat (44.0 vs. 33.8 %, P<0.001) but also less salt (3.9 vs. 5.5%, P<0.001) than standard sausages. Differences in chemical composition were consistent with the sensory evaluation results showing that Krškopolje sausages were scored lower for colour intensity, mature smell and saltiness and their texture perceived as softer, more juicy, crumbly and pasty (Figure 7).

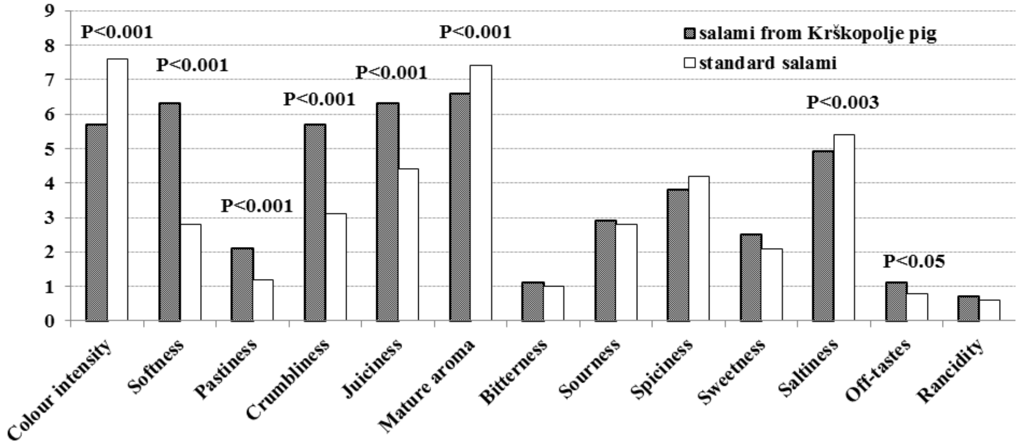


Figure 7: Differences in sensory profile of salami from Krškopolje pig and standard salami

Consumer preferences for salami from Krškopolje pigs

In a blind sensory test, standard salami was rated with a higher average rating scores compared to the salami from Krškopolje pig (P=0.003; Figure 8). On the contrary, respondents showed a significantly better expected acceptability for Krškopolje salami (P<0.001; Figure 8). When a sensory test with information about the product was applied (informed test), no difference in acceptability between the products was noted. According to the disconfirmation model, comparing results of blind and expected acceptability tests showed a negative disconfirmation for Krškopolje salami (the product is worse than expected; Figure 8). On the other hand, according to the assimilation model, higher scores of informed than of blind test for Krškopolje salami (P<0.001; Figure 8) are showing a positive assimilation and putting in evidence that information about the breed can have a positive impact on consumers' expectations about the tested product. Consumers showed higher acceptance when were informed about the product tasted. This finding highlights the importance of direct promotion and advertisement campaign that may give consumers the opportunity to know more about the breed.

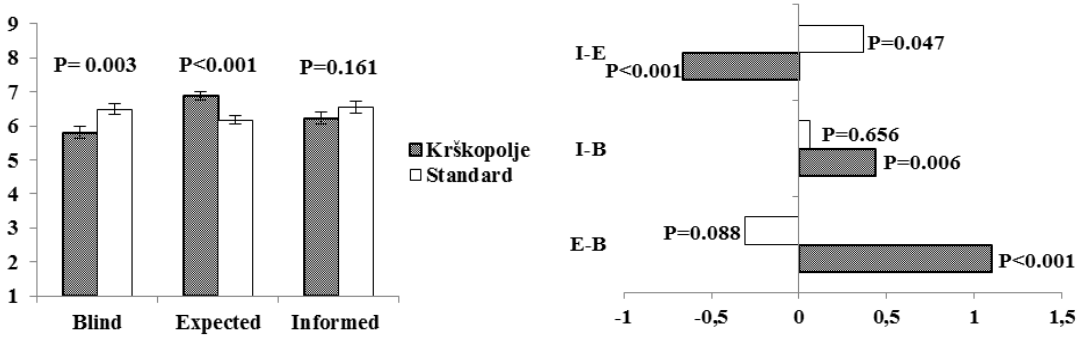


Figure 8: Results of sensory test and differences between expected (E) and blind (B), informed (I) and B, and I and E scores between standard salami and salami from Krškopolje pig

Conclusion

Preliminary results presented provide some basic information about the performances, productive traits and product quality of Slovenian local pig breed Krškopolje. Further studies are needed with regard to the growth potential according to the production system as well as for the purposes of developing adapted fattening systems. Krškopolje breed is facing a big challenge with the problem of high incidence of *RYR1* mutated allele that needs to be dealt with for the benefit of meat quality assurance. Studies with consumers show, that Krškopolje breed can have a positive impact on their expectations which highlights the importance of direct promotion.

Krškopoljska svinja u projektu TREASURE: od uzgoja do proizvoda

Marjeta Čandek-Potokar, Urška Tomažin, Martin Škrlep, Nina Batorek Lukač, Maja Prevolnik Povše, Peter Dovč, Zein Kallas, José M. Gil

Rezime

Krškopoljska svinja, slovenačka autohtona rasa, slabo je istražena i eksploatisana, kao što je slučaj kod mnogih autohtonih rasa svinja. Potrebno je više saznanja o različitim aspektima od karakterizacije rase, performansama i kvalitetu proizvoda, te društveno-ekonomskom značaju za razvoj održivih lanaca njihovih proizvoda. Da bi odgovorili na ove izazove, studije se sprovode u okviru projekta H2020 TREASURE koji se proteže od praćenja do procene proizvoda. U ovom radu prezentovani su prvi parcijalni i preliminarni rezultati za krškopoljsku svinju koji se odnose na procenu performanse rasta u različitim sistemima uzgoja, efekat mutacije RYR1 gena i senzornog kvaliteta i prihvatljivosti novog tradicionalnog proizvoda (suva kobasica u tipu salame) od Krškopoljskih svinja.

Ključne reči: autohtona rasa, krškopoljska svinja, performanse rasta, RYR1 mutacija, suva kobasica, test potrošača

Acknowledgment

The study was conducted within the project TREASURE, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634476. The content of this paper reflects only the author‘s view and the European Union Agency is not responsible for any use that may be made of the information it contains.

Authors would also like to acknowledge the core financing of Slovenian Agency of Research (grant P4-0133) and Krškopolje pig breeders for their cooperation.

References

BATOREK LUKAČ N., TOMAŽIN U., ŠKRLEP M., PREVOLNIK POVŠE M., ČANDEK-POTOKAR M. (2016): Effect of production system (organic vs. conventional) and roughage supplementation in diet on performance of growing-finishing Krškopolje pigs. Book of Abstracts of 9th International Symposium on Mediterranean Pig, Instituto Politécnico de Portalegre, Portalegre, Portugal, p. 36.

COUNCIL REGULATION EC (2007): Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Official Journal of the European Union, L 189/1.

FISHER P., MELLETT F.D., HOFFMAN L.C. (2000): Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. Meat Science, 54, 97–105.

KASTELIC A., ČANDEK-POTOKAR M. (2013): Application of quality labels in support of conservation of local breeds – a challenge for Slovenian Krškopolje pig. 8th International Symposium on the Mediterranean Pig, (Acta Agriculturae Slovenica), Biotechnical Faculty, Ljubljana, Slovenia, suppl. 4, 205–209.

MONIN G., LARZUL C., LE ROY P., CULIOLI J., MOUROT J., ROUSSET-AKRIM S., TALMANT A., TOURAILLE C., SELLIER P. (1999): Effects of the halothane genotype and slaughter weight on texture of pork. Journal of Animal Science, 77: 408–415.

OLSON, J.C., DOVER P.A. (1979). Disconfirmation of consumer expectations through product trial. Journal of Applied Psychology, 64(2), 179-189.

OLIVER, R.L. (1997). Satisfaction: A behavioral perspective on the consumer. New York, NY: McGraw-Hill.

SALMI B., TREFAN L., BLOOM-HANSEN J., BIDANEL J.P., DOESCHL-WILSON A.B., LARZUL C. (2010): Meta-analysis of the effect of the halothane gene on 6 variables of pig meat quality and on carcass leanness. Journal of Animal Science, 88, 2841–2855.

TOMAŽIN U., BATOREK LUKAČ N., ŠKRLEP M., PREVOLNIK POVŠE M., MEŽAN A., ČANDEK-POTOKAR M. (2016): Growth of Krškopolje piglets during lactation and the first rearing period. Book of Abstracts of 9th International Symposium on Mediterranean Pig, Instituto Politécnico de Portalegre, Portalegre, Portugal, p. 43.

TOMAŽIN U., ŠKRLEP M., BATOREK LUKAČ N., PREVOLNIK POVŠE M., ČANDEK-POTOKAR M. (2017a): Performance of Krškopolje pigs in extensive and intensive production system. 25th International Symposium Animal Science Days, September 20 to 22, 2017, Brandlucken, Austria.

TOMAŽIN U., BATOREK LUKAČ N., ŠKRLEP M., PREVOLNIK POVŠE M., OGOREVC J., DOVČ P., ČANDEK-POTOKAR M. (2017b): Meat quality of Krškopolje pigs as affected by *RYR1* genotype. 11th International Symposium “Modern Trends in Livestock Production”, 11th – 13th October 2017, Belgrade, Serbia.

GESTATIONAL STRATEGIES AFFECTING SOW REPRODUCTION AND PIGLET BIRTH WEIGHT

Giuseppe Bee

Agroscope Posieux, Posieux, 1725, Switzerland
Corresponding author : giuseppe.bee@agroscope.admin.ch
Review paper

Abstract: Nowadays it is becoming a great challenge for pig producers to deal with the increasing number of low birth weight piglets born from hyperprolific sows as underprivileged piglets suffer from increased pre-weaning morbidity and mortality primarily due to impaired in-utero development. As postnatal muscle growth and ultimately overall growth is determined not only by postnatal hypertrophy (increase in myofiber size) but also byprenatal hyperplasia (increase in myofiber number), the positive relationship between birth weight and myofiber number and myofiber number and growth efficiency, meat and carcass quality is of relevance. Therefore, nutritional strategies during gestation need to focus on improving muscle development of embryos and foetuses with the ultimate goal of minimizing the numbers of underprivileged low birth weight piglets. The first part of the review, will focus on myogenesis and its significance for postnatal growth performance, carcass characteristics, and pork quality. In addition, the inconsistency in the reported results with respect to low birth weight, postnatal growth, and carcass and pork quality will be discussed. The final part of the review will show known nutritional approaches to optimize the intrauterine environment via supplementing the sow during gestation with specific ingredients. In conclusion, some future research approaches will be proposed in order to further fill the knowledge gap with respect to prenatal development.

Key words: piglet, myofiber, birth weight, growth performance, growth efficiency, feeding strategy

Introduction

Both birth weight of the individual piglet and the intra-litter variation of birth weight are of considerable economic interest for pork production. Piglet's birth weight is highly correlated with the survival rate within the first days after birth. The latter dependson the ability of the newborns to ingest colostrum and of the dam to produce colostrum *(Theil et al., 2014; Quesnel et al., 2015).* Ultimately, this will affect the growth performance in the pre-weaning, nursery and grower-finisher period *(Quiniou et al., 2002; Pardo et al., 2013a; Jourquin et al., 2016).* The phenotype of the newborn piglet is determined by prenatal growth and development, which is under the control of intrinsic and extrinsic factors. These include maternal nutrition, maternal intestinal malabsorption, inadequate provision of amniotic and allantoic fluid nutrients, the ingestion of toxic substances, disturbances in maternal or fetal metabolic and homeostatic mechanisms, insufficiency or dysfunction of the uterus, endometrium, or placenta, environmental temperature and stress, and poor management (reviewed by *Wu et al. (2006)).*

Over the last decade, selection for improved prolificacy has resulted in a marked increase of piglets born per litter. In Switzerland, the number of piglets born alive has increased in the last 10 years from 11.5 to over 13 per litter and therefore over 30.6 piglets born alive and 26.3 piglets weaned *(SUISAG, 2014).* Slightly greater litter sizes have been reported in France with on average 13.6 piglets born alive *(IFIP-GTTT, 2015).* The selection for sow's ability to give birth to a greater number of piglets has led to an increased within-litter variation in piglet birth, as well as to an overall decrease in birth weight *(Foxcroft et al., 2007).* A possible cause for these observations is the increased competition among littermates for maternal nutrients in utero, because fetal and placental weight and birth weight have been shown to be inversely related with litter size *(Town et al., 2004).*

This non-exhaustivereview will focus on: 1) the consequences of impaired prenatal growth on myogenesis (prenatal skeletal muscle development), 2) the impact of differences in birth weight on postnatal development, carcass characteristics and meat quality traits and 3) possible feeding strategies in sows to affect fetal and early postnatal development of the offspring.

General Considerations on the Significance of Myogenesis for Postnatal Growth Performance

Embryonic Muscle Development

In the pig, myogenesis is a biphasic phenomenon and involves determination, migration, proliferation, differentiation, and fusion of myoblasts to form myotubes *(Picard et al., 2002).* In the first phase, lasting from d 35 to 55 of gestation, a primary generation of myotubes (the so-called primary myofibers) develop. In the second phase, which lasts until d 90 of gestation, the formation of a second generation of myotubes (secondary myofibers) occurs. Over 20 secondary myofibers appear around each primary myotube, using them as a scaffold *(Lefaucheur et al., 1995; Bérard and Bee, 2010; Bérard et al., 2010b).* Based on their findings, *Wigmore and Stickland (1983)* suggested that in the pigmyofiber hyperplasia ceases by around d 90 of gestation. Thus, the number of formed primary and secondary myofibers during gestation ultimately determines the total number of myofibers at birth. Recently, this hypothesis has been questioned as an increase in the total number of myofibers was observed within the first weeks after birth *(Loesel et al., 2009; Lopez et al., 2010; Bérard et al., 2011).* These observations give raise to the opportunity to develop feeding strategies that could affect hyperplasia after birth. Rather consistent among studies is the fact that the number of secondary myofibers, as well as the total number of myofibers, formed is lower in lighter compared with heavier newborn piglets *(Wigmore and Stickland, 1983; Rehfeldt and Kuhn, 2006; Bérard et al., 2010b; Pardo et al., 2013b). Wigmore and Stickland (1983)* gave for the lower hyperplasia in low birth weight newborn pigs the following explanation: since primary myofibers serve as a scaffold for the formation of secondary myofibers, the available smallersurface area of primary myofibers restrict the secondary myofiber formation.

POSTNATAL MUSCLE DEVELOPMENT

The increase in skeletal muscle weight during postnatal growth results from myofiber hypertrophy and elongation. Furthermore, the extent of myofiber hypertrophy and, thus, the capacity of the muscle to grow depends also on the total number of myofibers within a muscle. It has been shown that myofiber size is inversely correlated (r=-0.3 to -0.8) with myofiber number, which means that growth rate of the individual myofiber is lower when there are greater numbers of myofibers and greater when there are fewer myofibers *(Rehfeldt et al., 2000)*. On the other hand, both number and size of myofibers are positively correlated with the cross-sectional area of the muscle (r=0 to 0.6 and r=0.3 to 0.5, respectively). This raises the question whether hypertrophy or total number of myofibers is more important for lean tissue growth. Because myofiber hypertrophy islimited by genetic and physiological constraints, potential for lean tissue growth depends primarily on the number of the prenatally formed myofibers *(Rehfeldt and Kuhn, 2006)*. Based on this assumption, one can conclude that impaired postnatal growth can be expected in low birth weight piglets displaying low myofiber numbers.

LOW BIRTH WEIGHT AND ITS IMPACT OR LACK OF IMPACT ON POSTNATAL GROWTH PERFORMANCE, CARCASS AND MEAT QUALITY

Recent results from various experiments demonstrate the close relationship between birth eight, carcass characteristics, and meat quality traits. *Rehfeldt and Kuhn (2006)* determined that at birth, compared with the heavier littermates the lightest piglets exhibited the lowest percentages of muscle mass, total protein, total fat, the lowest *semitendinosus* muscle weight and total number of myofibers, whereas the percentages of internal organs, skin, bone, and total water were highest, compared to their heavier littermates. At 182 d of age, pigs of low birth weight were lighter, had lower meat percentages, and loin area was smaller compared to pigs of high birth weight, whereas the percentage of mental fat tended to be greater. The pigs of low birth weight exhibited the lowest myofiber numbers, the largest myofiber size, and the highest percentages of abnormal “giant” myofibers in both muscles under investigation. With respect to meat quality, higher drip losses were determined in the *longissimus* muscle of low birth weight pigs. *Rehfeldt and Kuhn (2006)* explained the difference in adipose tissue accretion between low and high birth weight pigs with the faster myofiber hypertrophy due to the low myofiber number and the plateau of myofiber growth is attained earlier compared to high birth weight pigs. Consistent with these earlier results, *Bee (2004)* reported larger myofibers and fatter carcasses in low compared with high birth weight pigs slaughtered at 105 kg body weight. *Gondret et al. (2005); (2006)* found that compared to heavy birth weight pigs, low birth weight pigs reached 12 d later the same slaughter weight of 112 kg. Not only was growth rate impaired, but also feed efficiency was inferior in low birth weight pigs. Accordingly, these pigs exhibited a fatter carcass, associated with markedly greater activity of enzymes involved in lipogenesis, such as fatty acid synthase and malic enzyme. Again, the total myofiber number was lower in the *semitendinosus* muscle, and the myofibers were larger in both the *semitendinosus* and *longissimus* muscles of low compared to high birth weight pigs. Of great importance with respect to consumer’s satisfaction with pork, was the finding that low birth weight pigs exhibited a lower score for loin meat tenderness compared with high birth weight pigs. These results also indicated that the birth weight effect on meat tenderness could be partly attributed to the myofiber hypertrophy, because tenderness score was negatively correlated (r= -0.34) with myofiber size of the *longissimus* muscle. However, other could only partly confirm the negative relationship between birth weight and carcass and meat quality traits (Bérard et al., 2008; Pardo et al., 2013a; Smit et al., 2013). Again, others did not observe any impact on performance and quality traits *(Beaulieu et al., 2010)*. The discrepancy between results of different studies regarding the impact of birth weight can be explained by the fact that different ranges of birth weights were studied (Table 1).

Table 1. Impact of birth weight range and litter size on differences in growth performance, carcass, and meat quality traits

Birth weight range (kg)	Litter size (pigs born alive)	Effects[1]	Study
1.27 vs. 1.76 kg	not specified	↓ pre-weaning growth ↑ adipose tissue yield	Bee (2004)
0.94 vs. 1.34 kg	not specified	↓ growth performance ↓ carcass quality ↓ water holding capacity	Rehfeldt and Kuhn (2006)
1.05 vs. 1.89 kg	not specified	↓ growth performance ↓ carcass quality ↓ meat tenderness	Gondret *et al.*(2006)
1.23 vs. 1.90 kg	≥ 14	↓ growth performance = carcass characteristics = meat quality	Bérard *et al.*(2008)
1.60 vs. 2.02 kg	≤ 10		
1.26 vs. 1.49 kg	> 10 and < 15[2]	↓ pre-weaning growth ↓ feed efficiency ↓ belly	Pardo *et al.*(2013a)
1.40 vs. 1.79 kg	> 10 and < 15[3]		
< 1.20 vs. > 1.70 kg	not specified	↓ growth performance = carcass characteristics ↑ intramuscular fat content	Beaulieu *et al.* (2010)
1.12 vs. 1.79 kg	>9 and < 16	↓ growth performance	Smit *et al.* (2013)

[1] Effects on growth performance, carcass characteristics and meat quality are reported against the high birth weight category = no effect; ↑ increased; ↓ impaired
[2] average litter birth weight of this experimental group was < 1.3 kg
[3] average litter birth weight of this experimental group was > 1.7 kg

Except for the study of *Bérard et al. (2008)* and *Pardo et al. (2013a)*, where the litter size from which the pigs were selected from was reported, this information is lacking or not clearly mentioned in the other studies. That litter size may have an impact on the effect of birth weight on growth, carcass characteristics and meat quality was recently suggested by *Bérard et al. (2010a)*. The authors reported that the superiority of pigs born with a high compared to a low birth weight regarding carcass and meat quality were less evident when pigs originate from large litters.

SOW REPRODUCTION AND PHENOTYPIC TRAITS OF OFFSPRING AS AFFECTED BY THE FEEDING STRATEGY DURING GESTATION

Commonly, gestating sows have access to a restricted diet. The daily energy requirements vary between 30.5 and 42.2 MJ DE for multiparous sows and between 25.6 and 36.3 MJ DE for gilts, respectively (Agroscope, 2015), depending on the gestation period, that can be divided into an early (d 1-28), middle (d 29-84) and late (d 85-115) gestation period. The greater energy allowance in late gestation targets the exponential fetal growth and development of the mammary gland *(Noblet et al., 1990; Dourmad et al., 2008)*. As fetal growth is largely affected by the nutrients fed to gestating sows, ideally, nutrient allowance should also take into account the number of fetuses in the uterus, which as previously mentioned, increased in the last decades. To our knowledge, nowadays in gestating sows the correct prediction of the litter size is not possible, which limits the possibility to feed the fetuses adequately. Nevertheless, various dietary approaches were made to positively affect fetal development, piglet vitality, birth weight and litter size.

IMPACT OF DAILY FEED ALLOWANCE IN DIFFERENT GESTATION PERIODS

Several authors investigated the effects of increasing the feed allowance of sows during gestation on sow reproduction, phenotypic traits of the offspring at birth and their postnatal performance *(e.g. Dwyer et al., 1994; Nissen et al., 2003; Bee, 2004; Heyer et al., 2004; Musser et al., 2004; Nissen et al., 2005; Lawlor et al., 2007; Cerisuelo et al., 2009)*. In all those studies, where the quantity of provided nutrients and the treatment window during gestation varied, the effect on sow's reproductive performance and litter birth weight were negligible. However, *Dwyer et al. (1994)* reported that by doubling feed allowance (5 vs 2.5 kg) from 25-80 d of gestation hyperplasia of secondary myofibers tended to increase (expressed as secondary-to-primary myofiber ratio) in the *semitendinosus* muscle of these offspring and in the grower period they grew faster and were more feed efficient. *Musser et al. (2004)* observed marked changes in fetal compartment when *ad libitum* feed was provided to sows during an important period of fetal myogenesis (d 28 to 56 of gestation). The elevated fetal IGF-I level could have been partly mediated effects of maternal feed intake on the aforementioned fetal myogenesis *(Oksbjerg et al., 2004)*. By contrast, limited or no impact on myofiber formation and postnatal growth were reported when compared to the control diet the amount of feed offered to gestating sows was increased by up to +100% in the treatment window varying from 0 to 85 d *(Nissen et al., 2003; Bee, 2004; Lawlor* *et al., 2007; Cerisuelo et al., 2009).* Negative long-term effects on the performance of their offspring were reported by *Heyer et al. (2004)* when sow's feed intake was increased by up to 100% compared to the control. Based on the low performance in the suckling period, *Heyer et al. (2004)* hypothesized that mammary gland development and therefore milk synthesis was impaired by the elevated feed allowance during gestation. In line with this hypothesis, *Dourmad (1991)* reported that increased feed intake throughout gestation negatively affected feed intake during lactation and concomitantly colostrum and/or mature milk synthesis *(Decaluwé et al., 2014).* However, *Nissen et al. (2003)* did not observe any relationship between feed intake in the gestation and lactation period as well as with mature milk yield. Taken together, the results of the various studies do not indicate that increasing feed intake in the first half of gestation affects reproduction or phenotypic traits of the offspring.

IMPACT OF SPECIFIC SUPPLEMENTS IN THE GESTATION DIET

DIETARY FAT AND FATTY ACID COMPOSITION

Instead of increasing the amount of feed, one can specifically increase the dietary energy density by adding additional fat. In addition this strategy has the advantage of having a low heat increment associated with digestion and metabolism *(Kleiber, 1961)*. Under certain environmental conditions such as heat stress and when average daily energy intake needs to be increased the lower heat increment might be of relevance. The main and most consistent effect of feeding additional fat during gestation is the increase in milk yield and depending on the fat source the modulation of the fatty acid profile *(Farmer and Quesnel, 2009; Quesnel et al., 2015)*. With respect to litter size and birth weight of the piglets, increasing the dietary energy content by adding fat to the gestation diet seems not to have any note worthy positive effects. Conversely, at the molecular level *Fainberg et al. (2014)* found that greater fat supply during gestation accelerates the muscle maturation of offspring, reflected in increased glycolytic metabolism and fibre cross sectional area in the *biceps femoris* muscle. Apart from the role as an energy source, the fatty acid composition of the dietary fat, especially the proportion of essential n-6 and n-3 polyunsaturated fatty acids (PUFA) attracted a great deal of interest on their possible effect on sow reproduction and foetal development. The reason for this increased awareness is the primordial role PUFA have on structural functions in the phospholipid bilayer of cell membranes, which ultimately affect membrane fluidity and intracellular signal transduction. *Gerfault et al. (1999)* found that whatever their chain length or their degree of un saturation, dietary fatty acids cross the placental barrier in the pig. Thus, attempts have been undertaken to use various sources of fats differing in their amounts of PUFA to affect litter size, birth weight and vitality of newborn pigs. As reviewed by *Tanghe* *and De Smet (2013)* and *Quesnel et al. (2015)* little evidence exist that feeding PUFA enriched diets increase litter size and litter weight. *Rooke et al. (2001b)* even reported that feeding salmon oil, a long chain n-3 rich fat source, to sows throughout pregnancy significantly decreased individual piglet birth weight. However, in the same study despite having no impact on litter size, pre-weaning mortality of offspring born from sows fed the salmon oil fortified gestation diet was reduced. Regarding number of total and live born piglets, *Corson et al. (2008)* found that a gestation diet enriched with n-6 PUFA fat (soy oil) offered in the first half of pregnancy compared with a gestation diet containing medium-chain fatty acids (palm oil) resulted in smaller and lighter litters at birth. The authors hypothesized that the fatty acids present in palm oil are more easily combusted, thus providing a readily available source of energy. In contrast, the diets supplemented with soy oil, tend to promote pro-inflammatory responses, which can have deleterious effects on foetal survival especially when offered in the first half of gestation. The authors could make this latter statement because when applying the same feeding strategy in the second half of gestation, the negative effect on litter weight was not observed.

Long chain n-3 fatty acid, especially docosa hexaenoic acid, can be found in high concentrations in the brain and is thought to act at the level of nerve growth, synaptogenesis and to be involved in interactive processes between nerve cells and cell signal transduction *(Innis, 2007)*. Since dietary fatty acid cross the placental barrier and during lactation affect the fatty acid composition of sow milk, the latter being the sole dietary source of the new born piglet, it was hypothesized that cognitive development and as a consequence behaviour of the new born piglet could be modulated by dietary means. Promising results were reported by *Rooke et al. (2001a)* who observed increased piglet vigour at birth when originating from sows receiving in the last third of gestation a tuna oil fortified compared to a soy oil supplemented diet. The greater vitality allowed them to reach the udder and suckle more quickly. However, the same authors reported in an earlier study that despite the proportions of long-chain *n*-3 fatty acid in tissues of new-born piglets were increased by feeding tuna oil, various vitality indicators such as heart rate at birth, onset of respiration and standing time were not improved *(Rooke et al., 1998)*. The reasons for these differences are unknown but may explain that the effects of n-3 PUFA supplemented gestation diets on pre- and post-weaning growth of the offspring are inconsistent and no clear evidence exits to support the fact that increased piglet vitality will positively affect postnatal growth. It is noteworthy to mention that many of the studies cited in various reviews were carried over a decade ago *(Tanghe and De Smet, 2013; De Vos et al., 2014; Quesnel et al., 2015)*. Because litter size increased dramatically over this time span and concomitantly the portion of less vital/underprivileged newborn piglets per litter increased, additional research on this important topic is warranted. Future research should focus on crucial factors, which affect the development of the growing foetus and, postnatally, impact growth performance such as type, timing and dietary oil source *(De Vos et al., 2014).*

DIETARY PROTEIN INTAKE

Availability, amount and quality of dietary protein play a key role for the developing embryo and foetus *(Metges and Hammon, 2005).* For instance, a 50%lower dietary protein supply (compared with requirement; 121 g/kg) during gestation reduced birth weight, impaired myogenesis, persistently restricted muscle growth potential and reduced the potential of postnatal lean growth of the offspring *(Rehfeldt et al., 2012a).* Feeding un physiologically elevated concentrations of dietary protein retards foetal growth as well but had little effect on foetal programming of postnatal muscle and adipose tissue phenotype of the progeny *(Rehfeldt et al., 2012b).* Surprisingly, either a protein-free diet throughout or during specific periods of gestation, and increased or decreased protein intake at different stages of pregnancy affected litter size at birth and the number of piglets born alive *(Pond et al., 1987; Rehfeldt et al., 2011).* However, the available data show also that unbalanced protein intake has consequences on body weight and backfat gain of the dams during gestation; low and high dietary protein supply significantly lowered the cumulative body weight gain and lowered the backfat gain, respectively *(Kusina et al., 1999; Rehfeldt et al., 2011).* It has been emphasized that these changes might affect mammary gland development and lactation performance *(Vadmand et al., 2015)* and weaning-to-oestrus interval. *Kusina et al. (1999)* showed that milk yield and pig weight gain increased as protein intake moderately increased during gestation and lactation. Not only the yield but also the composition of the milk seems to be partly affected by the protein intake. *Rehfeldt et al. (2011)* reported that colostrum lactose and fat levels tended to be lower when gestation diet contained either very low or very high protein levels, whereas no dietary effect was determined in crude protein and immunoglobulin concentrations.

FUNCTIONAL AMINO ACIDS

Based on a growing body of literature a new concept of functionality in addition to the classical concept of essentiality and non-essentiality with respect to amino acid requirement has been proposed. *Wu (2010)* defined functional amino acids as those amino acids that regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms. One of those amino acids, which with respect to reproduction and early postnatal growth attracted recently a lot of attention, is L-arginine (Figure 1). This amino acid is the common precursor for nitric oxide and polyamines, which are key regulators of angiogenesis, embryogenesis, and placental and foetal growth *(Wu et al., 2006).* As arginine is particularly abundant in porcine allantoic fluid and is associated with high rates of synthesis of nitric oxide and polyamines in the placenta during the first half of pregnancy, the hypothesis was formulated that increasing dietary L-arginine supply to sows may stimulate placental growth thereby promote conceptus survival, growth and development of some tissues. Results of various studies demonstrated that additional dietary supplementation with L-arginine positively affected the development of progeny *(Ramaekers et al., 2006; Mateo et al., 2007; Wu et al., 2010; Li et al., 2014).* Interesting was the study of *Ramaekers et al. (2006)* who showed that when sows were offered a diet supplemented with 25 g/d of L-arginine between d 14 to 28 of gestation litter size increased by 0.8 extra piglet/litter without any increase in within-litter birth weight variation. The positive impact of L-arginine supplementation during the peri-implantation period of gestation is due to the nitric oxide synthase-3 dependent production of nitric oxideby the conceptus trophectoderm and consequently the availability of arginine in conceptus tissues for synthesis of polyamines that are essential for conceptus survival and development *(Wang et al., 2014)*. *Mateo et al. (2007)* and *Wu et al.(2010)* found increased number of live-born piglets and total litter weight by up to 23 and 28%, respectively, when gilts were offered a 1 or 0.83% L-arginine supplemented diet also from d 30 (post-implantation) until parturition. One possible reason for this outcome is the enhanced placental angiogenesis through the arginine-nitric oxide pathway *(Hazeleger et al., 2007)* resulting in greater vascularization, which is a prerequisite for an efficientplacental transport of O_2 and nutrients from mother to embryo/foetus (Figure 1).

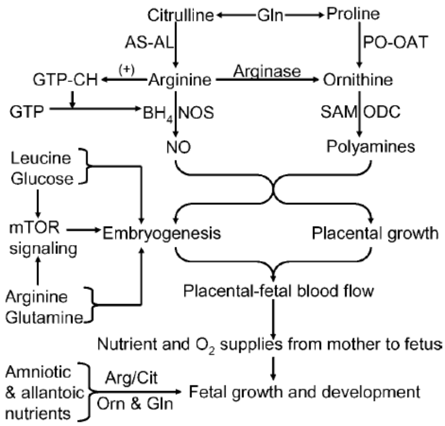


Figure 1. Roles of arginine, nitric oxide (NO), and polyamines in foetal growth
Both maternal under- and over-nutrition may impair placental synthesis of NO and polyamines, and therefore placental development and uteroplacental blood flows. This may result in reduced transfer of nutrients and O_2 from mother to foetus, and thus restrict foetal growth. The ornithine used for polyamine synthesis is derived from proline catabolism via proline oxidase in porcine placental and other tissues as well as from arginine hydrolysis via arginase in a variety of porcine tissues, including the small intestine, liver, and kidneys. Glutamine is a common substrate for the synthesis of both citrulline and proline in pigs [published in *Wu et al.* (2006)]
Arg = arginine; AS-AL= argininosuccinate synthase and argininosuccinatelyase; BH_4 = tetra-hydrobiopterin; Cit = citrulline; Gln = glutamine; mTOR = mammalian target of rapamycin; GTP-CH = GTPcyclohydrolase-I; ODC = ornithine decarboxylase; NOS = nitric oxide synthase; Orn = ornithine; PO-OAT = proline oxidase and ornithine aminotransferase; and SAM = S-adenosylmethionine.

The suggested greater foetal nutrient supply may further explain hyperplasia of primary myofibers in the *semitendinosus* muscle of 75 d old foetuses *(Bérard and Bee, 2010). Madsen et al. (2016b)* showed that the myofiber hyperplasia in the *semitendinosus* muscle of offspring of prolific sows fed an L-arginine fortified gestation diet around the peri-implantation period was greater compared to control sows. The greater number of formed myofibers resulted primarily from the greater number of secondary myofibers formed in the second wave of myofiber ontogenesis. Dietary supplementation with L-arginine may also benefit piglets, irrespective of the L-arginine given to the lactation sow *(Mateo et al., 2008)* or directly to the piglet *(Kim et al., 2004; Yao et al., 2008). Mateo et al. (2008)* found that on d 7 of lactation milk yield and the concentrations of most amino acids in milk were greater in response to arginine supplementation during lactation compared with the control. This increase could result from a positive effect of L-arginine on vascularity, enhanced blood flow and, therefore, the uptake of nutrients by the lactating mammary gland *(Rezaei et al., 2016)*. In addition, weight gain from birth to 21 d of age of these piglets was also greater. The study of *Kim et al. (2004)* and *Yao et al. (2008)* reported that artificially reared piglets fed a milk replacer supplemented with 0.4% L-arginine had greater plasma concentrations of insulin and growth hormone as well as protein synthesis, which may explain the faster growth from d 7 to 21 of age. *Madsen et al. (2016a)* could not confirm faster growth when L-arginine was add to the milk replacer of low birth weight pigs to cover at least the requirement of 1.08 g×kg body weight^{-1}×d^{-1}. However, the greater lactate dehydrogenase to citrate synthase and β-hydroxyacyl-CoA dehydrogenase ratio indicated that the relative importance of the glycolytic compared with the oxidative pathway in the the *semitendinosus* musclewas greater in these piglets compared to those of the control group. The greater glycolytic potential can be interpreted as greater muscle maturity.

Recent advances in research revealed that L-glutamine, a member of the arginine family of amino acids, is an abundant amino acid in physiological fluids and proteins, is utilized in multiple metabolic pathways (Figure 2) and has many regulatory functions (Figure 3). In recent years, glutamine has gained some interest in pregnancy as it is an abundant amino acid in fetal tissue proteins and a major energy substrate for the fetus *(Wu, 2010)*. Therefore, on can conclude that L-glutamine plays a key role in many metabolic processes such as cell proliferation, differentiation, and embryonic development *(Wu et al., 2011)*. The importance of glutamine for placental and fetal growth was deduced from the observation that among all amino acids, uterine and umbilical uptake of glutamine was the greatest *(Wu et al., 2015)*.To control energy intake of gestating sows, restricted feed allowance is a common management practice. Such a restricted feeding program limits maternal protein intake, resulting in protein deficiency, particularly during late gestation when absolute fetal growth is most rapid *(Noblet et al., 1985)*. In pigs, 60% of fetal growth occurs from d 90to 114 of gestation, which is a development stage where fetal glutamine requirement is increasingly elevated. In agreement, *Wu et al. (2011)* reported markedly lower concentrations of plasma glutamine in gilts at d 110 than at 10 d of gestation (0.30 ± 0.02 and 0.52 ± 0.04 mM; means ± SEM). The same authors hypothesized that glutamine deficiency may partly contribute to low birth weight pigs due to intra-uterine growth retardation. In support of this hypothesis, *Wu et al. (2011)* found that feeding gilts between d 90 and 114 of gestation a gestation diet fortified with 1% glutamine significantly increased maternal plasma level of glutamine (486 vs 354 μM), arginine (194 vs 176 μM), ornithine (82 vs 73 μM) and proline (277 vs 240 μM), the average birth weight (1.41 vs 1.33 kg) and litter birth weight of live-born piglets (14.7 vs 13.7 kg). Concomitantly, the percentage of low birth weight pigs (< 1.1 kg; 15.2 vs 24.8%), the variation in birth weight (11.0 vs 16.4%), and pre-weaning mortality of live-born piglets (6.1 vs 11.3%) decreased when compared

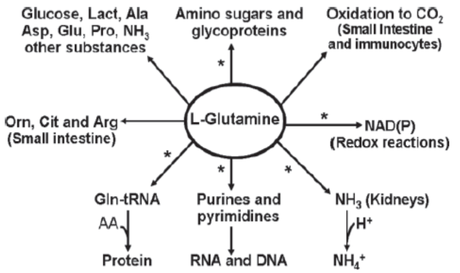


Figure 2. Multiple metabolic pathways for L-glutamine (Gln) utilization in animals

Synthesis of ornithine (Orn), citrulline (Cit), and arginine (Arg) from Gln occurs exclusively in the small intestine. Other metabolites of Gln include pyruvate, urea, and possibly glycin. The asterisk indicates that the reaction cannot be replaced by any other amino acid incells [published in Wu et al. (2011)].

Lact = lactate

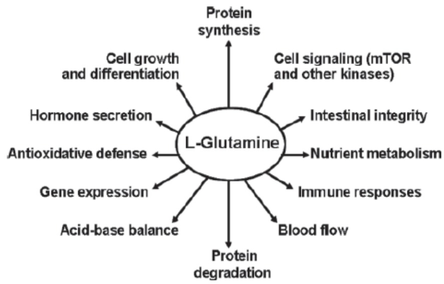


Figure 3. Regulatory functions of L-glutamine (Gln) in animals.

Gln can regulate multiple physiological processes in diverse cell types, including gene expression, cell signalling, and protein turnover. These effects of Gln are cell and tissue specific, indicating that interorgan cooperation is necessary for Gln to exert its beneficial actions on the whole body. [published in Wu et al. (2011)]

mTOR = mammalian target of rapamycin

with the isonitrogenous control. These results seem promising although it has to be taken into account that only gilts were used and the number of total born and born alive per litter were with 11.5 and 10.3, respectively, rather low.

It would be interesting to assess if this strategy would be as successful when prolific gilts and sows would be included.

In addition to the aforementioned amino acids, there is emerging evidence of the functional importance of branched-chain amino acids (leucine, isoleucine, and valine) throughout pregnancy. Their relevance are linked to the fact that these amino acids are substrates for the synthesis of glutamate, the immediate precursor of L-glutamine, and of arginine, which is important for the synthesis of citrulline, ornithine and proline *(Rezaei et al., 2013)*. Furthermore, branched-chain amino acids activate the mammalian target of rapamycin signaling pathway and protein synthesis and concomitantly decrease proteolysisand by that positively affect muscle growth *(Davis et al., 2010)*. Placental transport and fetal utilizationof branched-chain amino acidsis drastically reduced *(Lin et al., 2014)* whereas fractional rates of protein synthesis and its response to feeding are unaffected by intra-uterine growth restriction in newborn pigs *(Davis et al., 1997)*.Thus, there seems to be a possibility of accelerating lean mass growth in these fetuses through dietary intervention. Unpublished data from *Yuan et al. (2015)* suggest that this hypothesis could be valid because feeding gestating sows a branched-chain amino acid supplemented diet increased litter size, weights of piglets as well as of the placentas. In line with these finding, traits relevant for survival and growth of the concept us such as embryonic hepatic IGF-I level, estrogen receptor-α and progesterone receptor in the uterus, and IGF-II level in the placenta and expression of key enzymes involved in gluconeogenesis in embryonic livers were increased.

IMPACT OF L-CARNITINE IN SOW PRODUCTION

L-Carnitine is an important compound in mammals because itserves as an essential cofactor for mitochondrial fatty acid oxidation by transferring medium- and long-chain fatty acids as acyl-carnitine esters across the innermitochondrial membrane. Moreover, carnitine shuttles acylmoieties out of peroxisomes in the liver. It also regulates theintra-mitochondrial acyl-CoA/CoA ratio and acts as a CoA buffer in mammalian cell. Increased protein accretion and reduced backfat thickness with greater rates of palmitate oxidation, more rapid fluxthrough pyruvate carboxylase, and reduced fluxthrough branched-chain α-keto acid dehydrogenase suggests that growing pigs fed L-carnitine are more able to use fat for energy, divert carbon toward synthesis of amino acids, and spare branched-chain amino acids for protein synthesis *(Owen et al., 2001).*Various authors tested whether these changes in metabolism induced by L-carnitine would be beneficial for sow reproduction and for fetal development and growth. Supplementing the gestation diet with L-carnitine (100 mg/d) increased the number of viable fetuses (15.5 vs 10.8) and tended to increase their total weight (1450vs 989 g) but not the individual fetal weight at d 57 of gestation compared with controls *(Waylan et al., 2005).* When L-carnitine (125 mg/d) was fed for the whole gestation period, *Ramanau et al. (2004)* observed that L-carnitine-treated primiparous and second parity sows gave birth to larger (parity 1 and 2: 12.9 vs 10.2 and 13.5 vs 10.8, respectively) and heavier litters(parity 1 and 2: 16.8 vs 14.2 kg and 19.6 vs 17.3 kg, respectively) compared with control sows.The positive effect of L-carnitine (100 mg/d) on litter size was not confirmed in the study of *Musser et al. (1999).*

However, in the same study they observed increased total litter (15.5 vs 14.6 kg) and pig (1.53 vs 1.49 kg) birth weight. These findings concur with results of other studies where litter weight and pig weight increased by 1.0 to 2.6 kg and 70 to 110 g, respectively *(Eder et al., 2001; Ramanau et al., 2002; Musser et al., 2007; Ramanau et al., 2008)*.In addition, several studies concurred that L-carnitine supplementation of sows during pregnancy reduces the number of stillborn (from 0.76 to 0.49 per litter in *Musser et al., 1999*; from 0.97 to 0.68 per litter in *Ramanau et al., 2008*) and very low birth weight piglets (< 800 g: 0.9 vs 0.4%; Eder *et al.*, 2001). As reviewed by biochemical mechanisms underlying the favorable effect of L-carnitine on intra-uterine growth have not been fully elucidated. However, there is some evidence that L-carnitine influences the IGF-axis in sows and leads to greater placentae, which in turn improves intra-uterine nutrition, and stimulates oxidation of glucose in the fetuses (Figure 4). These effects may, at least in part, be responsible for the aforementioned heavier litters. IGF-1 is a key hormone forintra-uterine fetal development and promotes muscle development *(Oksbjerg et al., 2004)*. Data of *Waylan et al. (2005)* suggest that L-carnitine induced changes in the IGF-axis decreased IGF-II, IGFBP-3, and myogenin mRNA levels in porcine embryonic myoblasts and delayed their differentiation to existing myofibers and prolonged their proliferation. Ultimately, this could give riseto increased myofiber numbers at birth due to increased number

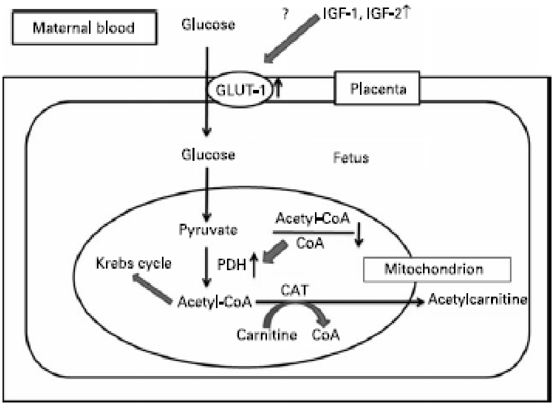


Figure 4. Proposed mechanism by which L-carnitine supplementation of sows increases birth weights of their offspring.

Carnitine supplementation of sows improves the development of the placenta and increases glucose transport protein (GLUT-1) concentration in the chorion, likely due to increased maternal plasma insulin-like growth factor (IGF)-1 and IGF-2 concentrations. This leads to an increased transfer of glucose from maternal to fetal blood. Carnitine moreover stimulates oxidation of glucose in fetal cells, which enhances the efficiency of energy production. This effect is based on the ability of carnitine to shuttle acetyl groups out of the mitochondrion as acetylcarnitine (formed by the action of carnitine acetyl transferase (CAT)), resulting in a reduction in the acetyl-CoA:CoA ratio, which in turn activates pyruvate dehydrogenase (PDH).[published in Eder (2009]) of embryonic myoblasts. In accordance, *Musser et al. (2007)* reported that piglets of L-carnitine-supplemented sows had a greater number of primary myofibers in the *semitendinosus* muscle than piglets of control sows. Surprisingly, in the second wave of myofiber formation the number of secondary myofibers was not increased as the secondary: primary myofiber ratio was lower.

Conclusion and outlook

It is a fact that due to targeted selection for reproduction performance and despite rather low heritability, sow prolificacy markedly increased in the last decade. When expressed per kg of gestation feed more piglets are born, which at first sight might suggest that sow production efficiency increased. However, with increasing litter size it is evident that the number of underprivileged low birth weight pigs increases as well. This sub-population of offspring are known to be less vital and more prone to perish within the first days and weeks of life. From a production point of view, this circumstance decreases reproduction efficiency but also causes some ethical challenges. Furthermore, there is enough evidence that low birth weight pigs will not express their innate genetic potential for growth, carcass and meat quality to the same extent as their heavier littermates, which ultimately will further decrease the overall production efficiency. One of the main causes for low birth weight is the impaired uterine environment, which affect fetal nutrient supply and result in intra-uterine growth retardation. As presented in this review, various promising feeding strategies that target the uterine environment and fetal nutrient supply have been proposed. One main issue is that the published experiments were not carried out with prolific or hyper prolific sows and therefore it isn’t clear to what extent the proposed strategies are effective. Furthermore, often only primiparous and not multiparous sows were used and only the impact of a given feeding strategy was studied in one parity. Thus, future studies should be designed, which specifically accounts for these facts.

The author believes that in the future one should aim at improving the “quality” (reduced heterogeneity) of the offspring rather than increase their number. We have evidence that for instance in the current Swiss Large White sow population there are prolific sows (> 15 pigs born alive), which are repeatedly able to give birth to large very homogenous litters, with almost none of the offspring displaying a birth weight <1.3kg. Thus, it appears that in the sow population the genetic potential exists to obtain large litters and concomitantly circumvent the problem of underprivileged low birth weight pigs. This potential should be considered in future selection programs. Nevertheless, feeding hyperprolific sows will remain a great challenge in swine production. As previously mentioned in this review various feeding strategies have been proposed. In the author’s opinion, future studies should target at improving fetal nutrient supply and thus fetal development in the post-implantation periods of gestation by combining the current knowledge on the effects of n-3 fatty acids, functional amino acids and L-carnitine. To exploit the full genetic potential of all littermates these kind of studies are necessary because it seems unlikely that postnatal feeding strategies exits which are effective in overcoming the effects of low birth weight.

What was not discussed in this review is the fact that besides optimizing intrauterine environment, both feeding and management strategies should also focus on increasing the survival chance and improve the early postnatal growth performance of underprivileged pigs. In this context, future research efforts should focus on colostrum, transient milk and mature milk production (yield and composition) and intake. In addition, from our study in the ECO-FCE project it is evident that future scientific work is required to improve the nutritional quality of milk replacer (offered either in the farrowing pen or in rescue decks) in order to boost piglet growth and ultimately efficiency.

Uticaj gestacionih strategija na reprodukciju i masu prasadi na rođenju

Giuseppe Bee

Rezime

Veliki izazov za proizvođače svinja, danas, predstavlja sve veći broj prasadi niskih telesnih masa na rođenju, koja potiču od hiperprolifičnih krmača, i koja, kao nerazvijena pate od povećanog morbiditeta i smrtnosti pre odbijanja, prvenstveno zbog slabog razvoja u uterusu. Pošto se postnatalni rast mišića i konačno ukupan rast ne određuje samo postnatalnom hipertrofijom (povećanjem veličine miofibera) već i biprenatalnom hiperplazijom (povećanjem broja miofibera), pozitivna veza između mase na rođenju i broja miofibera, kao i broja miofibera i brzine porasta, mesa i kvaliteta trupa je veoma relevantna. Prema tome, nutritivne strategije u toku gestacije bi trebalo da se usredsrede na poboljšanje razvoja mišića embriona i fetusa sa krajnjim ciljem da se minimizira broj prasadi sa niskim telesnim masama na rođenju. Prvi deo preglednog rada će se fokusirati na miogenezu i njen značaj za postnatalne performanse porasta, karakteristike trupa i kvalitet svinjskog mesa. Pored toga, biće diskutovano o nedoslednosti u izveštavanim rezultatima u pogledu niske težine pri rođenju, postnatalnog rasta i kvaliteta trupova i svinjskog mesa. Završni deo preglednog rada će pokazati poznate prehrambene pristupe za optimizaciju intrauterinog okruženja pomoću dopuna hrane za svinje tokom gestacije sa specifičnim sastojcima. U zaključku će

biti predloženi neki budući istraživački pristupi u cilju daljeg popunjavanja nedostatka znanja u pogledu prenatalnog razvoja.

Ključne reči:

References

AGROSCOPE (2015): Fütterungsempfehlungen und Nährwerttabellen für Schweine. Lehrmittel Zentrale, Zollikofen, Switzerland.
BEAULIEU A.D., AALHUS J.L., WILLIAMS N.H., PATIENCE J.F. (2010): Impact of piglet birth weight, birth order, and litter size on subsequent growth performance, carcass quality, muscle composition, and eating quality of pork. Journal of Animal Science 88, 2767-2778.
BEE G. (2004): Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. Journal of Animal Science 82, 826-836.
BÉRARD J., BEE G. (2010): Effects of dietary L-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. Animal 4, 1680-1687.
BÉRARD J., KREUZER M., BEE G. (2008): Effect of litter size and birth weight on growth, carcass and pork quality, and their relationship to postmortem proteolysis. Journal of Animal Science 86, 2357-2368.
BÉRARD J., KREUZER M., BEE G. (2010a): In large litters birth weight and gender is decisive for growth performance but less for carcass and pork quality traits. Meat Science 86, 845-851.
BÉRARD J., PARDO C.E., BETHAZ S., KREUZER M., BEE G. (2010b): Intra-uterine crowding decreases average birth weight and affects muscle fiber hyperplasia in piglets. Journal of Animal Science 88, 3242-3250.
BÉRARD J., KALBE C., LÖSEL D., TUCHSCHERER A., REHFELDT C. (2011): Potential sources of early-postnatal increase in myofibre number in pig skeletal muscle. Histochemistry and Cell Biology 136, 217-225.
CERISUELO A., BAUCELLS M.D., GASA J., COMA J., CARRION D., CHAPINAL N., SALA R. (2009): Increased sow nutrition during midgestation affects muscle fiber development and meat quality, with no consequences on growth performance. Journal of Animal Science 87, 729-739.
CORSON A.M., LAWS J., LITTEN J.C., DODDS P.F., LEAN I.J., CLARKE L. (2008): Effect of dietary supplementation of different oils during the first or second half of pregnancy on the glucose tolerance of the sow. Animal 2, 1045-1054.
DAVIS T.A., FIOROTTO M.L., BURRIN D.G., POND W.G., NGUYEN H.V. (1997): Intrauterine growth restriction does not alter response of protein synthesis

to feeding in newborn pigs. American Journal of Physiology - Endocrinology and Metabolism 272, E877-E884.

DAVIS T.A., SURYAWAN A., ORELLANA R.A., FIOROTTO M.L., BURRIN D.G. (2010): Amino acids and insulin are regulators of muscle protein synthesis in neonatal pigs. Animal 4, 1790-1796.

DE VOS M., CHE L., HUYGELEN V., WILLEMEN S., MICHIELS J., VAN CRUCHTEN S., VAN GINNEKEN C. (2014): Nutritional interventions to prevent and rear low-birthweight piglets. Journal of Animal Physiology and Animal Nutrition 98, 609-619.

DECALUWÉ R., MAES D., COOLS A., WUYTS B., DE SMET S., MARESCAU B., DE DEYN P.P., JANSSENS G.P.J. (2014): Effect of peripartal feeding strategy on colostrum yield and composition in sows. Journal of Animal Science 92, 3557-3567.

DOURMAD J-Y., ETIENNE M., VALANCOGNE A., DUBOIS S., VAN MILGEN J., NOBLET J. (2008): InraPorc: A model and decision support tool for the nutrition of sows. Animal Feed Science and Technology 143, 372-386.

DOURMAD J.Y. (1991): Effect of feeding level in the gilt during pregnancy on voluntary feed-intake during lactation and changes in body-composition during gestation and lactation. Livestock Production Science 27, 309-319.

DWYER C.M., STICKLAND N.C., FLETCHER J.M. (1994): The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. Journal of Animal Science 72, 911-817.

EDER K. (2009): Influence of l-carnitine on metabolism and performance of sows. Br J Nutr 102, 645-654.

EDER K., RAMANAU A., KLUGE H. (2001): Effect of L-carnitine supplementation on performance parameters in gilts and sows. Journal of Animal Physiology and Animal Nutrition 85, 73-80.

FAINBERG H.P., ALMOND K.L., LI D., RAUCH C., BIKKER P., SYMONDS M.E., MOSTYN A. (2014): Impact of maternal dietary fat supplementation during gestation upon skeletal muscle in neonatal pigs. BMC Physiology 14, 1-12.

FARMER C., QUESNEL H. (2009): Nutritional, hormonal, and environmental effects on colostrum in sows. Journal of Animal Science 87, 56-65.

FOXCROFT G.R., BEE G., DIXON W., HAHN M., HARDING J., PATTERSON J., PUTMAN T., SARMENTO S., SMIT M., TSE W-Y., TOWN S.C. (2007): Consequences of selection for litter size on piglet development. In Paradigms in Pig Science (eds. J Wiseman, MA Varley, S McOrist and B Kemp), pp. 207-229, Nottingham Univ. Press, Nottingham, UK.

GERFAULT V., MOUROT J., ETIENNE M., MOUNIER A. (1999): Influence de la nature des lipides dans le régime de gestation de la truie sur ses performances et la composition corporelle des porcelets à la naissance. In pp. 191-197.

GONDRET F., LEFAUCHEUR L., JUIN H., LOUVEAU I., LEBRET B. (2006): Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. Journal of Animal Science 84, 93-103.

GONDRET F., LEFAUCHEUR L., LOUVEAU I., LEBRET B., PICHODO X., LE COZLER Y. (2005): Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livestock Production Science 93, 137-146.

HAZELEGER W., RAMEAKERS P., SMITS C., KEMP B. (2007): Influence of nutritional factors on placental growth and piglet imprinting. In Paradigms in Pig Science (eds. J Wiseman, MA Varley, S McOrist and B Kemp), pp. 309-327.

HEYER A., ANDERSSON H.K., LINDBERG J.E., LUNDSTRÖM K. (2004): Effect of extra maternal feed supply in early gestation on sow and piglet performance and production and meat quality of growing/finishing pigs. Acta Agriculturae Scandinavica, Section A — Animal Science 54, 44-55.

IFIP-GTTT (2015): Gestion technique des troupeaux de truies. In gestion technique des troupeaux de truies (Ed. IFIP).

INNIS SM (2007): Dietary (n-3) fatty acids and brain development. Journal of Nutrition 137, 855-859.

JOURQUIN J., MORALES J., BOKENKROGER C.D. (2016): Impact of piglet birth weight increase on survivability and days to market, a simulation model. In Journal of Animal Science, pp. 34-34. Des moines.

KIM S.W., MCPHERSON R.L., WU G. (2004): Dietary arginine supplementation enhances the growth of milk-fed young pigs. Journal of Nutrition 134, 625-630.

KLEIBER M. (1961): The fire of life. An introduction to animal energetics. John Wiley & Sons, Inc., New York: London

KUSINA J., PETTIGREW J.E., SOWER A.F., WHITE M.E., CROOKER B.A., HATHAWAY M.R. (1999): Effect of protein intake during gestation and lactation on the lactational performance of primiparous sows. Journal of Animal Science 77, 931-941.

LAWLOR P.G., LYNCH P.B., O CONNELL M.K., MCNAMARA L., REID P., STICKLAND N.C. (2007): The influence of over feeding sows during gestation on reproductive performance and pig growth to slaughter. Archives Animal Breeding 50, 82-91.

LEFAUCHEUR L., EDOM F., ECOLAN P., BUTLER-BROWNE G.S. (1995): Pattern of muscle fiber type formation in the pig. Developmental Dynamics 203, 27-41.

LI X.L., BAZER F.W., JOHNSON G.A., BURGHARDT R.C., FRANK J.W., DAI Z.L., WANG J.J., WU Z.L., SHINZATO I., WU G.Y. (2014): Dietary supplementation with L-arginine between days 14 and 25 of gestation enhances embryonic development and survival in gilts. Amino Acids 46, 375-384.

LIN G., WANG X., WU G., FENG C., ZHOU H., LI D., WANG J. (2014): Improving amino acid nutrition to prevent intrauterine growth restriction in mammals. Amino Acids (Vienna) 46, 1605-1623.

LOESEL D., KALBE C., REHFELDT C. (2009): L-Carnitine supplementation during suckling intensifies the early postnatal skeletal myofiber formation in piglets of low birth weight. Journal of Animal Science 87, 2216-2226.

LOPEZ J.M.R., PARDO C.E., BEE G. (2010): Hyperplastic muscle growth occurs from birth to weaning in pigs. Journal of Animal Science 88 (suppl. 2), 364-364.

MADSEN J.G., KREUZER M., BEE G. (2016a): Potential to improve muscle maturation in low birth weight piglets fed milk replacers supplemented with either L-arginine or L-carnitine for 21 days. (unpublished).

MADSEN J.G., KREUZER M., BEE G. (2016b): Impact of dietary L-arginine supply during early gestation on myofiber development in newborn pigs exposed to intra-uterine crowding. (unpublished).

MATEO R.D., WU G., MOON H.K., CARROLL J.A., KIM S.W. (2008): Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets. Journal of Animal Science 86, 827-835.

MATEO R.D., WU G., BAZER F.W., PARK J.C., SHINZATO I., KIM S.W. (2007): Dietary L-arginine supplementation enhances the reproductive performance of gilts. Journal of Nutrition 137, 652-656.

METGES C.C., HAMMON H. (2005): Nutritional programming: prenatal nutritional effects on the regulation of growth and metabolism. Journal of Animal and Feed Sciences 14, 15-30.

MUSSER R., DRITZ S., DAVIS D., TOKACH M., NELSSEN J., GOODBAND R., OWEN K. (2007): Effects of L-carnitine in the gestating sow diet on fetal muscle development and carcass characteristics of the offspring. Journal of Applied Animal Research 31, 105-111.

MUSSER R.E., DAVIS D.L., DRITZ S.S., TOKACH M.D., NELSSEN J.L., MINTON J.E., GOODBAND R.D. (2004): Conceptus and maternal responses to increased feed intake during early gestation in pigs. Journal of Animal Science 82, 3154-3161.

MUSSER R.E., GOODBAND R.D., TOKACH M.D., OWEN K.Q., NELSSEN J.L., BLUM S.A., DRITZ S.S., CIVIS C.A. (1999): Effects of L-carnitine fed during gestation and lactation on sow and litter performance. Journal of Animal Science 77, 3289-3295.

NISSEN P.M., DANIELSEN V.O., JORGENSEN P.F., OKSBJERG N. (2003): Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. Journal of Animal Science 81, 3018-3027.

NISSEN P.M., SØRENSEN I.L., VESTERGAARD M., OKSBJERG N. (2005): Effects of sow nutrition on maternal and foetal serum growth factors and on foetal myogenesis. Animal Science 80, 299-306.

NOBLET J., DOURMAD J.Y., ETIENNE M. (1990): Energy utilization in pregnant and lactating sows: modeling of energy requirements. Journal of Animal Science 68, 562-572.

NOBLET J., CLOSE W., HEAVENS R., BROWN D. (1985): Studies on the energy metabolism of the pregnant sow. British Journal of Nutrition 53, 251-265.

OKSBJERG N., GONDRET F., VESTERGAARD M. (2004): Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domestic Animal Endocrinology 27, 219-240.

OWEN K.Q., JIT H., MAXWELL C.V., NELSSEN J.L., GOODBAND R.D., TOKACH M.D., TREMBLAY G.C., KOO S.I. (2001): Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine. Journal of Animal Science 79, 3104-3112.

PARDO C.E., KREUZER M., BEE G. (2013a): Effect of average litter weight in pigs on growth performance, carcass characteristics and meat quality of the offspring as depending on birth weight. Animal 7, 1884-1892.

PARDO C.E., BÉRARD J., KREUZER M., BEE G. (2013b): Intrauterine crowding in pigs impairs formation and growth of secondary myofibers. Animal 7, 430-438.

PICARD B., LEFAUCHEUR L., BERRI C., DUCLOS M.J. (2002): Muscle fibre ontogenesis in farm animal species. Reproduction Nutrition Development 42, 415-431.

POND W.G., YEN J.T., MERSMANN H.J. (1987): Effect of severe dietary protein, nonprotein calories or feed restriction during gestation on postnatal growth of progeny in swine. Growth 51, 355-371.

QUESNEL H., GONDRET F., MERLOT E., FARMER C. (2015): Sow influence on piglet colostrum intake and neonatal survival. Inra Productions Animales 28, 295-304.

QUINIOU N., DAGORN J., GAUDRÉ D. (2002): Variation of piglets' birth weight and consequences on subsequent performance. Livest. Prod. Sci 78, 63-70.

RAMAEKERS P., KEMP B., VAN DER LENDE T. (2006): Progenos in sows increases number of piglets born. In Journal of Animal Science, pp. 394-394.

RAMANAU A., KLUGE H., SPILKE J., EDER K. (2002): Reproductive performance of sows supplemented with dietary L-carnitine over three reproductive cycles. Archives of Animal Nutrition 56, 287-296.

RAMANAU A., KLUGE H., SPILKE J., EDER K. (2004): Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. Journal of Nutrition 134, 86-92.

RAMANAU A., KLUGE H., SPILKE J., EDER K. (2008): Effects of dietary supplementation of L-carnitine on the reproductive performance of sows in production stocks. Livestock Science 113, 34-42.

REHFELDT C., KUHN G. (2006): Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. Journal of Animal Science 84, E113-E123.

REHFELDT C., FIEDLER I., DIETL G., ENDER K. (2000): Myogenesis and postnatal skeletal musle cell growth as influenced by selection. Livest. Prod. Sci 66, 177-188.

REHFELDT C., LEFAUCHEUR L., BLOCK J., STABENOW B., PFUHL R., OTTEN W., METGES C.C., KALBE C. (2012a): Limited and excess protein intake of pregnant gilts differently affects body composition and cellularity of skeletal muscle and subcutaneous adipose tissue of newborn and weanling piglets. European Journal of Nutrition 51, 151-165.

REHFELDT C., STABENOW B., PFUHL R., BLOCK J., NUERNBERG G., OTTEN W., METGES C.C., KALBE C. (2012b): Effects of limited and excess protein intakes of pregnant gilts on carcass quality and cellular properties of skeletal muscle and subcutaneous adipose tissue in fattening pigs. Journal of Animal Science 90, 184-196.

REHFELDT C., LANG I.S., GORS S., HENNIG U., KALBE C., STABENOW B., BRUSSOW K.P., PFUHL R., BELLMANN O., NURNBERG G., OTTEN W., METGES C.C. (2011): Limited and excess dietary protein during gestation affects growth and compositional traits in gilts and impairs offspring fetal growth. Journal of Animal Science 89, 329-341.

REZAEI R., WU Z., HOU Y., BAZER F.W., WU G. (2016): Amino acids and mammary gland development: nutritional implications for milk production and neonatal growth. Journal of Animal Science and Biotechnology 7, 1-22.

REZAEI R., WANG W., WU Z., DAI Z., WANG J., WU G. (2013): Biochemical and physiological bases for utilization of dietary amino acids by young pigs. J Anim Sci Biotechnol 4.

ROOKE J.A., BLAND I.M., EDWARDS S.A. (1998): Effect of feeding tuna oil or soyabean oil as supplements to sows in late pregnancy on piglet tissue composition and viability. British Journal of Nutrition 80, 273-280.

ROOKE J.A., SINCLAIR A.G., EDWARDS S.A. (2001a): Feeding tuna oil to the sow at different times during pregnancy has different effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. British Journal of Nutrition 86, 21-30.
ROOKE J.A., SINCLAIR A.G., EDWARCS S.A., CORDOBA R., PKIYACH S., PENNY P.C., PENNY P., FINCH A.M., HORGAN G.W. (2001b): The effect of feeding salmon oil to sows throughout pregnancy on pre-weaning mortality of piglets. Animal Science 73, 489-500.
SMIT M.N., SPENCER J.D., ALMEIDA F.R., PATTERSON J.L., CHIARINI-GARCIA H., DYCK M.K., FOXCROFT G.R. (2013): Consequences of a low litter birth weight phenotype for postnatal lean growth performance and neonatal testicular morphology in the pig. Animal 7, 1681-1689.
SUISAG 2014. 2014 - Zahlen und Projekte. In.
TANGHE S., DE SMET S. (2013): Does sow reproduction and piglet performance benefit from the addition of n-3 polyunsaturated fatty acids to the maternal diet? Veterinary Journal 197, 560-569.
THEIL P.K., LAURIDSEN C., QUESNEL H. (2014): Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal 8, 1021-1030.
TOWN S.C., PUTMAN C.T., TURCHINSKY N.J., DIXON W.T., FOXCROFT G.R. (2004): Number of conceptuses in utero affects porcine fetal muscle development. Reproduction 128, 443-454.
VADMAND C.N., KROGH U., HANSEN C.F., THEIL P.K. (2015): Impact of sow and litter characteristics on colostrum yield, time for onset of lactation, and milk yield of sows. Journal of Animal Science 93, 2488-2500.
WANG X., FRANK J.W., XU J., DUNLAP K.A., SATTERFIELD M.C., BURGHARDT R.C., ROMERO J.J., HANSEN T.R., WU G., BAZER F.W. (2014): Functional role of arginine during the peri-implantation period of pregnancy. II. Consequences of loss of function of nitric oxide synthase NOS3 mRNA in ovine conceptus trophectoderm. Biology of Reproduction 91, 59, 51-10.
WAYLAN A.T., KAYSER J.P., GNAD D.P., HIGGINS J.J., STARKEY J.D., SISSOM E.K., WOODWORTH J.C., JOHNSON B.J. (2005): Effects of L-carnitine on fetal growth and the IGF system in pigs. Journal of Animal Science 83, 1824-1831.
WIGMORE P.M., STICKLAND N.C. (1983): Muscle development in large and small pig fetuses. Journal of Anatomy 137 (Pt 2), 235-245.
Wu G. (2010): Functional amino acids in growth, reproduction, and health. Advances in Nutrition: An International Review Journal 1, 31-37.

WU G., BAZER F.W., WALLACE J.M., SPENCER T.E. (2006): Board-invited review: intrauterine growth retardation: implications for the animal sciences. Journal of Animal Science 84, 2316-2337.

WU G., BAZER F.W., BURGHARDT R.C., JOHNSON G.A., KIM S.W., LI X.L., SATTERFIELD M.C., SPENCER T.E. (2010): Impacts of amino acid nutrition on pregnancy outcome in pigs: mechanisms and implications for swine production. Journal of Animal Science 88, E195-204.

WU G., BAZER F.W., JOHNSON G.A., KNABE D.A., BURGHARDT R.C., SPENCER T.E., LI X.L., WANG J.J. (2011): TRIENNIAL GROWTH SYMPOSIUM: Important roles for L-glutamine in swine nutrition and production. Journal of Animal Science 89, 2017-2030.

WU X., XIE C., ZHANG Y., FAN Z., YIN Y., BLACHIER F. (2015): Glutamate-glutamine cycle and exchange in the placenta-fetus unit during late pregnancy. Amino Acids (Vienna) 47, 45-53.

YAO K., YIN Y.L., CHU W., LIU Z., DENG D., LI T., HUANG R., ZHANG J., TAN B., WANG W., WU G. (2008): Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. Journal of Nutrition 138, 867-872.

YUAN T.L., ZHU Y.H., SHI M, LI T.T., LI N., WU G.Y., BAZER F.W., ZANG J.J., WANG F.L., WANG J.J. (2015): Within-litter variation in birth weight: impact of nutritional status in the sow. Journal of Zhejiang University-Science B 16, 417-435.

ALENTEJANO AND BÍSARO PIGS: TRADITION AND INNOVATION – THE TREASURE PROJECT

Rui Charneca[1], Amadeu Freitas[1], José Martins[1], José Neves[1], Miguel Elias[1], Marta Laranjo[2,3], José Nunes[1]

[1]Universidade de Évora, Escola de Ciências e Tecnologia, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), apartado 94, 7006-802 Évora, Portugal
[2]ICAAM, apartado 94, 7006-802 Évora, Portugal
[3]Instituto de Investigação e Formação Avançada (IIFA), Universidade de Évora, apartado 94, 7002-554 Évora, Portugal
Corresponding author: Rui Charneca, rmcc@uevora.pt
Review paper

Abstract: The Alentejano pig (AL) and the Bísaro pig (BI) are the two main autochthonous swine breeds of Portugal. Originated from different ancestors they have a similar history across the last century. One hundred years ago AL was the most common swine breed in Portugal, but in the second half of the 20th century both Portuguese pig breeds undertook a strong population decline that almost lead to their extinction. However, since the late 80's of last century local breeds were rescued and their populations recovered over the last 30 years. Breeding recovering led to the protection of pork itself and dry products by European protections PDO's and PGI's. In the framework of TREASURE project, a study is in progress aiming to investigate the opportunity of a crossbreeding program involving both breeds with the goal to obtain new products with potential commercial interest and to improve the knowledge on the pure breeds and to protect them against future threats. This paper includes a revision about AL and BI breeds and presents some preliminary original data from Treasure project study.

Key words: Alentejano, Bísaro, autochthonous pigs, reproduction, production.

Origin and geographical distribution

The Alentejano pig (AL) and Bísaro pig (BI) are the main local Portuguese pig breeds. The AL pig belongs to the Mediterranean group *(Porter, 1993)* and share the origin from the *Sus mediterraneus* with nowadays Iberian pig. In the southern Portugal and Spain, mainly Alentejo, Extremadura and Andalucía regions, the extensive swine production is historically associated to the use of AL and

Iberian breeds perfectly adapted to the environment and to the use of natural resources namely the Quercus forest known as Montado in Portugal and Dehesa in Spain *(Freitas, 2014)*. The BI pig belongs to the Celtic group *(Porter, 1993)*, originated from the Celtic wild boar. It presents similar characteristics to the Celta pig breed *(Carolino et al., 2009)*, being both breeds manly raised in the northern regions of the Iberian Peninsula (Minho and Trás-os-Montes e Alto Douro in Portugal and Galicia in Spain).

Production systems

The AL pig is mostly raised under extensive conditions in an integrated agro-sylvo-pastoral system where the "montanheira", the fattening period based on acorns, from Quercus forest since late October to the end of February is a strategic element of the production system. The herd size is, presently on average, of 35 sows *(ANCPA*, data base*)*. Most Bísaro pig were raised in small nucleus, but the number of sows per farm has increased from 10 sows in 2003 *(Alves, 2003)* to 30 sows more recently *(Santos Silva and Tirapicos Nunes, 2013)*. The animals, are reared in intensive outdoor or semi-extensive systems *(Santos Silva and Tirapicos Nunes, 2013)*.

Traditionally, AL herds were separated in 3 categories: breeding sows, growing pigs and fattening pigs. Formerly the breeding sows (even when in lactation) and growing pigs, graze during day and were supplement with cereal grains or leguminous even seasonal agriculture by-products only when necessary *(Póvoas Janeiro, 1951)*. Nowadays, in order to improve and to standardize performance most sows and pigs receive concentrated balanced feeds. The BI pigs feeding has always been based on the feeds produced at the farms, and nowadays the pigs diet is a mix of cereals complemented with other farm products (tubers, horticultural by-products, fruits). Some farmers use commercial complete feeds for specific phases, as lactation or weaned piglets *(ANCSUB, 2017)*.

In AL farms, now that the old traditional concrete facilities ("malhadas") are almost abandoned, in most cases farrowing occurs in outdoors conditions, in a "camping" environment with farrowing huts. In BI breed the farrowing and lactation were also in man-made facilities (with stone and/or wood) but there is also a tendency to increase the camping system in the last years *(Santos Silva and Tirapicos Nunes, 2013)*. Traditionally, the AL piglets are usually weaned with 45-60 days of age and 10-14 kg of LW (live-weight) *(Freitas, 2014)*. The weaning of the BI piglets occurs at 40-45 days of age. Traditional consumption of roast piglets is common. Seventy per cent of the weaned piglets are slaughtered at young age, weighing 8–12 kg *(Santos Silva and Tirapicos Nunes, 2013).*

The AL piglets born between April and September go to “Montanheira” the following year *(Santos Silva and Tirapicos Nunes, 2013)* while piglets born during Autumn and Winter season go to other markets (roasted piglets, fresh meat), either they can be used to replace cull animals in farms or, in many cases they come from crossbreed AL x Duroc. The slaughter age and weight dependents on the product/market target. Considering pork market pigs are usually slaughtered with 8-14 months of age and 90-100kg LW. For protected (IGP) dry-cured products the average age and weights are 15-24 months and 120-140kg live-weight, respectively. Finally, for the ham industry both in Portugal and also in Spain (main destination) the slaughter age and weight vary from 14-18 months and weights of 140-170kg LW *(Freitas, 2014)*. Typically, Bísaro pig have two growing phases. The first one of moderate growth until 70–75 kg LW and a finishing phase with variable diets depending on the availability of food resources on each farm (flour, fruits, vegetables, chestnuts and acorns). During finishing period of Bísaro pigs, the rate of growth depends on feeding management and availability of pasture. The animals can be slaughtered from 120 to 180 kg LW, depending on tradition of each region *(Santos Silva and Tirapicos Nunes, 2013)*.

Historical evolution, demographics and products

Alentejano and Bísaro breeds were the main pig raised in Portugal at the beginning of the XX. In fact, by the 50’s of that century the AL pig represented about 45% of the total pig population in Portugal *(Carvalho, 1964)*. However, due to several reasons both breeds suffered a clear decline in their populations that almost lead to their extinction on the 80’s. The reasons were various and most of them common for both breeds: emigration and migration (to littoral regions) phenomena, new dietary habits and diet health concepts, sanitary problems (mostly African swine fever), introduction of exotic lean breeds (sometimes promoted by state policy) and their crosses with our breeds *(Carolino et al., 2009; Freitas, 2014; Tirapicos Nunes, 1998)*. From that decade onwards, a recovery of both breeds and their traditional production systems was recorded, enhanced by grants of several agents with the purpose of saving them. Presently, there were 6559 sows and 436 boars registered in the Alentejano breed Herd book distributed by 162 breeders (February 2017, ANCPA database). The correspondent values for Bísaro breed are 5460 females, 520 boars and 189 breeders (March 2017, ANCSUB records). During last decade of XX Century tree breeders’ associations were registered (AL:1990 - ACPA and 1991 – ANCPA; BI: 1994 - ANCSUB). Presently (*DGADR, 2017*), the AL breed has 27 protected products (4 PDO’s and 23 IGP’s) whereas the BI breed (or crosses) has 17 protected products (16 PGI’s and 1 PDO).

Breeds reproductive and productive characteristics

Unlike other European pig breeds, both AL and BI breeds were not submitted to genetic improvement programs *(Gama et al., 2013)*. Therefore, any performance improvement is mainly connected to empirical selection made by farmers and/or some improvement on production management (e.g. nutritional).
The gestation length in BI sows is similar to the recorded in other pig breeds (115 days; *Outor-Monteiro et al., 1998*), however AL breed presents a shorter gestation length (111-112d; *Nunes, 1993*; *Charneca et al., 2012*).

The AL breed present low prolificacy (7-8 piglets per litter; *Marques, 2001*; *Charneca et al., 2012*). The BI breed can be considered a medium prolific breed with reported values for litter size from 8 to 12 (*Carolino et al., 2009; ANCSUB, 2017*). In both breeds the pre-weaning mortality is high, 24-27% in AL (*Marques et al., 1996; Charneca et al., 2012*) and higher than 20% in BI (*Outor-Monteiro et al., 1998*).

AL sows produces less colostrum than commercial genotypes but colostrum is richer in IgG (*Charneca et al., 2015*). The composition of BI colostrum and milk was reported by *Lopes et al. (1998).*

The AL piglets born with 1,1 – 1,2kg LW (*Marques, 2001; Charneca et al., 2012*) and their growth rate (average daily gain- ADG) varies between 130 - 163g/d until 21 days (*Marques, 2001; Charneca et al., 2012*). The LW of BI piglets at birth is 1,3 to 1,4kg (*Carolino et al., 2009; Outor-Monteiro et al., 1998*). The values found for growth rate of BI piglets in nursing period (45d) range between 220-260g/d (*Outor-Monteiro et al., 1998; ANCSUB report, 2006*). In the post-weaning period, *Freitas (1998)* reported ADG values of 320 to 360g/d in AL piglets between 20 and 50kg LW.

The AL growth performance in pre-finishing phase (60-100kg) most reported values of ADG are between 400-600g/d *(Freitas et al., 2007; Martins et al., 2012; Martins et al., 2015)*. During fattening period "montanheira" when the daily intake of acorns can reach 10kg, depending on the animal density and fruits production *(Santos Silva and Tirapicos Nunes, 2013)* a higher growth rate can be observed 700-950g/d *(Nunes, 1993; Freitas, 1998).* An interesting knowledge on this breed is that it presents a compensative growth when submitted to feeding restriction before finishing period *(Freitas, 1998)*. In the BI pig the growth rate between 35 and 100kg LW is ~592/d in indoor facilities but it is lower (460g/d) in outdoor conditions *(Figueiredo et al., 2007)*. At higher weights, between 100 - 140kg LW the observed ADG is ~530g/d *(Santos e Silva et al., 1999)*. More recent results in a study concerning the type of pig facilities *Araújo et al. (2016)* reported an ADG between 25-80kg LW around 555g/d and from 80-120kg about 520g/d.

TREASURE project study

Justification and goals

Before intensification of indoors pig production, AL and BI were the main pig breeds in Portugal. It can be considered that Tagus river, including confined fields, separate each breed homeland. In the South, AL was predominant, while the dominance of BI in Northern territories was evident. Nevertheless, for some time they cohabited in Ribatejo region. In the contact zones, according to ancient testimonies *(Bernardo Lima, 1865; Miranda do Vale 1949)* the cross between both breeds was a common practice, and the result (animal, meat and products) was rather appreciated. However, no scientific data is available about the crossed animals or their products. The study and possible use of crosses between these pig breeds, whose former name "Ribatejano Pig" (RI) we have resumed, can help to increase the revenue of pig producers by creating new and economically interesting products, but it can also help to maintain or increase the two pure breed populations, therefore contributing to the conservation of animal biodiversity. The trial aimed to study the performance, the carcasses and meat quality, and also the quality of processed dry cured products of Ribatejano (RI) pigs obtained by the cross between AL males x BI females (ALBI piglets) and between BI males x AL females (BIAL piglets). Besides the crossbred, AL and BI pure breed pigs were used as controls in the trial.

Reproductive results

The mating, gestation, farrowing and lactation supervision of AL and BI females mated on crossbreeding allowed us to observe that, as expected, AL sows had a shorter gestation (111 vs 114d) and smaller litter size (6.7 vs 10 born alive piglets) than BI *(Charneca et al., 2016)*. Colostrum intake of piglets was similar between genotypes being on average of 289g for ALBI and 281g for BIAL piglets. The mortality rate until 28d averaged 12%, value much lower than the reported for both breeds in pure line *(Charneca et al., 2016)*. The reasons for the low mortality rate in the study may be related to a closer supervision during the lactation period, mainly during the first days post-farrowing and/or by a heterotic effect on the piglets' viability. The BI females weaned more piglets than AL, 8.5 vs 6 piglets. The growth rate of crossbred piglets and weight at 28d of age was similar between genotypes with average values of 195g and 6.8kg, respectively *(Charneca et al., 2016)*.

Productive results

First period (occurred mostly during spring season)

On this phase 20 castrated males of each genotype (AL, BI, ALBI and BIAL) were reared outdoor (similar conditions to those of observed in private farms), animals were feed *ad libitum* in group, and followed from to about 30kg to 65kg LW. The ADG of AL pigs (344g/d) was lower than all other genotypes that were similar among them (401- 414g/d). Overall, carcass length, carcass yield, and lean cuts weight were higher in BI than AL pigs, with intermediate values for both crosses. Conversely, fat cuts percentage, ZP fat depth and average backfat thickness were higher in AL than in BI, and ALBI and BIAL pigs. At 65kg LW, RI crosses presented intermediate characteristics between fatter (AL) and leaner (BI) genotypes *(Neves et al., 2016).*

Second period (occurred mostly during summer season)

After the first slaughter, the 10 remaining pigs per genotype remained in the same outdoor system but began to be fed individually, in order to control individual feed intake. The final slaughter weight was ~150kg. The preliminary results of this period showed no differences in growth rate, and ADG varied between 550 and 601g/d. In the carcasses the only clear differences between AL and the other genotypes are fat related traits. The AL carcasses had higher percentage of fat cuts, average backfat thickness and ZP fat depth. Again, RI animals (ALBI and BIAL) present, in most cases, intermediate values between the 2 pure breeds. After animal slaughtering a traditional dry-cured product *(Alves et al., 2017)* of pig (“paio”), was made using meat and fat of animals of each genotype.

The so far observed and analyzed results of the Treasure experiment show that the RI pig can be raised in outdoor conditions, during summer season without performance loss, the carcasses present equal or intermediate values of those from the pure breed animals what can be an advantage in some markets and/breeding seasons when the pure breed animal is not so valorized. Regarding our particular interest of the BIAL cross because most AL sows are in Alentejo region we forecast future studies of this RI pig eventually in a more comparative study with the most usual cross with Duroc made by farmers.

Alentejano i bisaro svinje: tradicija i inovacija - Treasure projekat

Rui Charneca, Amadeu Freitas, José Martins, José Neves, Miguel Elias, Marta Laranjo, José Nunes

Rezime

Alentejano svinja (AL) i bisaro svinja (BI) su dve glavne autohtone rase svinja u Portugaliji. Od različitih pretaka, oni imaju sličnu istoriju tokom prošlog veka. Pre sto godina AL je bila najčešća rasa svinja u Portugaliji, ali su u drugoj polovini 20. veka obe portugalske rase svinja doživele snažan pad populacije koji gotovo dovodi do njihovog izumiranja. Međutim, od kraja 80-tih godina prošlog veka, lokalne rase su spašene i njihova populacija se oporavila u posljednjih 30 godina. Oporavak uzgoja dovodi do zaštite svinjskog mesa i suvih proizvoda od svinjskog mesa pod evropskim programima zaštite PDO i PGI. U okviru TREASURE projekta, u toku je studija sa ciljem da se ispita mogućnost programa ukrštanja koji uključuju obe rase sa ciljem dobijanja novih proizvoda sa potencijalnim komercijalnim interesima i poboljšanjem znanja o čistim rasama i njihovoj zaštiti od budućih pretnje. Ovaj rad obuhvata reviziju znanja i podataka o AL i BI rasama kao i neke preliminarne originalne podatke iz studije Treasure projekta.

Ključne reči: Alentejano, Bisaro, autohtone svinje, reprodukcija, proizvodnja.

Acknowledgements

This work has been made in the scope of TREASURE project. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634476 (Project acronym: TREASURE). The content of this paper reflects only the author’s view and the European Union Agency is not responsible for any use that may be made of the information it contains.

This work was funded by National Funds through FCT- Fundação para a Ciência e a Tecnologia under the Project UID/AGR/00115/2013.

M. Laranjo acknowledges a Post-Doc research grant from FCT (SFRH/BPD/108802/2015).

References

ALVES C.M.G. (2003): O porco Bísaro: Recuperação e valorização. Suinicultura, 60, 52-63.

ALVES S.P., FERNANDES M.J., FERNANDES M.H., BESSA R.J.B., LARANJO M., SANTOS A.C.A., ELIAS M., FRAQUEZA M.J. (2017): Quality and acceptability of dry fermented sausages prepared with low value pork raw material. Journal of Food Processing and Preservation, 41: n/a, e12823. doi:10.1111/jfpp.12823

ANCSUB (2006): Associação Nacional de Criadores de Suínos de Raça Bísara. Boletim informativo nº7, Julho 2006. Available at: http://www.porcobisaro.net/dados/BOLETIM7.pdf

ANSUB (2017): Associação Nacional de Criadores de Suínos de Raça Bísara. http://www.porcobisaro.net/dados/index.php. Consulted in 28 June 2017.

ARAÚJO J.P.P., CERQUEIRA J.O.L., PIRES P., AMORIM I., DURÃO J., CADAVEZ V., SANTOS E SILVA J., DOMINGUEZ R., BERMÚDEZ R., LORENZO J.M. (2016). Growth performance on Bísaro pigs: hoop barn model vs confinement. Book of Abstracts of IX International Symposium on the Mediterranean Pig, 3-5 November, Portalegre, Portugal, 33.

BERNARDO LIMA, S. (1865): História Natural e Económica do Porco. O Arquivo Rural, VIII, 91-96.

CAROLINO N., SANTOS E SILVA J., VICENTE A.A., TIRAPICOS NUNES, J. (2009): La Producción Porcina a Campo: Portugal. In: *Produccion Porcina a Campo – Un Modelo Alternativo e Sostenible.* Eds. Miguel Ángel Aparício Tovar and Carlos R. Gonzalez Araújo, Caja Rural de Extremadura, 67-86.

CARVALHO, J. O. (1964): Contribuição para o Estudo Económico da Montanheira. Edition: Junta Nacional dos Produtos Pecuários, Lisboa.

CHARNECA R., NUNES, J., LE DIVIDICH, J. (2012): Reproductive and productive traits of sows from Alentejano compared to sows Large-White x Landrace genotype, Revista Portuguesa de Zootecnia (edição electrónica), Ano XIII nº 1, 10-29.

CHARNECA R., REQUETIM C., FREITAS A., NEVES J., MARTINS J.M., NUNES J.T. (2016): First reproductive and productive results on “Ribatejano pig”. Book of Abstracts of IX International Symposium on the Mediterranean Pig, 3-5 November, Portalegre, Portugal ,73.

CHARNECA R., VILA-VIÇOSA M.J., INFANTE P., NUNES, J., LE DIVIDICH J. (2015): Colostrum production of Alentejano and Large-White × Landrace sows: consumption, passive immunity and mortality of piglets, Spanish Journal of Agricultural Research, 8, 317-25.

DGADR (2017): Portuguese Traditional Products. Available in: https://tradicional.dgadr.pt/en/. Consulted in 28 june 2017.

FIGUEIREDO F.O., SANTOS E SILVA J., ABREU J.M.F., PIRES DA COSTA J.S. (2007) : Influence du système d'alimentation et de logement (traditionnel et de plein air) sur la performance des porcins de la race Bísaro. Options Méditerranéennes: Série A. Séminaires Méditerranéens,76, 95-104.

FREITAS A. (1998): Influência do nível e regime alimentar em pré-acabamento sobre o crescimento e desenvolvimento do porco alentejano e suas repercussões sobre o acabamento em montanheira e com alimento comercial. PhD Dissertation, Universidade de Évora. Portugal.
FREITAS A. (2014): A raça suína Alentejana: passado, presente e futuro. In: “Las razas porcinas Iberoamericanas: un enfoque etnozootécnico”. Capítulo III. Instituto Federal de Educação, Ciência e Tecnologia (IF Baiano). Salvador. Brasil.
FREITAS A.B., NEVES, J., LANÇA M., CHARNECA R., TIRAPICOS NUNES J. (2007): Influence of the feeding level on growth and carcass characteristics of Alentejano pigs. Options Méditerranéennes – Serie A: Séminaires Méditerranéennes, 76, 105-108.
GAMA L.T., MARTÍNEZ A.M., CAROLINO I., LANDI V., DELGADO J.V., VICENTE A.A., VEGA-PLA J.L., CORTÉS O., SOUSA C.O. (2013): Genetic structure, relationships and admixture with wild relatives in native pig breeds from Iberia and its islands, Genetics, Selection, Evolution : GSE, 45, 1, 18-.
JANEIRO J.P. (1944): A Suinicultura em Portugal: Subsídios biométricos para o estudo do gado suino nacional, Boletim Pecuário, XII, nº 2, 3-192.
LOPES A.F., OUTOR-MONTEIRO D., PINHEIRO V.M.C. (1998): Contribution to the Characterization of Colostrum and Milk Composition of the Bísara Breed Sows. Revista Portuguesa de Zootecnia, Ano V, nº 2, 43-49.
MARQUES P. (2001). Características produtivas e reprodutivas do Porco Alentejano. Influências genéticas e ambientais. PhD Dissertation. Universidade de Évora. Portugal.
MARQUES P., TIRAPICOS NUNES J.L., COLAÇO J., ALMEIDA J.A. (1996): Influence de la mortalité dans la productivité de la truie Alentejana, Produzione Animale, vol. IX – III Série – Numero Speciale, 19-22.
MARTINS J.M., NEVES J.A., FREITAS A., NUNES, J.L. (2012): Effect of long-term betaine supplementation on chemical and physical characteristics of three muscles from the Alentejano pig. Journal of the Science of Food and Agriculture, 92, 10, 2122-2127.
MARTINS J.M., NEVES J.A., FREITAS A., TIRAPICOS J.L. (2015): Rearing system and oleic acid supplementation effect on carcass and lipid characteristics of two muscles from an obese pig breed, Animal, 9, 10, 1721-1730.
MIRANDA DO VALE J. (1949): Suínos. In: Gado Bissulco. Colecção a Terra e o Homem. Livraria Sá da Costa. 35-78.
NEVES J.A., MARTINS J.M., SERRANO A., ABECASSIS I., FREITAS A., NUNES J.T., CHARNECA R., ALBUQUERQUE A. (2016): The Ribatejano pig – rebirth of a local pig population? First results on growth, carcass and meat parameters. In IX International Symposium on the Mediterranean Pig - Book of Abstracts pp. 74, 3-5 November, Portalegre, Portugal.

NUNES. J.L.T. (1993): Contributo para reintegração do porco Alentejano no Montado. PhD Dissertation. Universidade de Évora. Portugal.
OUTOR-MONTEIRO D., CARDOSO M.A.S., MESTRE R.B. (1998): Contributo para a caracterização produtiva de suínos da raça Bísara. Actas das 1as Jornadas Internacionais de Suinicultura. 26-28 Março, Vila Real, Portugal. 163-70.
PORTER V. (1993): Spain and Portugal. In: *Pigs: A Handbook to the Breeds of the World* 1st edition, Eds. V Porter & TJ Mountfield (eds). Cornell University Press, Ithaca, United States, 137-40.
PÓVOAS JANEIRO (1951): Ritmo de crescimento, em peso, dos leitões Alentejanos, até às 8 Semanas. Revista de Medicina Veterinária, XLVI, 495-502.
SANTOS SILVA J., TIRAPICOS NUNES J.L. (2013): Inventory and characterization of traditional mediterranean pig production systems. Advantages and constraints towards its development. Acta Agriculturae Slovenica, Supplement 4, 61 – 67.
SANTOS E SILVA J., FERREIRA-CARDOSO J., BERNARDO A., PIRES DA COSTA J. (1999): Conservation and development of the Bísaro pig. Characterisation and zootechnical evaluation of the breed for production and genetic management. In: Quality of Meat and fat in pigs as affected by genetics and nutrition Ed. European Association for Animal Production by Caspar Wenk , ETH Zurich, Nº 100, 2000.
TIRAPICOS NUNES J. (1998): Produção Extensiva – Porco Alentejano. Actas das 1as Jornadas Internacionais de Suinicultura, Vila real, 26 – 28 Março, 153 – 162.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

TREASURE - MANGALITSA LOCAL PIG BREED IN SERBIA

Čedomir Radović[1], Milica Petrović[2], Marija Gogić[1], Radomir Savić[2], Nenad Parunović[3], Dragan Radojković[2], Nikola Stanišić[1]

[1]Institute for Animal Husbandry, Autoput 16, RS-11080 Belgrade-Zemun
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, RS-11080 Belgrade-Zemun,
[3]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000, Belgrade, Republic of Serbia
Corresponding author: cedomirradovic.izs@gmail.com
Review paper

Abstract: The main objective of this paper is to present the results of the research of the Swallow belly Mangalitsa genotype in the last six decades. According to the research, females reach the full maturity the age of 9-10 months, but they are mated at the age of 1-1.5 years. Average age at first farrowing is 556 days. Reproductive ability is poorly expressed, with a strong maternal instinct. Fertility of the Mangalitsa is relatively poor because it gives birth to 1-12 piglets, on average 4 to 5 piglets, with an average body weight of 1.25 kg with a variation of 0.8 to 1.8 kg. The suckling period is about 50 days (from 47 to 53 days). At lactation duration of 60 days, the piglet weight at the weaning ranges from 6-13 kg (average 9.61 kg) for piglets born in the spring, and from 7-15 kg (average 9.50 kg) for piglets born in fall. Depending on the rearing system, the start of fattening and final body weight, gains range 268 g to 830 g. The fat thickness (average measurements) at the ridge was 10.2 cm, the middle of the back 7.9 cm and the rump 8.1 cm, in previous studies, while in recent studies these values of fat thickness are somewhat lower, with the pre-slaughter body weight also being lower (the ridge 6.18 cm, the middle of the back 4.38 cm and at the rump 5.19 cm). The recent research of the *Longissimus dorsi* muscle shows an intramuscular fat content of 13.5%, protein content of 21.1% with specific qualitative properties pH^{45}=6.11; pH^{24}=5.50; CIE L*=40.13; a*=11.77; b*=3.73). In the *musculus longissimus lumborum and thoracis*, Mangalitsa (Swallow-bellied) pigs show higher levels of monounsaturated fatty acids (MUFA 55.1%) and lower levels of saturated fatty acids (SFA 35.3%) in comparison with Swedish Landrace pigs.

Key words: autochthonous breed, Swallow belly Mangalitsa, reproductive traits, growth, carcass and meat quality

Introduction

Autochthonous breeds of farm animals are breeds that originated in the territory of the Republic of Serbia and which have economic, scientific and cultural significance for our country. They contain in their genes information from the environment, they have been created over a long period of time, under the influence or without the influence of man. Their genes represent safety in the production of food in future times, in which the greater importance will be attributed to the resistance and adaptability of the breed. Bearing this fact in mind, autochthonous breeds represent the TREASURE that we need to preserve for future generations. Pig production in the Republic of Serbia has a long tradition. During the 19th century, pigs were the main export product. In that time, the pig farming was based on local indigenous breeds with the dominant breeds Šiška and Šumadinka. Šiška and Šumadinka was the most primitive breed of pigs, created by domestication of wild pigs Sus scrofa ferus (*Belić, 1951*). Today in Serbia there are three local indigenous pig breeds: Mangalitsa, Moravka and Resavka while Šiška and Šumadinka breeds are lost in their original form. Mangalitsa was very popular in Vojvodina (especially in Srem) and Hungary in the period from the 19th century until the fifties, and recently farming of this breed has been restored. In the Republic of Serbia there are three Mangalitsa breed strains, the Swallow-belly strain (Srem black Mangalitsa or Buđanovci pig), white and Subotica strain. In Hungary and Romania there is also so called red strain of this breed. Swallow belly Mangalitsa developed in the area of Srem near Ruma, village of Buđanovci *(Belić 1949)*. Mangalitsa is a native primitive breed, originated from former Šumadinka breed and is so called "fat" pig breed type. In addition to their genetic merit for agro-biodiversity, they are the basis for a sustainable local pig production chain, and are particularly important for regions where arable land and grain production are limited. Autochthonous breeds of pigs provide security for the sustainable production of food in the future and form part of the genetic and cultural heritage. Since in Serbia the largest population is the Swallow-belly strain, the aim of this paper is to describe this variety, from the literary sources available, to the most important production traits.

Description of the breed and population size

Exterior: The head is relatively small in length and width, with ears that are not too large and always facing in the direction of the snout. The length of the ear is 2/3 of the length of the head. The body is short and rather rounded, while the ridge is of the medium width and usually seamlessly crosses into the backline (*Belić 1951).* The cheast is wide and deep. The back, loin and sides are of the medium width. Shoulders and ham are quite well expressed. The legs are of medium length with plenty of thin bones and often soft pastern bones. The skin is dark in colour, with dense, luminous and curly bristles that are shorter in the Swallow-belly strain. The colour of the bristles can be from gray-yellow to reddish (ginger). *Briznej (1948)* states that there are two varieties of which one from the western breeding region is called "Buđanovac" variety named after the village Buđanovci, which has a greater part of the pigmented body (entire head, body and the sides of the body and the legs from the outer-lateral side to the claws). The second variety - "Otok" and "Lasa" named after the village of Otok in the western part of the Srem region, with the legs pigmented only to the hock joint and the lower part of the papilla is white. The Otok variety has regularly strongly developed bristles or "feathers" which the "Buđanovac" variety lacks ot are not so developed (*Briznej 1948)*. Claws, teats and snout tip are pigmented *(Belić, 1951).* The number of animals of mangalits breed has increased over years (Table 1). In 2016, only on the territory of Central Serbia, 321 sowes and 32 boars were registered (in the records of the Main Breeding Organization).

Table 1. Population size

Year	2004	2008	2009	2012	2013	2014	2014*	2015*
Population	55-100	400-1000	400-1000	1000-2000	100-1000	300-1000	300-1000	300-1000
Nº sows	19	200	350	600	90(90#)	203 (153#)	247(247#)	345 (345#)
Nº boars	9	20	20	50	2	24	35	50

Source of data-DAD-FAO (www.dad.fao.org) access 29/06/2016
#Registered animals in Herdbook.
*Source of data of Institute for Animal Husbandry *(Main Breeding Organisation),* Annual Report (2014, 2015)-animals under the control of productivity.

Mangalitsa is very resistant and well adapted to extensive conditions of housing, where the needs are only for a simple shelter from rain and snow. It is located in the municipalities of Subotica, Sremska Mitrovica, Bačka Palanka, Vršac, Pančevo, Ub, Obrenovac, Ljig, Valjevo, Novi Sad, Kuzmin, Šid, Surčin and Kovilj (Krčedinska ada). It can also be found in traces on Stara Planina Mountain (Municipality of Dimitrovgrad), around Čačak and Kraljevo. When it comes to Mangalitsa, which is the most common of all the indigenous breeds in Serbia, it is necessary to make efforts to increase the number of animals in the population and control its productivity. This is the only way the breed can be preserved as an important genetic resource in pig production and breeding not only of Serbia, but also the entire region (*Petrović et al. 2007*).

Reproductive performance

Mangulica is a late maturity breed. It reaches the full maturity at the age of 9-10 months, but is not mated until the age of 1-1.5 years, and it is fully grown and mautre at the age of 3-4 years (*Briznej 1948*). In controlled herds in the four years (2011-2014), the average age at first farrowing was 556 ±176.65 days (*Radović et al. 2015*). Reproductive ability is poorly expressed, with a strong maternal instinct. The number of dairy nipples is most often 10. Fertility of the Mangalitsa females is relatively poor because it gives birth to 1-12 piglets, on average 4 to 5 piglets with an average of 1.25 kg body weight, varying from 0.8 to 1.8 kg (*Belić 1951*). Pigs at birth have characteristic stripes, which disappear in 10 days in white strain piglets, and in swallow belly strain in 3-4 months. Low productivity of the Mangalitsa is also reflected in the low farrowing index (1.21-1.81 litters/year). The poor rearing conditions and uncontrolled mating contribute to the poor reproductive parameters of this breed of pigs in Serbia (Table 2). However, the variability of fertility traits indicates the possibility for genetic improvement. Regardless of the low fertility properties, this breed should be preserved as an important genetic resource and be included in the selection program (*Petrović et al., 2013*). It is also necessary to have a plan of mating in a more precise manner so as to avoid inbreeding.

Table 2. Reproductive traits

Reference /	**Annual Report**	**Annual Report**	**Study**	**Study**	**Study**
Reproductive traits	UNIBG, 2010 (Mean±SD)	IAH, 2015 (Mean±SD)	Brinzej (1949)	Szabó (2002)	Petrović et al. (2013) (Mean±SD)
Number of sows recorded	28 litters	70 sows (85 litters)	10 litters	74 litters	53 sows (129 litters)
Sow parity[1]	2.04	3.45 (508.92±127.56 age at first farrowing)			
Litters/sow and year		1.21		1.81	1.77
Litter weight (kg)			6.99		
Piglets/litter	5.32±1.78 (1-9)	4.96±1.81	6.20	6.64	4.60±1.65
Piglets alive/litter	4.82±2.31 (0-9) [1of#]4.45±2.23 [2of]4.50±1.69 [3of]5.11±2.89	4.73±1.78			
Piglets weaned/litter	4.92±2.24 (0-9)[24 litters]	4.48±1.84		5.90 (88.90%)	4.09±1.91
Duration of lactation (d)	50 days	47.05±8.38			52.57±15.24
Weaning to conception interval (d)					92.31±61.66 period farrowing-mating => WCI=92.31-52.57=39.74)

[1] Keep records classified according to this parameter, #-order of farrowing

BW – body weight of sow must indicate also the stage i.e. weaning, end gestation

Observing the size of the litter, there are no significant differences between the number of piglets in the litter established in the middle of the 20th century *(Briznej 1949)* and the beginning of the 21st century *(Szabó 2002*). Research of *Petrović et al. (2013)* as well as the *Annual Report (2009 and 2014)* show that a somewhat lower number of piglets per litter has been registered, which may be the result of inbreeding. *Petrović et al. (2013)* found the average number of piglets in the litter of 4.60, of which 4.09 weaned pigs with a suckling period of 52.57 days. The same group of authors calculated the duration of the weaning-conception period obtained by subtracting the farrowing-mating period (92.31 days) and the duration of lactation (52.57 days), which amounted to 39.74 days (Table 2).

Growth traits

Literary data on the production performance of the breed are shown in various production systems (open and closed, extensive, semi-intensive and intensive system of rearing), of nutrition and gender (Table 3 and 4).

Table 3. Production system, nutrition, gender and number of animals in the trial

	Literature source			
	Brinzej (1949)	Belić and Mitić (1954)	Radović et al. (2017)	Brinzej (1956)
Number of pigs	10 litters (62 piglets)	74 animals in each group	12	32
Pol	♂/♀	♂/♀	castrated ♂ animals and ♀ animals	castrated ♂ animals (after weaning) and castrated ♀ animals (one month before the beginning of the fattening)
Production system/ Nutrition	Milk and supplemental diet (peas and wet barley) from 4 weeks of age	Two weeks after birth, the piglets are fed at will, barley and fresh green alfalfa during the summer or alfalfa flour in winter.	Semi-Intensive System (grazing and maize)	Intensive nutrition with maize and barley

Depending on the intensity of the rearing system and the nutrition, there are differences in the rate of growth within and between gender of piglets/fatteners (Table 4). Differences in the growth characteristics exist among groups of piglets born in different seasons. The birth weight varies in the range of 700 to 2000 g, which is similar to today's allochthonous breeds. The average daily gain during the suckling period is around 120-130 g, which depends on the lactation period, the piglets gender and the season of birth. In the intensive rearing system, mangalitsa animals can achieve high average daily gains of 830 g, as demonstrated by the results of the research by *Brinzej (1956)*. Unlike the above mentioned research, study by *Radović et al. (2017)* conducted on fatteners grown in semi-intensive system, show much lower potential in terms of the growth rate (about 267 g). Possible reasons for this difference in gain are differences in the intensity of rearing, in the final body weight, but also in the differences in the genetic structure of fatteners, since the time distance of the conducted researches is about 60 years.

Table 4. Body weight at birth and gain

<table>
<tr><td rowspan="2">Trait</td><td colspan="7">Literature source</td></tr>
<tr><td colspan="2">Brinzej (1949)</td><td colspan="3">Belić and Mitić (1954)</td><td>Radović et al. (2017)</td><td>Brinzej (1956)</td></tr>
<tr><td rowspan="5">Body weight (BW)</td><td rowspan="5">At birth</td><td rowspan="5">1130g</td><td colspan="2">♂</td><td>♀</td><td rowspan="5">133*</td><td rowspan="5">158
(132-174)*</td></tr>
<tr><td rowspan="2">At birth (g)</td><td>1205</td><td>1170</td></tr>
<tr><td>1220</td><td>1270</td></tr>
<tr><td rowspan="2">At weaning (kg)</td><td>9.61</td><td>9.97</td></tr>
<tr><td>9.50</td><td>9.54</td></tr>
<tr><td rowspan="3">Average daily gain (ADG),g</td><td>1-4 weeks</td><td>120</td><td colspan="2" rowspan="3">137^{spring}
138^{autumn}</td><td rowspan="3">146^{spring}
137^{autumn}</td><td rowspan="3">267,86</td><td rowspan="3">830</td></tr>
<tr><td>5-8 weeks</td><td>130</td></tr>
<tr><td>Total suckling period</td><td>125</td></tr>
</table>

*slaughter BW

Brinzej (1949) states in his study that the average body weight of piglets at birth was 1130 g (females - 1080 g while males have a slightly higher birth weight of 1180 g); average daily gain of piglets in the first 4 weeks of life is 120 g, from 5 to 8 weeks of age - 130 g, i.e. for the total suckling period of 8 weeks on average it is 125 g; from 5-8 weeks, the average daily gain is from 84-153 g, with the female animals showing gain of 77-150 g and a males 100-157 g. According to *Belić and Mitić (1954)*, the body weight of the male piglets at birth is 1205 g, with variations of 800-1900 g for piglets born in the spring, and 1220 g with variation of 700-1900 g for piglets born in autumn, whereby on the day 60 (weaning), the body weight ranged from 6 to 13 kg (average 9.61 kg) for piglets born in the spring, and in the range of 7-15 kg (average 9.50 kg) for piglets born in autumn. The average daily gain from birth to weaning at body weight of 1.22-9.48 kg was 137 g for piglets born in the spring and 138 g for piglets born in autumn. At birth, the average weight of female piglets is 1170 g, with variations of 700-1800 g for piglets born in the spring, and 1270 g with an interval of variation of 700-2000 g for piglets born in autumn, whereby on day 60 (weaning), body weight ranged between 6-13 kg (average 9.97 kg) for piglets born in the spring, and 7-14 kg (average 9.54 kg) for piglets born in autumn. The average daily gain from birth to weaning at body weight of 1.22-9.48 kg is 146 g for piglets born in the spring and 137 g for piglets born in autumn. *Radovic et al. (2017)* examined animals with pre-slaughter body weight of 133 kg, with a gain of 267.86 g (the animals were 30 kg at the start of the trial, and the final body weight reached 150 kg) and the fat thickness of 50.0 mm. Contrary to this study, *Brinzej (1956)* examined animals with pre-slaughter weight of 158 kg (132-174 kg) and obtained the following results: gain of 830 g (59 to 158 kg).

Carcass and meat quality traits

The Mangalitsa is an extremely fatty pig breed (*Teodorović and Radović, 2004*). While adipose tissue is about 65–70% of the carcass, lean meat is less than 35% *Rátky et al (2013)*. The carcass side quality traits vary depending on the nutrition and the housing system (Table 5). The slaughter weight is different and ranges from about 101 to 150 kg. In the earlier period, pigs were fattened to higher body weights, which affected the higher content of fat tissue in the carcass. Today consumers' demands are focused on the higher meat content of the carcass sides, which caused pigs to be fattened to lower body weights, similar to those in the intensive rearing system (about 100-110 kg). The slaughter yield ranges from 76 to 83%. The fat thickness varied in the presented researches depending on the body weight of the pigs at slaughter and the location on the carcass on which it was measured. *Petrović et al. (2010)* found the thickest fat on the ridge - 61.85 mm while the lowest was measured in the middle of the back - 43.78 mm. In the research of *Petrović et al. (2012)* the maximum values of fat thickness at withers determined was 85 mm and the lowest value was 48 mm, while for fat thickness at the middle of the back (between 13th and 15th vertebrae) max. value was 55 mm and the lowest measured value was 27 mm.

Table 5. Carcass traits

Reference	Petrović et al. (2010)	Brinzej (1956)	Petrović et al. (2012) LSM	Parunović et al. (2012)* LSM±SE	Petrović et al. (2014) LSM±SE
Nº pigs recorded	10	32	23	22 CO-12+FR-10	16 (balanced ratio of gender)
Production system (extensive, intensive, mixed…)[2]	semi-intensive	intensive	open & closed	conventional (CO) & free range (FR)	farm free range (conventional mixture)
Type of housing/ no. of animals per group	10			CO (six pigs per cage, 4 m^2 per animals) FR	surface of 150 m^2: 110 m^2 open + 40 m^2 cover section (4.8 m^2 of surface area per animal)
Slaughter weight (kg)	101,22	158	103.83	CO 102.06±3.70 FR 98.06±4.06	107.14±2.85 337.1±7.83$^{\text{age (days)}}$
Carcass weight (kg)	73,90		82.31 warm; 80.22 cold	CO 80±0.43$^{\text{warm}}$ FR 76.8±0.47$^{\text{warm}}$ CO 78.1±0.46$^{\text{cold}}$ FR 74.7±0.49$^{\text{cold}}$	
Carcass yield (% live weight)	73			CO 77.4±0.46 FR 73.9±0.51	
Carcass length (cm) os pubis-atlas os pubis-1st rib	88,74 72,80		92.78 76.26	CO 89.3±0.63 FR 89.2±0.69	

*CO-conventional mixture, FR-pasture, acorns and grains; After reaching a 60 kg of live weight both groups fed with conventional mixture.

Table 6. Total mass in four major carcasses parts and backfat thickness

Reference	Brinzej (1956)	Petrović et al. (2010)	Petrović et al. (2012)	Parunović et al. (2012)*
Ham weight (kg)		7.760	open 8.25 closed 7.83	
Shoulder weight (kg)		4.170	open 4.45 closed 4.82	
Loin weight (kg)		6.268	open 6.52 closed 6.62	
Belly-rib (kg)		4.73	open 4.60 closed 4.38	
Backfat thickness (cm)	ridge 10.2; loins 7.90; rump 8.10	ridge 6.18; loins 4.38; rump 5.19	ridge 6.17; loins 4.27; rump 4.78	CONV #6.19; 5.46; 6.01 FR #5.84; 5.18; 5.65

*CONV-conventional mixture, FR-pasture, acorns and grains; after reaching a 60 kg of live weight both groups fed with conventional mixture.
#Above the *M. gluteus medius* at the carcass split-line, on the three positions.

Petrović et al. (2012) found in the open system the thigh weight (Table 6) of 8.25 kg (with 3.75 kg of muscle tissue), while in the closed system the weight of the thigh is 7.83 kg (with 3.75 kg muscle). The weight of the shoulder is 4.45 kg in the open system (2.13 kg muscle tissue) and 4.82 kg (muscle tissue 2.19 kg) in the closed system. The wight of back-loin section in the open system is 6.52 kg with 1.76 kg of muscle, in the closed system 6.62 kg with 1.91 kg of muscle tissue. The backfat thickness at the ridge is 6,17 cm, at the middle of the back 4,27 cm and on the sides 4.78 to 5.37 cm (*Petrović et al., 2012*). *Parunović et al. (2012)* state that the thickness of backfat in conventional nutrition at three points respectively is: 6.19; 5.46; 6.01, and in the free farm system 5.84; 5.18; 5.65 cm.

Table 7. Meat quality traits longissimus dorsi muscle

	Study	**Study**	**Study**	**Study**	**Study**
Reference	Stanišić et al. (2015)[#] Mean±SD	Radović et al. (2017)[##] LSM±SE	Petrović et al. (2007) LSM±SD	Parunović et al. (2012)[###] LSM±SE	Tomović et al. (2016) Mean±SD
Nº pigs recorded	7	12	13:10	12:10	15
Production system (extensive, intensive, mixed…)	intensive	intensive	open (O) & closed (C)	conventional (CON) & free range (FR)	intensive
pH 45		6.11±0.13	O 6.04±0.10 C 6.32±0.32	CON-6.12±0.05 FR-5.89±0.06	
pH 24	5.47±0.66	5.50±0.06		CON-5.80±0.06 FR-5.41±0.06	5.56
CIE L*	38.19±1.92	40.13±1.48			48.39
CIE a*	10.58±2.50	11.77±1.04			10.13
CIE b*	2.68±0.88	3.73±0.45			4.14
Drip loss %					
Cooking loss	29.60±1.82				
Tenderness (kg)	5.05±1.13				
Water holding capacity	13.20±2.15				

WBSF: Warner-Bratzler shear force test; IMF intramuscular fat content

[#]Water Holding Capacity measured according to the method of Weiss et al. (1953). Cooking loss was determined in the following manner: a sample size of 3 x 4 x 2 cm is weighed and placed into a beaker of boiling water and cooked for 10 minutes; the difference in mass of the sample before and after cooking is the mass loss during the heat treatment, expressed as a percentage. The samples used to determine the mass loss and the cooking were used to determine the meat cutting force (kg): muscles are cut into pieces the size of 1 x 1 cm in the direction of extension of the muscle fibers; tenderness of meat, expressed forcibly cutting, measured Volodkevich instrument (Volodkevich, 1938); read more value on the instrument representing more cutting force values, and firmer flesh. Surface color just the cut of meat from the color stabilization time of 30 min (the samples were stored in contact with air at 4 ° C) was measured by a portable Minolta colorimeter CR-400 (light source D65, the geometry of the observation angle 0). Values are based on a spectrum of color CIEL* a* b* (CIE, 1976).

Meat value pH *musculus longissimus* (MLD) and *m. semimembranosus* (SM) was determined 45 minutes (pH_1) and 24 hours post mortem (pH_2) by pH-meter (Hanna, HI 83141). The color of MLD was determined 24 hours *post-mortem* measured (between 3. and 4. rib, from caudo-cranial point of view) using Chromameter CR-400 (Minolta Co. Ltd, Tokyo, Japan).
###pH measurment-Testo 205 pH meter (±0.02 pH, ±0.4°C, Germany, 2007).

In *Longissimus dorsi muscle* of fatteners kept in closed system *Petrovic et al. (2012)* established higher water content (70.71:68.49%; *p*=0.0069), lower content of total fats/lipids (5.45:8.09%; *p*=0.0081) and higher ash content compared to animals reared in the open housing system (1.09:1.02%; *p*=0.0392). Lower values for the share of water (64.3%), protein (21.1%) and ash (0.95%) with a fat content of 13.5% in *musculus longissimus lumborum et thoracis* (MLLT) are reported in the research of *Parunović et al. (2013)* compared to research by *Petrović et al. (2012)* and *Tomović et al. (2016).* In the study of *Parunović et al. (2013)*, Mangalitsa (Swallow-bellied and White Mangalitsa) pigs showed higher levels (55.1% and 58.0%) of MUFA (*P* <0.001) in the *MLLT* than Swedish Landrace pigs (44.9%). A higher percentage of unsaturated fatty acids, which are purportedly less harmful to human health, were measured in WM and SBM breeds, whereas the percentage of saturated fatty acids was proven to be significantly higher in Swedish Landrace pigs (*Parunović et al. 2013)*.

Conclusion

Mangalitsa is a late and extremely fatty pig breed with low fertility, long suckling period and a very weak-slow growth. From the research it can be seen that there is lower or higher variability for individual characteristics and therefore potential for selection-improvement of these properties. With such features, its cost-effectiveness is in low-investment in housing facilities with as large areas for pasturing and acorn nutrition, preferably if an organic breeding system is possible and the production of traditional high-value products (ham, kulen and sausages) and their marketing as highly valuable organic products or products protected by a geographical indication. Only the presence of local breeds of domestic animals makes the production of food safe in the dynamic change of the production environment, especially in the predicted and expected climatic changes. It should not be forgotten that precisely native/autochthonous breeds are adapted to different environmental conditions, resistant to various illnesses and modest in terms of housing and nutrition. Due to all this, it is necessary to work primarily on increasing the number of the population, increasing the number of breeders in the organic production system and the formation of Gene Bank (semen), because it does not exist in Serbia. It is also necessary to continue research with different breeding systems and their impact on the quality of meat and meat products of the Mangalitsa pigs.

TREASURE – Mangulica, lokalna rasa svinja u Srbiji

Čedomir Radović, Milica Petrović, Marija Gogić, Radomir Savić, Nenad Parunović, Dragan Radojković, Nikola Stanišić

Rezime

Glavni cilj ovog rada prikaz rezultata istraživanja genotipa Swallow belly Mangalitsa u poslednjih šest decenija. U skladu sa istraživanjima plotkinje polnu zrelost dostižu sa uzrastom od 9-10 meseci ali se pripuštaju sa uzrastom od 1-1.5 godine. Average age at first farrowing is 556 days. Reproduktivna sposobnost je slabo izražena, sa snažnim materinskim instinktom. Plodnost mangulice je relativno slaba jer jer prasi između 1-12 prasadi, prosečno 4 do 5 prasadi sa prosečnih 1.25 kg telesne mase sa variranjem od 0.8 do 1.8 kg. Dojni period je oko 50 dana (od 47 do 53 dana). Pri trajanju laktacije od 60 dana telesna masa prasadi pri zalučenju bila je u intervalu od 6-13 kg (prosek 9,61 kg) za prasad rođena u proleće, i u intervalu od 7-15 kg (prosek 9,50 kg) za prasad rođena u jesen. U zavisnosti od sistema uzgija, početka tova i završne telesne mase prirasti su bili od 268 g do 830 g. Debljina slanine (prosečne mere) na grebenu je bila 10.2 cm, sredini leđa 7.9 cm i na krstima 8.1 cm u ranijim istraživanjima dok su u novijim istraživanjima te vrednosti debljine slanine nešto niže, s tim da je telesna masa pri klanju niža (greben 6.18 cm, sredini leđa 4.38 cm i na krstima 5.19 cm). Novija istraživanja mišića *Longissimus dorsi muscle* pokazala su sadržaj intramuskularne masti od 13.5%, sadržaj proteina od 21,1% sa specifičnim kvalitativnim osobinama (pH^{45}=6.11; pH^{24}=5.50; CIE L*=40.13; a*=11.77; b*=3.73). U *musculus longissimus lumborum thoracis* grla lasaste mangulice imala su veći udeo mononezasićenih masnih kiselina (55.1%) i niži nivo zasićenih masnih kiselina (SFA 35.3%) u odnosu na grla rase švedski landras.

Ključne reči: autohtona rasa, Swallow belly Mangalitsa, reproduktivne osobine, porast, kvalitet polutki i mesa

Acknowledgment

"This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634476 (Project acronym: TREASURE). The content of this paper reflects only the

author‘s view and the European Union Agency is not responsible for any use that may be made of the information it contains."

References

BELIĆ J. (1949): O intenzitetu porasta prasadi lasaste mangulice od prvog dana do tri meseca starosti. Godišnjak poloprivrednog fakulteta, Naučna knjiga, 2, 117-157.

BELIĆ J. (1951): Specijalna zootehnika (ovčarstvo i svinjarstvo), Naučna knjiga, Beograd.

BELIĆ J., MITIĆ N. (1954): Broj prasadi jorkšira, bele i lasaste mangulice i njihovih meleza i porast istih do 2 meseca starosti. Arhiv za poljoprivredne nauke, VII, 16, 3-20.

BRINZEJ M. (1948): O lasastoj mangulici s obzirom na njen uzgoj u šumi. Stočarstvo, II, 12, 293-299.

BRINZEJ M. (1949): O prirastu prasadi lasaste mangulice. Stočarstvo, 1, 28-31.

BRINZEJ M. (1956): Poznavanje klaoničke vrijednosti lasaste mangulice. Stočarstvo, X, 11-12, 516-522.

DAD-FAO (www.dad.fao.org) access 29/06/2016

INSTITUTE OF ANIMAL HUSBANDRY (2015): Annual Report, 2015.

PARUNOVIĆ N., PETROVIĆ M., MATEKALO-SVERAK V., TRBOVIĆ D., MIJATOVIĆ M., RADOVIĆ Č. (2012): Fatty acid profile and cholesterol content of m: longissimus of free-range and conventionally reared mangalitsa pigs. South African Journal of Animal Science, 42, 2, 101-113.

PARUNOVIĆ N., PETROVIĆ M., MATEKALO-SVERAK V., RADOVIĆ Č., STANIŠIĆ, N. (2013): Carcass properties, chemical content and fatty acid composition of the *musculus longissimus* of different pig genotypes. South African Journal of Animal Science, 43, 2, 123-136.

PETROVIĆ M., MIJATOVIĆ M., RADOVIĆ Č., RADOJKOVIĆ D., JOSIPOVIĆ S. (2007): Genetic resources in pig breeding – carcass quality traits of breeds Moravka and Mangalitsa. Biotechnology in Animal Husbandry, 23:421-428.

PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., MIJATOVIĆ M., RADOJKOVIĆ D., ALEKSIĆ S., STANIŠIĆ N., POPOVAC M. (2010): Quality traits of carcass sides and meat of Moravka and Mangalitsa pig breeds. Biotechnology in Animal Husbandry, 26:21-27.

PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R. (2012): Composition of carcass sides and quality of meat from swallow-belly mangalitsa reared in two systems. Biotechnology in Animal Husbandry, 28, 2, 303-311.

PETROVIĆ M., SAVIĆ R., PARUNOVIĆ N., RADOJKOVIĆ D., RADOVIĆ Č. (2013): Reproductive traits of pigs of Mangalitsa breed. 8th International

Symposium on the Mediterranean Pig, Slovenia, Ljubljana, October 10th−12th, 2013. Acta agriculturae Slovenica, Supplement 4, 89–92, Ljubljana.

PETROVIĆ M., WÄHNER M., RADOVIĆ Č., RADOJKOVIĆ D., PARUNOVIĆ N., SAVIĆ R., BRKIĆ N. (2014): Fatty acid profile of m. longissimus dorsi of Mangalitsa and Moravka pig breeds. Archiv Tierzucht 57, 17, 1-12.

RADOVIĆ Č., PETROVIĆ M., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R., STANIŠIĆ N., GOGIĆ M. (2017): Carcass and pork quality traits of indigenous pure breeds (Mangalitsa, Moravka) and their crossbreads. Indian Journal of Animal Research, 51, 2, 371-376.

RADOVIĆ Č., PETROVIĆ M., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R., GOGIĆ M., STANIŠIĆ N. (2015): Measures of in-situ protection, reintroduction and reproductive parameters of indigenous pig breeds in Republic of Serbia. Reproduction in domestic animals, 50, 3, 85-85.

RÁTKY J., EGERSZEGI I., TOTH P., KEONUCHAN S., NAGAI T., KIKUCHI K., MANABE N., BRÜSSOW K.P. (2013): Saving Genetic Resources of Native Pigs in Occidental and Oriental Countries — Practical Examples of the Characterization and Utilization of Native Pigs in Hungary and Laos. Journal of Reproduction and Development, 59, 5, 437–441.

STANIŠIĆ N., RADOVIĆ Č., STAJIĆ S., ŽIVKOVIĆ D., TOMAŠEVIĆ I. (2015): Fizikalno-kemijska svojstva mesa svinja pasmine mangulica. Meso, 1, XVII, 126-129.

UNIVERZITET U BEOGRADU, POLJOPRIVREDNI FAKULTET (2010): Izveštaj o obavljenim poslovima koordinacije za mere selekcije u svinjarstvu u 2009 (Annual Report 2010), 1-47.

SZABÓ P (2002): Theriogenological results of alternative pig breeds. (Alternatív sertésfajták szaporodásbiológiai eredményei) In: Innováció, a tudomány és a gyakorlat egysége az ezredforduló agráriumában, Debrecen, 97-102.

TOMOVIĆ V., STANIŠIĆ N., JOKANOVIĆ M., KEVREŠAN Ž., B. ŠOJIĆ, ŠKALJAC S., TOMAŠEVIĆ I., MARTINOVIĆ A., DESPOTOVIĆ A., ŠUPUT D. (2016): Meat quality of Swallow-Belly Mangulica pigs reared under intensive production system and slaughtered at 100 kg live weight. Hemijska Industrija, 70, 5, 557–564.

TEODOROVIĆ M., RADOVIĆ I. (2004): Svinjarstvo. Univerzitet u Novom Sadu, Poljoprivredni fakultet, 1-286.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

PIG WELFARE AT DIFFERENT PRODUCTION SYSTEMS

Zoran Luković, Dubravko Škorput, Danijel Karolyi

University of Zagreb, Faculty of Agriculture, 10000 Zagreb, Croatia
Corresponding author: Zoran Luković, lukovic@agr.hr
Review paper

Abstract: Animal welfare is increasing interest worldwide. Pig farming is one of the most intensive of all livestock production systems. Pigs are very adaptable animals and could be reared at different production systems. In many countries in region pigs are still reared in three production systems: farm enterprises, full-time family farms, and part-time family farms. In intensive production systems pigs are housed mainly indoors, while pigs in less intensive systems often could be found in outdoor or semi-outdoor environment. Rearing pigs in outdoor environment allows studying natural behaviour of pigs, as one of the most important criteria for animal welfare assessment. Although it is generally considered that pigs kept outdoors have fewer problems with welfare, there are some critical points needed to be considered. Malnutrition of different categories of pigs and exposure to parasites and infectious diseases are the most common reasons for concern about outdoor pig production systems. According to the principles of five freedoms, differences between the different production systems will be discussed and divergences from the EU recommended resources will be highlighted.

Key words: pigs, welfare, five freedoms, production systems

Introduction

Animal welfare is increasing interest worldwide. Animal welfare is a diverse area often described by the five freedoms *(FAVC, 1979)*. Pig farming is one of the most intensive of all livestock production systems. Pigs are very adaptable animals and could be reared at different production systems *(Uremović et al., 2001a)*. Consumers often have a favourable perception of some alternative production systems, like outdoor or deep litter, considering it more humane, sustainable and environmentally friendly *(Edwards, 2005)*. Of course, pigs kept outdoor could also face some welfare problems such as thermal stress, parasite pressure, and competition for food. Additionally, absence of one particular indicator of poor welfare, for example when growth rate is good, cannot be taken to mean that there is no welfare problem *(Broom, 1986)*. No matter which production system is considered, welfare of pigs should be improved according to consumer demands and their willingness to pay higher price for products obtained from animals reared in welfare friendly systems *(Borgen and Skarstad, 2007)*.

Changes in animal agriculture over the last half of the 20th century have drastically altered farming practices and management. On the large, commercial operations, pigs are primarily confined indoors in industrialized facilities *(HSI, 2014)*. Large scale farms with more thousand breeding sows in one location became the dominant production type. Less intensive pig production systems are generally diverse worldwide. So, they differ by pig genotype used, environmental conditions, and other natural resources (food, manipulative materials). Outdoor pig farming is defined as a system that allows the pigs outside access including contact with soil and growing plants in which animals can express their natural behaviour *(Miao et al., 2004)*.

Assessment and comparison of welfare of pigs in different production system often use resource-based method described by five freedoms criteria as suggested by *Brambell (1967)*. Another approach to determine welfare of pigs in different systems is to use animal-based indicators, like feeding and housing principles *(Temple et al., 2012)*. So, in the further chapters each freedom will be discussed according to different production systems: intensive and alternative, mainly deep litter and outdoor. Of course, between those two main systems is plenty of different systems with some characteristics of the first or the second one. Aim of this paper is to determine the main critical points in welfare of pigs at different production systems.

Freedom from hunger and thirst

Freedom from hunger and thirst should be ensured by providing fresh water and pig category specific diets *(EC Directive, 2001)*. Water supply should be ad libitum, and pigs need to feed more than once a day. Appropriate quantity and quality of food are the one of most important conditions for success in any kind of livestock production. In the intensive confined systems, different categories of pigs are fed by complete food mixtures created mainly by professional nutritionist. Sows should be provided with sufficient quantities of high-fibre and high-energy food *(EC Directive, 1991)*. Beside positive effect on sows, *Bernardino et al. (2016)* noted that high fibre diets during pregnancy influence on less aggression among piglets prior to weaning.

In less intensive system, quality of food often depends on farmer's knowledge about nutritional needs of pigs. *Luković et al. (2017)* showed that very small number of pig owners consulting professionals of animal nutrition to prepare diets for their pigs. *Wellbrock (2008)* reports that part-time family farmers provided their pigs with kitchen leftovers, green grass and potatoes, bread and whey, and did not comply with the EU recommended feed compositions. In rough outdoor environment with limited amount of food, malnutrition of pigs is a common case, especially visible in sows as low body condition after lactation *(Luković et al., 2017)*. Because of group feeding of all categories, there is no way to feed sows according to body condition.

At all production systems, pigs should need to have constant availability to fresh water. Even a smaller shortage of drinking water can lead to dehydration and for a longer time to reduction of production traits in pigs *(Fraser et al., 1990)*. In some outdoor systems, pigs of local breeds kept in the forest during all year used water from the forest's creek passed through. The potential problem of using natural sources of drinking water (creeks, ponds) is freezing during the winter because of extremely low temperatures or extreme droughts in the summer when creeks can dry up *(Luković et al., 2017)*. Under these conditions, water should be additionally provided to pigs from other sources (tanks).

Freedom from discomfort

Discomfort can be avoided by assuring adequate environmental conditions. All pigs should have access to clean, dry, and thermally comfortable areas. Comfortable areas for pigs in intensive system are often connected with floor type *(Uremović et al., 2001b)*, while poorly maintained or slippery flooring are still common causes of physical injuries *(Kilbride et al., 2008)*. Further, young animals are especially sensitive to low temperature, so too cold environment could be cause of high mortality rate in piglets. In fully slatted floor, width of slats and openings need to be in accordance with EU regulation *(EC Directive, 1991)*, thus avoiding injuries in pigs.

Outdoor pigs need shelters for protection against sun in the summer months and cold in winter time. Additionally, outdoor pigs showed natural behaviour wallowing in the mud, mainly for cooling, sunburn protection and the removal of ecto-parasites *(Bracke and Spoolder, 2011)*. The practice of nose-ringing outdoor pigs has been questioned on ethical grounds, although nose rings are widely used commercially to reduce the pasture damage that is caused by indiscriminate rooting of the paddock *(Edge et al., 2005)*. Although, some alternatives to use of nose rings were suggested, the only effective way to reduce pasture damage is to assure enough large area for outdoor pigs and to rotate pastures.

Freedom from pain, injury and diseases

Using of preventive measures, rapid diagnosis and immediate treatments is the best way to ensure freedom from pain, injury and diseases *(Salajpal et al., 2013)*. Pig facilities in intensive systems should assure comfortable environment for pigs and freedom from injury of any kind. There is some evidence that leg injuries are more likely to occur on concrete, barren or fully-slatted floors than on straw-bedded, concrete floors *(Scott et al., 2006)*.

Pain is mainly related to some procedures, like tail docking, teeth clipping and castration, where it may not be carried out routinely, except castration of male fattening pigs. All interventions should be carried out by trained persons, what is sometimes problem at small units, especially at part-time family farms. In outdoor pigs, of local less productive genotypes, procedures like tail docking and teeth clipping were not used at all *(Luković et al., 2017)*. From the point of view of disease in pigs at different production systems, fact is that pigs could get sick from the same diseases regardless production system. In intensive confined systems, there is higher incidence of respiratory and digestive diseases, mainly because overcrowding of facilities and bed environmental conditions (temperature, humidity, gasses). On the other hand, in outdoor systems pigs are at risk from infectious diseases like swine fever, brucellosis, leptospirosis etc. *(Salajpal et al., 2013)*. There is also some higher incidence of endo and ecto-parasitism in outdoor pigs in comparison to indoor ones. In study by *Guy et al. (2002)* it was concluded that for the finishing systems used in this study, pig welfare was enhanced in both outdoor paddocks and straw yards compared to fully-slatted pens.

One additional problem of small pig units in relation to farm enterprises is absence of any herd health risk plan what is very important especially in the frame of protection of local pig breeds of small population size *(Luković et al., 2017)*.

Freedom to express natural behavior

One of the criteria for assessment of animal welfare is possibility to express natural behaviour *(Špinka, 2009; Kittawornrat and Zimmerman, 2010)*. Pigs in intensive production systems don’t have possibility to express some form of natural behaviour as pigs reared outdoor. This is obvious that pigs reared in barren industrial environment don’t have access to manipulative materials (straw, soil, wood), and foraging or feeding behaviour differs completely in relation to pigs in natural environment. Pigs with straw were more active, spending a large proportion of time manipulating straw, and they don’t spend time in behaviour directed at other pigs *(Scott et al., 2006)*. *Scott et al. (2007)* also noted that in the absence of straw, significantly more investigatory behaviours were directed towards pen components, with a similar tendency in behaviours directed at pen-mates.

Lack of space and the artificial group structure of pigs in intensive systems can negatively influence social interactions. Social behaviour related to grouping or mixing of pigs in different production stages is also often disturbed resulting in aggression among animals. To ensure sow welfare, housing design must, at the very least, ensure unimpeded access to necessary resources, opportunity to avoid or escape from potential aggressors, and avoidance of chronic physiological stress *(Weng et al., 1998)*. The results indicate that a minimum space of between 2.4 and 3.6 m^2 per sow was necessary in the conditions of this experiment to promote good welfare.

Provision of sows with nesting material before parturition and potential to show maternal behaviour is one of the key advantages of alternative production systems in comparison to industrial pig farming. The results of *Akos and Bilkei (2004)* indicate that, although an outdoor environment may better satisfy the ethological needs of the animals, indoor production systems allow the breeding female a longer life and higher production level. Continental climate with high temperature fluctuations may present a risk factor for successful low-investment outdoor sow systems *(Uremović et al., 2003)*.

Freedom from fear and distress

To prevent fear and distress, any conditions which may cause mental suffering should be avoided *(EC Directive, 2001)*. All procedures with piglets should be applied by trained persons with aim to reduce stress as much as possible. In intensive production systems pigs are moved from one to another place several times during lifetime, and this movement could be important source of stress, especially in time of weaning *(Sutherland et al., 2014)*. Weaning time is probably one of the most critical period in life of piglets because of more stressors at the same time, including handling at loading and unloading, mixing with unfamiliar pigs, feed and water withdrawal, exposure to a new environment, vibrations and noise, etc. Lactation length is usually longer in outdoor production system than in intensive systems, but prolonged lactation in some cases is unfavourable from sow's welfare point of view. This is obvious in group feeding of pigs, where sows after two months of lactation have a serious problem with body condition, and subsequent return to oestrus *(Luković et al., 2017)*.

One potentially new welfare problem should be discussed considering highly productive sows, where high level of production, i.e. litter size could be also stress for sow and piglets *(Baxter et al., 2013)*. Welfare issues related to litter size in pigs are complex, affecting sows and piglets. Management interventions that are used when litter size routinely exceeds the ability of individual sows to successfully rear all the piglets could be additional triggers of stress, and consequently triggers of reduced welfare.

Conclusions

There is a plenty of room to improve pig welfare in both, intensive and alternative production systems. Criteria of welfare defined as five freedom indicate critical points in all pig categories. Although, alternative production systems assure better welfare of pigs related to freedom to express natural behaviour, there are still many problems in welfare of all categories of pigs. Malnutrition of different categories of pigs and exposure to parasites and infectious diseases are the most common reasons for concern about outdoor pig production systems. Further education of the farmers at large and especially small family farms is necessary to improve welfare of pigs.

Dobrobit svinja u različitim proizvodnim sistemima

Zoran Luković, Dubravko Škorput, Danijel Karolyi

Rezime

Dobrobit životinja je sve veći interes širom sveta. Uzgajanje svinja je jedan od najintenzivnih sistema stočarske proizvodnje. Svinje su veoma prilagodljive životinje i mogu se odgajati u različitim proizvodnim sistemima. U mnogim zemljama u regionu svinje se još uvek gaje u tri proizvodna sistema: poljoprivredna preduzeća, porodične farme sa punim radnim vremenom i porodične farme sa delimičnim radnim vremenom. U intenzivnim proizvodnim sistemima svinje se smeštaju uglavnom u zatvorenom prostoru, dok se svinje u manje intenzivnim sistemima često mogu naći u otvorenom ili poluotvorenom okruženju. Uzgoj svinja u prirodnom okruženju omogućava proučavanje prirodnog ponašanja svinja, kao jedan od najvažnijih kriterijuma za procenu dobrobiti životinja. Iako se uopšteno smatra da svinje na otvorenom imaju manje problema sa dobrobiti, potrebno je razmotriti neke kritične tačke. Neuhranjenost različitih kategorija svinja i izloženost parazitima i zaraznim bolestima su najčešći razlozi za zabrinutost oko sistema za proizvodnju svinja na otvorenom. Prema principima pet sloboda, razmatraće se razlike između različitih proizvodnih sistema i istaknuće se razlike u odnosu na resurse preporučene EU.

Ključne reči: svinje, dobrobit, pet sloboda, proizvodni sistemi

Acknowledgment

The study has partly received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634476 (project acronym TREASURE). The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains. Authors also wish to acknowledge the financial support received from Ministry of Agriculture (Project No 2016-14-8).

References

AKOS K., BILKEI G. (2004): Comparison of the reproductive performance of sows kept outdoors in Croatia with that of sows kept indoors. Livest. Prod. Sci. 85, 293–298.

BAXTER E. M., RUTHERFORD K. M. D., D'EATH R. B., ARNOTT G., TURNER S. P., SANDOE P., MOUSTSEN V. A., THORUP F., EDWARDS S. A., LAWRENCE A. B. (2013): The welfare implications of large litter size in the domestic pig II: Management factors. Anim. Welf. 22, 219–238.

BERNARDINO T., TATEMOTO P., MORRONE B., RODRIGUES P. H. M., ZANELLA A. J. (2016): Piglets born from sows fed high fibre diets during pregnancy are less aggressive prior to weaning. PLoS One 11, 12: e0167363. https://doi.org/10.1371/journal.pone.0167363.

BORGEN S. O., SKARSTAD G. A. (2007): Norwegian pig farmers' motivations for improving animal welfare. Br. Food J. 109, 891–905. Available from: http://www.emeraldinsight.com/doi/10.1108/00070700710835705

BRACKE M. B. M., SPOOLDER H. A. M. (2011): Review of wallowing in pigs: Implications for animal welfare. Anim. Welf. 20, 347–363.

BRAMBELL F. W. R. (1967): Report of the Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. Her Majesty's Stationary Office: Great Britain.

BROOM D. M. (1986): Indicators of Poor Welfare. Br. Vet. J. 142, 524–526.

EC Directive (1991): Council Directive 1991/630/EEC Minimum standards for the protection of pigs. Consolidated Text. Office for Official Publications of the European Union.

EC Directive (2001): Commission Directive 2001/93/EC laying down minimum standards for the protection of pigs. Official Journal of the European Union.

EDGE H. L., BULMAN C. A., EDWARDS S. A. (2005): Alternatives to nose-ringing in outdoor sows: The provision of root crops. Appl. Anim. Behav. Sci. 92, 15–26.

EDWARDS S. A. (2005): Product quality attributes associated with outdoor pig production. Livest. Prod. Sci. 94, 5–14.

FAWC (1979): Farm animal welfare council, press statement. FAWC, Tolworth.

FRASER D., PATIENCE J. F., PHILIPS P. A., MCLEESE J. M. (1990): Water for piglets and lactating sows: Quantity, quality and quandaries. In: Recent Advances in Animal Nutrition. Eds Haresign W., Cole D. J. A., Butterworths, London, UK, 137-160.

GUY J. H., ROWLINSON J. P., CHADWICK P., ELLIS M. (2002): Health conditions of two genotypes of growing-finishing pig in three different housing systems: Implications for welfare. Livest. Prod. Sci. 75, 233–243.

HSI (2014): An Human Society International report: The Welfare of Animals in the Pig Industry. http://www.hsi.org/assets/pdfs/hsi-fa-white-papers/welfare_of_animals_on_the_pig.pdf. Accessed June 25, 2017.

KILBRIDE A. L., GILLMAN C. E., GREEN L. E. (2008): Prevalence of foot lesions, limb lesions and abnormal locomotion in pigs on commercial farms in Britain and risks associated with flooring. The Pig Journal 61, 8.

KITTAWORNRAT A., ZIMMERMAN J. J. (2010): Toward a better understanding of pig behaviour and pig welfare. Animal Health Research Reviews 12, 1, 25-32.

LUKOVIĆ Z., IVŠAC I., ŠKORPUT D., SALAJPAL K., KAROLYI D. (2017): The welfare of Turopolje pig in outdoor system. In: 52nd Croatian and 12th International Symposium on Agriculture. February 12-17, Dubrovnik, 544-548.

MIAO Z. H., GLATZ P. C., RU Y. J. (2004): Review of production, husbandry and sustainability of free-range pig production systems. Asian-Australasian J. Anim. Sci. 17, 1615–1634.

SALAJPAL K., KAROLYI D., LUKOVIĆ Z. (2013): Sanitary Aspects of Outdoor Farming Systems. Acta Agric. Slov. Suppl. 4, 109–117.

SCOTT K., CHENNELLS D. J., CAMPBELL F. M., HUNT B., ARMSTRONG D., TAYLOR L.,. GILL B. P., EDWARDS S. A. (2006): The welfare of finishing pigs in two contrasting housing systemss: Fully-slatted versus straw-bedded accommodation. Livest. Sci. 103, 104–115.

SCOTT K., TAYLOR L., GILL B. P., EDWARDS S. A. (2007): Influence of different types of environmental enrichment on the behaviour of finishing pigs in two different housing systems. 2. Ratio of pigs to enrichment. Appl. Anim. Behav. Sci. 105, 51–58.

SUTHERLAND M. A., BACKUS B. L., MCGLONE J. J. (2014): Effects of transport at weaning on the behavior, physiology and performance of pigs. Animals 4, 657–669.

ŠPINKA M. (2009): Behaviour of pigs. In: The Ethology of Domestic Animals. 2nd edition. Ed Jensen P. CAB International, Wallingford, Oxon, UK, 177-191.

TEMPLE D., COURBOULAY V., MANTECA X., VELARDE A., DALMAU A. (2012): The welfare of growing pigs in five different production systems: assessment of feeding and housing. Animal 6, 4, 656-667.

UREMOVIĆ Z., UREMOVIĆ M., LUKOVIĆ Z., KATALINIĆ I. (2001a): Effect of feeding and housing system in fattening pigs on environmental pollution. Agronomski glasnik 63, 5, 215-231.

UREMOVIĆ Z., VINKOVIĆ B., UREMOVIĆ M., GOJMERAC T., LUKOVIĆ Z., KATALINIĆ I. (2001b): Influence of floor types in piglots on production results, microclimate and pollution with waste material. Stočarstvo 55, 3, 179-186.

UREMOVIĆ M., UREMOVIĆ Z., LUKOVIĆ Z., KONJAČIĆ M. (2003): The Influence of Genotype and Production Conditions on the Fertility of Sows in Outdoor System. Agric. Conspec. Sci. 68, 245–248.

WELLBROCK W. (2008): Pig welfare in Croatia: A critical reflection on the EU pig welfare directives. MSc Thesis, Wageningen University, Wageningen.

WENG R. C., EDWARDS S. A., ENGLISH P. R. (1998): Behaviour, social interactions and lesion scores of group-housed sows in relation to floor space allowance. Appl. Anim. Behav. Sci. 59, 307–316.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

NOVEL ASPECTS OF THE TAIL LENGTH IN PIGLETS CONSIDERING TO THE OCCURRENCE OF VERTEBRAL ABNORMALITIES

Thomas Kunze, Martin Wähner

Anhalt University of Applied Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany
Corresponding author: Thomas Kunze, thomas.kunze@hs-anhalt.de
Original scientific paper

Abstract. To cease up docking the tails in piglets, it is important to obtain knowledge about the basic anatomy of tails and the reasons for tail biting. In this investigation, different parts of the body of the piglets were measured, such as the tail length. The data was collected in three pig farming enterprises in Germany in April 2015. 1,273 piglets from 100 sows were examined. The animals tested were between the ages of two to three days. The genetic situation differed across the three enterprises. Sows are DanBred, PIC and a product of the cross breeding of German Large White and German Landrace while the boars had the genetic of Duroc, Piétrain, German Large White or TOPIGS. The following parameters were studied: The tail length, the weight, the body length as well as the diameter of the tail. Abnormalities of the tails were observed. The mean tail length in piglets is 8.50 cm ± 0.94 cm. The parameter has a range of 8 cm. The tail length is influenced by the weight of the piglets ($p \leq 0.001$), the number of the litter of the sow ($p \leq 0.001$) and the enterprise ($p \leq 0.001$). Differences in tail length could be found between, as well as within, the litters. There are correlations between the presence of abnormalities and the length of a tail of a piglet. With a higher level of an abnormality, the mean tail length decreases significantly.

Key words: tail, length, piglet, variability, abnormality

Introduction

The expression of the phenotype of the tail in newly born piglets, which means the length and the occurrence of vertebral abnormalities, was rarely the focus of breeding work up until now. However, this has changed and this part of the body is becoming much more important. Tail biting has become a huge cause for concern. Primarily in group- housing, the tail of the animals is an object to play with. According to *Hempler (2012)* tail biting is a behavioural disorder with a great complexity of causes. It appears especially in intensive group-housing of fattening pigs. Concerning this, the increasing intensity of pig farming in many countries of Europe is a contradiction *(Statistisches Bundesamt, 2013 und 2015).* Docking the tails in piglets is the method often used to deal with tail biting. It has, however, no influence on the origin of the problem. Nevertheless, it is a very controversial method *(Hempler, 2012).* Tail biting occurs in organic and free-range pigs, too *(Walker und Bilkei, 2006).* In legal terms, it is conditionally allowed to dock the tails *(Richtlinie 2008/120/EG des Rates, 2008 und TIERSCHG, 2006).* However, a waiver of this procedure is simply not possible *(Wörle, 2010).* This is justified in his heterogeneous nature *(Kompetenzkreis Tierwohl, 2015a)*. The result is a claim for a completion of tail docking long-term or rather systematically *(Kompetenzkreis Tierwohl, 2015b).* In some countries of Europe it is already forbidden to cut the tails, e.g. in Sweden *(Pollmann und Meyer, 2014).* In Germany, such a prohibition lies ahead *(Wörle, 2014).*

The situation in the production of pigs nowadays forces us to search for alternatives. For achieving socially accepted livestock farming, a willingness to change by the farmers, the politicians and the consumers is necessary *(Wildraut und Mergenthaler, 2016)*. Based upon their willingness to change the result may be drastic *(Bmel, 2015).* Furthermore, in order to achieve these goals, much more research is needed. They are worried about the genetics of the animals, e.g. *(Ble, 2016)*. Against this background, general information is needed for the phenotypic expression of the conformation about the tail on pigs. First assertions are possible by the results of this investigation.

Materials and Methods

The study was conducted in April and May of 2015, in three pig farming enterprises. These enterprises were located in Thuringia, Saxony and Saxony-Anhalt. Thereby 1,273 piglets from 100 sows were available. The data was measured two to three days after the birth of the piglets. 752, 258 and 263 piglets are from the companies 1, 2 or 3.

The genetic situation between the three farms differed. There were sows, which were progeny of the crossing between German Large White and German Landrace, and some had the genetic origins DanBred or PIC. Boars had the genetic origin of Duroc, Piétrain, German Large White or TOPIGS. In one enterprise (No. 3), piglets could be assigned to their fathers. In this enterprise, every boar had the genetic origin Piétrain. Seven litters are from boar 1, 11 litters from boar 2 and two litters from boar 3.

In addition to the genetic, the number of the litter of the sows and the amount of piglets per sow were recorded. The mean number of the litter of the sow was 3.85 ± 1.67. There were averagely 13.46 ± 2.36 piglets per sow. These piglets had an age of 3.11 ± 1.08 days at time of measurement. The age was counted as complete, after the day of birth and day of data collection. Regarding to this parameters, significant differences occur between the separate farms. Between two day and four day old piglets, there are no significant differences for the parameter tail length. On this basis all piglets were comparable with each other. The sex of the piglets was documented. The weight was collected with a digital or hanging scale. Further, the body length was measured with a tape measure starting at the tail up to a imaginary line between the ears of the piglet. These points guaranteed a good reproducibility. The values for the tail length or the diameter of the tail were gauged with the help of a folding rule (figure 1).

Tail length was recorded from the direct end of the body to the tip of the tail. The diameter was also measured directly at the end of the body of the piglet. A consistently position of the piglets and the same perspective of time at measuring was important to ensure comparable data.

Figure 1. Data collection for parameters: Tail length (left), diameter of the tail (centre) and body length (right)

The form of the tail was classified in four groups. The classification depends on the occurrence of an abnormality of the caudal vertebra. Tails with a normal form were assigned to the category “0”. Piglets with a slightly angle of the tail ($\ll 90°$ from normal form) were the category “1”. With tails of an angulation of approx. 90°, piglets got assigned to the group “2” and of $\gg 90°$ they were categorized to the group “3”. Examples for the classification of group 1 and 3 were illustrated in figure 2.

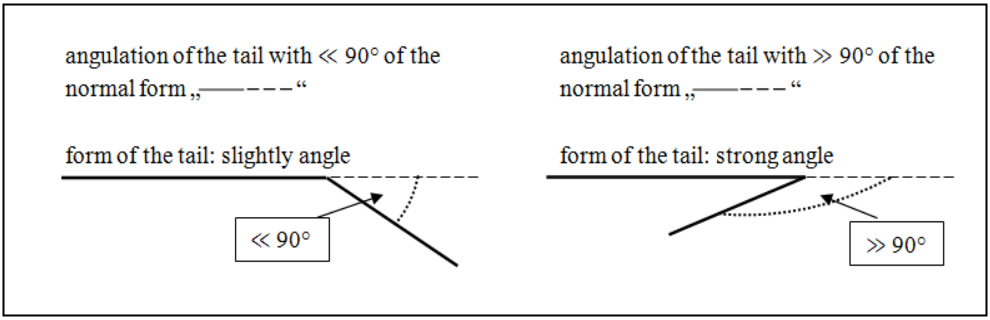


Figure 2. Examples for the categorisation of the form of the tail in piglets

In figure 3, original pictures from the investigation are shown. The left picture contains a group-2-form of the tail with an angulation of approx. 90°. At the second image there is a tail of a piglet with a kink of ≫ 90°.

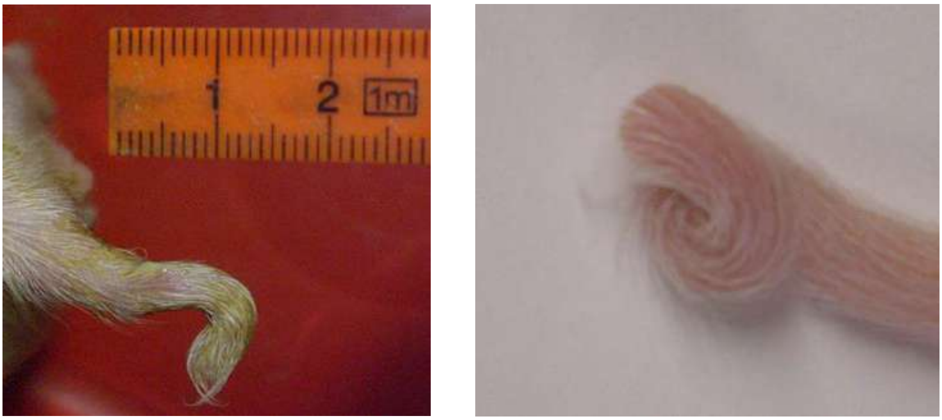


Figure 3. Tails of piglets with an angle of approx. 90° (left) and ≫ 90° (right)

For statistical processing of the data, Microsoft Excel (version 2007) and IBM SPSS (version 22.0) were used. For simple comparison of means, oneway anova has been applied. For determination of significantly differences, Post- Hoc-Test LSD was progressed. A level of significances of $p \leq 0.05$ was defined for the complete analysis. Furthermore mean values ($\bar{x}$), minima (Min) and maxima (Max), standard deviation (s) and correlations (PEARSON) were calculated.

The influences of different fix factors, such as farm or number of the litter of the sow on the dependent variable tail length was tested with univariate variance analysis GLM- Univariate. As covariables, weight of piglets and litter size were used. The displaying of the results was made as LSM ± SE.

Model:

$$Y_{i,j,k} = \mu + F_i + NL_j + b\,(W_k - \overline{W}) + b\,(LS_k - \overline{LS}) + e_{i,j,k}$$

$Y_{i,j,k}$:	Observed value, dependent variable tail length of piglets
μ:	Mean
F_i:	Fix factor farm (i= 1,2,3)
NL_j:	Fix factor number of litter- effects of classes (j= 1,2,3)
b (W):	Covariable weight of piglets
b (LS):	Covariable size of litter
$e_{i,j,k}$:	Residual error

The number of the litter of the sow is classified in class I: parity number one and two; class II: parity number three and four and class III: parity number five and more.

Results and Discussion

The mean tail length in piglets is 8.50 ± 0.94 cm. The value varies from 3.40 up to 11.40 cm. There are in corresponding a range of 8.00 cm. The diameter of the tail is 0.92 ± 0.13 cm. The minimum is 0.40 cm and the maximum 1.40 cm for this parameter. The average body length amounts 28.94 ± 2.82 cm (table 1).

Table 1. Results for different selected body parameters (n = 1,273)

Parameter	**$\bar{x} \pm s$**	**Min – Max**
Tail length (cm)	8.50 ± 0.94	3.40 – 11.40
Body length (cm)	28.94 ± 2.82	16.50 – 38.50
Birth weight (kg)	1.65 ± 0.42	0.53 – 3.40
Diameter of the tail (cm)	0.92 ± 0.13	0.40 – 1.40

Between piglets with a Piétrain or a Duroc father, significant differences in the length of the tails occur. The tails of piglets with Duroc blood are very little, usually 0.06 cm shorter. Concurrently, the litters by a Piétrain father are more homogenous regarding to the level of the tail length. It can be expected that the variation of the length of the tails in piglets varies between different genetics. *Shelton (1977)* confirmed the same in sheep. Various means and ranges occurred in different pure- and crossbreeding.

The piglets´ weight ($p \leq 0.001$) influences the tail length of the piglets as well as the farm ($p \leq 0.001$) and the number of the litter of the sow ($p \leq 0.001$), which are highly significant. The size of the litter shows a significant influence ($p \leq 0.05$).

Piglets of a first or second litter of a sow have a tail length of 8.28 ± 0.09 cm (LSM ± SE). In higher litter numbers, tail length is significantly longer. With at least the third litter of the sow the tails of the piglets have an average length of 8.62 ± 0.04 cm. Some characteristics are dependent on each other. The litter size and the birth weight of the piglets are correlating, which is known, as negative.

Between the litters in farm one, differences in the mean tail length occurred. The general average value in this enterprise are 8.47 ± 0.94 cm. Piglets of 60 sows were recorded. The lowest mean value for a group of full siblings is 7.15 cm (sow 31). The highest average has an amount of 9.65 cm (sow 26). The difference between these two extreme values is 2.50 cm. That is pointing on a relatively high phenotypic variability of this parameter (figure 4).

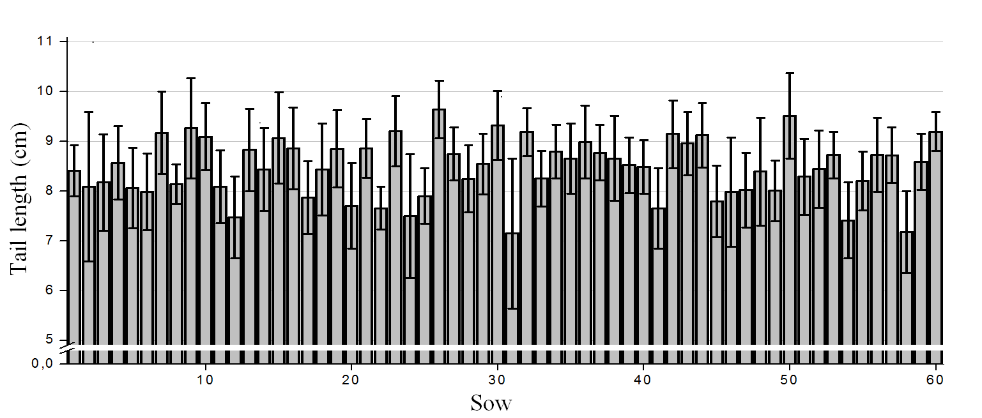


Figure 4. Mean tail length of piglets in dependence to the sow (n=752; farm I)

The same situation appears in farm three. Three boars were brought in. They are all of the Piétrain breed. Independent of the used boars, the mean of the tail lengths per litter of the sows differ to each other. The differences are not significant. When crossing boar I, the average tail length of the piglets in seven litters ranged from 6.61 to 8.77 cm. The length in eleven litters of sows crossed with boar II is 7.01 to 9.27 cm. Boar III was mated with two sows and the tail lengths of the piglets have a total of 7.51 cm to 9.13 cm. As you can see, the two litters of boar III are also showing differences in the tail length of the piglets. Based on these values, variability in the length of the tails is deducible (figure 5).

In general, the amount between the sows in this farm varies by approx. 2 cm depending on the used boar. The values suggest a maternal influence on the length of the piglets' tail.

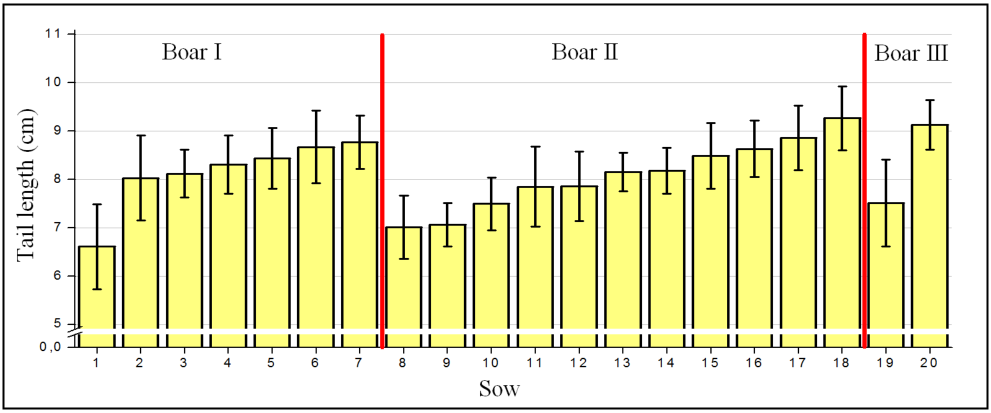


Figure 5. Mean tail length of piglets in dependence to the sow and the boar (n=263; farm III)

Diverse phenotypic correlations have been calculated (table 2). Therefore, the tail length; the diameter of the tail; the body length and the weight of the piglets were the main focus points of the observation that were carried out. The tail length correlates to the mentioned parameters with approx. r=0.600. The heavier the piglets are, the longer the tail size. The relations are highly significant ($p \leq 0.01$). With an increasing litter size, the birth weight and the body length of the piglets will be decreasing. Therefore, the influence of the litter size is indirectly related to the tail length of the piglets.

Table 2. Phenotypic correlations in piglets (n = 1,235)

Parameter	Body length	Tail length	Diameter of the tail
Body weight	0.825**	0.608**	0.692**
Body length	-	0.598**	0.682**
Tail length		-	0.554**

** significant at $p \leq 0.01$

As a special feature, the occurrence of abnormalities in the caudal vertebral was recorded. In 4.01% of all cases such characteristics were determined. Without having an abnormality, the tails have a length of 8.55 ± 0.90 cm. With a slight angle, the length is reduced to 7.61 ± 1.23 cm. In comparison to the normal, it is nearly one centimetre (0.94 cm) shorter. *Beer (1964)* indicated that they were a shorter length. This difference is significant. With an increasing level of the abnormalities, tails are becoming even shorter. The results point out that the appearance of abnormalities in the area of the tail could differentiate between several races.

Concerning the longest tails of each group, the extreme values are also decreasing. With regard to the normal tails, the maximum tail length with an abnormality was 1.60 cm shorter (table 3).

Table 3. Tail length in piglets in accordance to the level of caudal vertebral abnormalities (n= 1,273)

Form of the tail		**%**	**Tail length (cm)**	
			$\bar{x} \pm s$	**min- max**
Normal	(class 0)	95.99	$8.55^a \pm 0.90$	4.00 – 11.40
Angle ≪ 90°	(class I)	1.34	$7.61^b \pm 1.23$	4.60 – 9.80
Angle approx. 90°	(class II)	1.41	$7.17^b \pm 0.63$	5.80 – 8.10
Angle ≫ 90°	(class III)	1.26	$6.90^b \pm 1.44$	3.40 – 8.60
Total	(n= 1,273)	100.00	8.50 ± 0.94	3.40 – 11.40

^{a,b} significant at $p \le 0.05$

James (2006) detected sheep with “anury” and “brachyury”. Anury describes sheep without a tail whereas brachyury means the animal has a very short tail. This was discovered in long-tailed races. In his research, the phenotypic phenomenon anury and brachyury appeared with a frequency of 0.1 up to 11.1%. Often, the occurrence was combined with other physical defects. These are especially musculoskeletal defects. No tailless or those with extremely short tails could be observed in the animals of this investigation in piglets direct after birth. However, the common occurrence of caudal vertebral abnormalities and shorter average tail lengths are hints, for a related situation in the species of sheep and swine, again.

Conclusion

The tail length in neonatal piglets has an average total of 8.50 cm. There is a huge variability for this parameter. The range between the shortest and longest tails (3.40 or rather 11.40 cm) is 8.00 cm. The piglets of Piétrain breed fathers have a slightly longer tail than Duroc blood piglets.

The birth weight ($p \le 0.001$); the number of the litter of the sow ($p \le 0.001$) and the size of the litter ($p \le 0.05$) influence the length of the tail. Influences on the tail length are obvious in farm III. Both, the mother and the father are assignable to the full siblings of the several litters. Variabilities are found in the single piglets as well as the litters.

Correlations of the tail length with other parameters were calculated. The relations are on a medium level and high significant ($p \leq 0.01$). The tail length correlates to the body weight with $r=0.608$, the body length with $r=0.598$ and the diameter of the tail with $r=0.554$.

In 4.01% of the piglets, a caudal vertebral abnormality was observed. Piglets with an abnormality have shorter tails than piglets with normal formed tails. Moreover, with a higher level of an angulation of the tail, the length is becoming shorter. There are indications regarding differences in the frequency of the occurrence of abnormalities relating the genetic situation of the mother.

Novi aspekti dužine repa kod prasadi uzimajući u obzir pojavu vertebralnih abnormalnosti vretenca

Thomas Kunze, Martin Wähner

Rezime

Da bi se zaustavilo sečenje repa kod prasadi, važno je upoznati se sa osnovnom anatomijom repa i razlozima za griženje repa. U ovom istraživanju, izmereni su različiti delovi tela prasadi, kao što je dužina repa. Podaci su prikupljeni na tri gazdinstva koja se bave odgojem svinja u Nemačkoj u aprilu 2015. godine. Ispitivano je 1.273 prasadi od 100 svinja. Ispitane životinje su bile uzrasta dva do tri dana. Genetička situacija se razlikovala u tri preduzeća. Krmače su bile DanBred, PIC i proizvod ukrštanja nemačke velike bele i nemačkog landrasa, dok su nerastovi imali genetiku duroka, pijetrena, nemačke velike bele ili TOPIGS-a. Ispitivani su sledeći parametri: dužina repa, težina, dužina tela, kao i prečnik repa. Opažene su abnormalnosti repa.

Prosečna dužina repa kod prasadi je 8,50 cm ± 0,94 cm. Parametar ima opseg od 8 cm. Dužina repa je pod uticajem težine prasadi ($p \leq 0.001$), broj legla krmače ($p \leq 0.001$) i objekta ($p \leq 0.001$). Razlike u dužini repa se mogu naći između, kao i unutar, legla.

Postoje korelacije između prisustva abnormalnosti i dužine repa praseta. Sa višim nivoom abnormalnosti, srednja vrednost dužina repa se značajno smanjuje.

Ključne reči: rep, dužina, prasad, varijabilnost, abnormalnost

References

BLE (2016): Presseinformation. Aus der Forschung in den Stall – Tierschutz-Symposium mit ersten Ergebnissen. http://www.ble.de/DE/08_Service/03_Pressemitteilungen/2016/160421_Tierschutz-Symposium.html. gesehen am: 29.04.2016.

BMEL (2015): Wege zu einer gesellschaftlich akzeptierten Nutztierhaltung. Gutachten. Wissenschaftlicher Beirat für Agrarpolitik beim Bundesministerium für Ernährung und Landwirtschaft. S. 337.

BEER, J. (1964): Erblich bedingte Missbildungen beim Schwein. Sonderdruck aus „Monatshefte für Veterinärmedizin". 19. Jahrgang 1964, Heft 15, S. 581; VEB Gustav Fischer Verlag, Jena.

HEMPLER, J. (2012): Schwänze kupieren – Können wir darauf verzichten?. Landwirtschaftskammer Niedersachsen. https://www.lwk-niedersachsen.de/download.cfm/file/426,830236e4-0508-b9d5-498ec015c6e12f15~pdf.html; gesehen am: 21.04.2015.

JAMES, P.J. (2006): Genetic alternatives to mulesing and tail docking in sheep: a review. Australien Journal of Experimental Agriculture, 2006, 46, S. 10 – 12.

KOMPETENZKREIS TIERWOHL (2015a): Zwischenbericht des Kompetenzkreises Tierwohl an das Bundesministerium für Ernährung und Landwirtschaft vom 22. Januar 2015.

KOMPETENZKREIS TIERWOHL (2015b): Empfehlungen des Kompetenzkreises Tierwohl an das Bundesministerium für Ernährung und Landwirtschaft zum Thema Schwanzbeißen bei Schweinen vom 23. September 2015.

POLLMANN, C. und MEYER, C. (2014): Wie läuft es in anderen Ländern? Ausstieg aus dem Schwanzkupieren bei Schweinen in Norwegen. ; aus Bauernblatt 05.07.2014, S.55; http://www.lksh.de/fileadmin/dokumente/Bauernblatt/PDF_Toepper_2014/BB_27_05.07/55-56_Pollmann_Meyer.pdf; gesehen am: 28.04.2015.

RICHTLINIE 2008/120/EG DES RATES (2008): RICHTLINIE 2008/120/EG DES RATES vom 18. Dezember 2008 über Mindestanforderungen für den Schutz von Schweinen.

SHELTON, M. (1977): Studies on tail lengths of Rambouillet and Mouflon sheep. The Journal of Heredity 68, S. 128 – 130, 1977.

STATISTISCHES BUNDESAMT (2013): Tiere und tierische Erzeugung – Landwirtschaftliche Betriebe mit Haltung von Schweinen zum 1. März 2013 nach Bestandsgrößenklassen und Bundesländern; https://www.destatis.de/DE/ZahlenFakten/Wirtschaftsbereiche/LandForstwirtschaftFischerei/TiereundtierischeErzeugung/Tabellen/StrukturSchweineBetriebe.html;jse ssionid=43F3384A84B56AA657DD9D10B7734838.cae3; gesehen am: 22.04.2015.

STATISTISCHES BUNDESAMT (2015): Tiere und tierische Erzeugung; https://www.destatis.de/DE/ZahlenFakten/Wirtschaftsbereiche/LandForstwirtschaftFischerei/TiereundtierischeErzeugung/Tabellen/BetriebeSchweineBestand.html; Betriebe mit Schweinen und Schweinebestand für Mai 2015 und November 2015. gesehen am: 23.05.2016.

TIERSCHG (2006): Tierschutzgesetz vom 18. Mai 2006, geändert durch Artikel 8 Absatz 13 des Gesetzes vom 3. Dezember.

WALKER, P.K. und BILKEI, G. (2006): Tail-Biting in outdoor pig production. The Veterinary Journal 171, S.367-369

WILDRAUT, C. und MERGENTHALER, M. (2016): Bewertung und Einordnung der Tierhaltung in Deutschland aus Sicht von Landwirten. Deutsches Hochschulforum. 21. April 2016 in Osnabrück.

WÖRLE, H. (2010): Schwanzkupieren bleibt 'Mittel der Wahl'. agrarheute.com vom Deutschen Landwirtschaftsverlag GmbH; http://www.agrarheute.com/schwanzkupieren?suchbegriff2=Kupieren%20Schw%E4nze; gesehen am: 21.04.2015.

WÖRLE, H. (2014): Niedersachsen: Ringelschwanz-Prämie kommt. agrarheute.com vom Deutscher Landwirtschaftsverlag GmbH; http://www.agrarheute.com/ringelschwanz-praemie; gesehen am: 21.04.2015.

QUALITY OF MEAT FROM NATIVE PIGS

Władysław Migdał[1], Čedomir Radović [2], Vladimir Živković[2], Emilia Gwiazda[1], Łukasz Migdał[3], Anna Migdał[4], Maria Walczycka[1], Ewelina Węsierska[1], Marzena Zając[1], Joanna Tkaczewska[1], Piotr Kulawik[1], Katarzyna Krępa-Stefanik[1]

[1]Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland
[2]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[3]Department of Genetics and Animal Breeding, [4]Institute of Veterinary Sciences, Faculty of Animal Sciences, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland
Corresponding author: w.migdal@ur.krakow.pl
Original scientific paper

Abstract: Production of traditional meat products, characterized by very good organoleptic properties demands specific raw material which is meat from pigs with slaughter weight 120 kg, intramuscular fat content higher then 3% and bred by extensive system. Some of breeds which can fulfil this requirements are native breeds like Mangalitza, Moravka, Złotnicka Spotted, Złotnicka White and Pulawska pigs. Aim of this study was analysis of raw meat from Mangalitza, Moravka, Złotnicka Spotted, Złotnicka White and Pulawska pigs – quality of meat and its technological value~~s~~ were evaluated. Meat from analysed pigs had proper chemical composition, favourable n3/n6 fatty acids profile, low drip loss, proper pH. The composition of loin of: Puławska, Złotnicka, Mangalica and Moravka races was similar in protein, ash and carbohydrates contents. The content of intramusular fat in loins of examined races ranged from 3.0% (Złotnicka White) to 5,1% (Moravka). In this study there was confirmed good meat quality and its usefulness for production of traditional products.

Key words: pigs, Złotnicka Spotted, Złotnicka White, Puławska, Mangalitza, Moravka, meat, quality

Introduction

The market of meat products is differentiated, beside the high yield efficiency goods there are also produced the low yield usually considered as luxury articles. The last group also includes raw, ripening products, characterised by specific, very good organoleptic properties, namely Parma hams, Iberico hams or Polish raw smoked meat products, e.g. Lublin loin or Podlaski kumpiak. The production of raw-ripening meat products requires a specific raw material - meat of heavy pigs with slaughter body weight of over 120 kg, with intramuscular fat content exceeding 3%, and which should be fed traditionally using extensive methods. Breeds which meat has long been used to manufacture such products include: Iberico, Casertana, Alentejana and Mangalica *(Pugliese and Sirtori, 2012)*. Polish national husbandry is still posses swine breeds which were used in the past and can still be used for production of high quality raw ripening products, although populations of these breeds are small *(Szulc et al., 2011)*. These pigs include the following native breeds: Złotnicka Spotted, Złotnicka White and Puławska *(Babicz et al., 2009; Buczyński et al., 2005)*. In Republic of Serbia there are three native pig breeds: Mangalitza, Moravka and Resavka. Mangalitsa is typical fat breed which is farmed mainly in Hungary and also in Serbia and Croatia. There is 65-70% of fat in carcass halves and approx. 30-35% of meat *(Egerszegi et al., 2003)*. Results of recent studies *(Egerszegi et al., 2003)* show this amount of meat is sufficient for production of high quality ham. Moravka is breed of combined production with more meat in carcass halves and significantly less fat *(Petrović et al., 2010).*

The Złotnicka Spotted, Złotnicka White and Puławska breeds has not been improved by crossing with other breeds. Due to its small size, the conservation breeding aims to maintain biodiversity and is not focused on selection towards increasing production. That is why Złotnicka Spotted, Złotnicka White and Puławska pigs maintained good meat quality suitable for the manufacture of traditional products. This was confirmed by experiments carried out by *Buczyński et al., (1997)*; *Kapelański et al., (2006); Grześkowiak et al., (2009)* and *Szulc et al., (2012)* which revealed that meat of the Złotnicka Spotted breed was characterised by lack of quality changes, small free drip loss and proper pH. Meat used for manufacturing raw and raw-ripening products must be characterised by pH 5.6-5.8, approximately 3.5% intramuscular fat as well as good profile of muscle fibres. It is evident from experiments carried out so far that the size and quantity of individual muscle types exert a decisive impact on muscle “functional character” and, hence, on its quality as well as technological and culinary value *(Cameron et al., 1998)*.
Aim of this study was analysis of raw meat products from Mangalitza, Moravka, Złotnicka Spotted, Złotnicka White and Puławska pigs.

Materials and Methods

Animals

The performed investigations used 30 carcasses of fatteners from the following five genetic groups (purebreed fatteners):

- Złotnicka Spotted (ZS) - (n= 6),
- Złotnicka White (ZW) - (n=6),

- Puławska (Pul) (n=6),
- Mangalitza (Ma) (n=6)
- Moravka (Mo) (n=6).

Animals were divided into five experimental groups keeping the sex ratio at 1: 1. Złotnicka Spotted (ZS), Złotnicka White (ZW) and Pulawska (Pul) pigs were bred in Poland and Mangalitza (Ma) and Moravka (Mo) were bred in Serbia. All the analyses on pigs' meat were performed in Poland.

Experimental animals with the average weight of 20 kg were selected and the experiment was terminated when the animals attained the slaughter weight of about 120 kg (113.0 – 123.6 kg). They were fed *ad libitum* with complete mixed rations and had constant access to water. At the final of fattening, the animals were slaughtered. The half-carcasses were cooled down using the mono-gradual system at the temperature of about 4^{o} C. After 24 h of meat ageing, samples of *m. longissimus dorsi* (LD) were collected from half-carcasses.

Meat quality

24 hours (pH_{24}) after the slaughter, pH was measured in the *longissimus dorsi* (LD) muscle at the last rib by means of an integrated electrode pH meter Radiometr PHM 80 Portable. Samples from the lumbar section of the LD muscle were collected for laboratory investigations. The following items were estimated in the raw meat samples:

- water content according to the standard *PN-ISO 1442:2000*,
- fat content according to the standard *PN-ISO 1444:2000*,
- protein content by Kjeldahl method *(PN-75/A-04018)* with TECATOR apparatus
- total ash content according to the standard *PN-ISO 936:2000*,
- total carbohydrates content was calculated assuming that the all total solids and water stand for 100%
- fatty acid profile – was obtained by gas chromatography method. The fat samples were extracted with chloroform-methanol (2:1, v/v) according to the method of *Folch et al. (1957)*. Then 1 g of meat samples was mixed with 15 mL chloroform–methanol mixture and homogenized for 10 min at 5000 rpm, and after 5 min pause – 5 min at 1000 rpm using homogeniser MPW-120. The mixture was then filtered through filter paper tothe regular cylinder and completed with extraction mixture up to 15 mL. Next, 3 mL of 0.74% KCl solution was added to 15 mL of filtrate. The alcohol–water phase was removed, and the chloroform phase was washed 3 times using 2 mL solution of chloroform:methanol: 0.74% KCl (3:48:47, v/v/v). Subsequently the chloroform phase was recovered, dehydrated with anhydrous sodium sulphate (Na2SO4) and dried using nitrogen at 45^{o}C. To

the sample (about 10 mg) were added 0.5 ml 0.5 N KOH in methanol and heated at 85ºC. Next 1 ml 12% BF3 in methanol were added and the sample was again heated at 85ºC. After cooling in room temperature 1 ml hexane and 5 ml saturated solution of NaCl were added. Fatty acid methyl esters profile in one µl samples at the split ratio of 10:1 were separated by gas chromatography on a TRACE GC ULTRA gas chromatograph, equipped with 30 m capillary column SUPELCOWAX 10 of 0.25mm inner diameter and coating thickness of 0.25 µm (30 m × 0.25 mm × 0.25 um). Operating conditions were as follows: helium was used as a carrier gas, flow 1 ml/min, split flow 10 ml/min, injector temperature 220ºC, detector temperature 250ºC, initial column temperature 160ºC.

- meat weight cooking loss. Samples were heated to reach the internal temperature of 75ºC in the geometric centre of the sample. The results were computed from the difference between the weight before and after cooking *(Baryłko–Pikielna, 1975),*
- the measurements of colour of meat samples were obtained in CIELab system. Lightness [L*], redness [a*] and yellowness [b*] of meat were determined using a Konica Minolta CM – 600d spectrophotometer. Values of [a*] and [b*] were used to calculate the saturation value - chroma [C*].

Statistical analysis

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

Results and Discussion

In table 1 is presented the basic chemical composition of examined loins of different pigs' breeds, whereasin table 2 the fatty acid profile of intramuscular fat of analysed pigs.

Table 1. The chemical composition of loin of analysed pigs' breeds

Chemical componet [%]	Breed of fatteners					SEM
	Złotnicka Spotted (ZS)	Złotnicka White (ZW)	Puławska (Pul)	Mangalitza (Ma)	Moravka (Mo)	
Water	72.7	73.7	73.6	73.3	72.9	0.36
Total solids	27.3	26.3	26.4	26.7	27.1	0.36
Protein	22,1[a]	21.7[a]	21.4[ab]	20.7[b]	20.2[b]	0.68
Fat	3,4[ab]	3.0[a]	3.2[a]	4.0[b]	5.1[c]	0.95
Ash	1.2	1.1	1.2	1.3	1.2	0.08
Carbohydrates	0.6	0.5	0.6	0.7	0.6	0.08

a,b,c – Mean values in the same columns designated by the different letters differ significantly at $P \leq 0.05$
SEM- standard deviation

The loins of sustainable breeds were characterised by a proper chemical composition. The level of total solids ranged from 26.3% (Złotnicka White breed) to 27.3% (Złotnicka Spotted), and the level of protein from 20.2% (Moravka) to 22,1%(Złotnicka Spotted), respectively. The contents of protein in longissimus dorsi of Pulawska and Zlotnicka White were lower that those obtained by *Babicz et al., (2013 and 2013a)* and *Grześkowiak et al., (2009)* in the meat of two, above mentioned breeds. The contents of ash and carbohydrates in analysed pigs' muscles were similar and did not differed statistically, whereas the meats statistically significantly differed in fat content.The fat level in Zlotnicka white pig meat was 3,0%, in loin of Pulawska pig 3,2% whereas in loin from Mangalitza 4,0% and as much as 5,1% in loin of Moravka pig. The levels of intramuscular fat content of assessed loins of different pig breeds can be recognised as optimal. According to *Wood et al., (1999), Daszkiewicz et al., (2005)* oraz *Tyra & Mitka (2015)* to obtain the optimal taste, juiciness and tenderness of meat the amount of fat should be at least 2,5-3,0% Intramuscular fat (IMF) is an important marker of meat quality, because higher contents of intramuscular fat exert a positive influence on sensory characteristics, technological and culinary usefulness of meat *(Wood et al., 1999; Buczyński et al., 2005; Świtoński et al., 2010). Schwörer et al., (2000)* reported that the intramuscular fat content below 1% influences on lowering of meat flavour. After the thermal treatment of such (above) meat it becomes dry and fibrous. The smaller amounts of intramuscular fat (1.19 ÷ 2.20 %) and at the same time lower caloric value of *l.dorsi* of Pulawska pig was described by *Piórkowska et al. (2010) and Kasprzyk et al. (2013).* Meat of Mangalica and Moravka pigs contained higher amounts of fat from 4.0 to 5.1%, respectively. Similar results for above two breeds were obtained by *Petrović i in. (2010).* Their research revealed that tere was less total fat and cholesterol contents in musculus longissimus dorsi of Moravka than in Mangalitsa.

It is generally accepted that traditional, local breeds produce a higher IMF content *(Serra et al., 1998; Rosenvold and Andersen 2003; Florowski et al., 2006; Park et al., 2007; Pugliese and Sirtori, 2012).* As *Serrano et al. (2008)* indicated, muscles of the Spanish breed Iberico are characterised by a particularly high content of fat (8.8%). Intramuscular fat considerable ranging from 3.32 to 4.27% in the *m. longissimus dorsi* was reported for the native Italian breed of Nero Siciliano *(Pugliese et al., 2004).* On the other hand, *Čandek-Potokar et al. (2003)* determined the content of IMF in the Slovenian breed of Krškopolje at 3%. The fat content above 2.5% can influence on lower consumers' score because of meat high marbling *(Czarniecka-Skubina et al., 2007).*

The fat content in loin was assessed as high but desirable because of meat flavor and consumers' demands concerning traditional products obtained from that kind of meat in Hungary, Serbia, Croatia and Romania. It is considered that high intramuscular greasing is essential to obtain optimum of flavor, juiciness and tenderness of meat and is decisive for food acceptability *(Florowski et al., 2005).* The desirability level of such product is formed by the shares of basic chemical components present in the IMF fat. The levels of IMF fat are different and influenced by many factors i.e. species, breed, age, muscle kind and muscle's physical activity. The problem of too low levels of IMF concerns mainly pork obtained from breeds and lines of high productivity *(Tyra & Mitka, 2015).*

The content of IMF of Polish bred pig's breeds (Polish Landrace and Polish Large White) is much lower than 2% *(Tyra i Żak, 2010).* As far as the primitive pigs' breeds (for this group belong pigs analysed in this experiment) the problem with IMF fat level is not observed *(Tyra & Mitka, 2015).* Beside the level of IMF the composition of the individual fatty acids –the fatty acids profile – is important. The fatty acids profile depends mainly on feeding mixtures and way of feeding. In table 2 there is presented the fatty acids profile of IMF of loins for analysed native breeds. The differences in IMF's fatty acids profiles were caused by different ways of breeding. Polish fatteners were bred with full portion mixtures with barley, triticale, corn middlings, post-extraction soya and post-extraction rapeseed meals, whereas the fatteners of Mangalitsa and Moravka were fed with corn silage, wheat, soya and corn middlings. It should be pointed out the high levels of C16:0 and C18:0 in IMF of Mangalitsa and Moravka. Also in IMF of above breeds there was observed wide, adverse from nutritional point of view, ratio of PUFA n6/n3. Therefore, the *n*-6/*n*-3 ratio was higher than dietary recommendations in all cases *(British Nutrition Foundation, 1994). Ruiz et al. (1998)* and *Andrés et al. (2001)* concluded that free-reared pigs fed on pasture and acorns showed higher levels of MUFA than those fed on concentrates. *Parunović et al. (2012b)* found that free-range Mangalitsa pigs showed a higher PUFA content in the *musculus longissimus* than pigs reared indoors and fed conventionally. The varying fatty acid compositions of adipose tissue and muscle have profound effects on meat quality *(Wood et al., 2008; Parunović et al.2013).*

Table 2. The fatty acids profile of *m. longissimus dorsi* (LD) of analysed pigs' breeds

Fatty acid	Breed of fatteners					SEM
	Złotnicka Spotted (ZS)	Złotnicka White (ZW)	Pulawska (Pul)	Mangalitza (Ma)	Moravka (Mo)	
C-10:0	0.07	0.10	0.07	0.12	0.12	0.02
C-12:0	0.06	0.07	0.08	0.09	0.09	0.01
C-14:0	0.99	1.34	1.06	1.43	1.57	0.25
C-14:1	0,02	0.03	0.02	0.03	0.04	0.008
C-15:0	0.05	0.02	0.04	0.03	0.03	0.01
C-16:0	24.28[ab]	27.51[a]	20.51[b]	27.78[a]	28.48[a]	3.33
C-16:1*n9*	0.39	0.27	0.23	0.26	0.29	0.061
C-16:1*n7*	3.80	4.42	3.41	3.81	4.68	0.51
C-17:0	0.13[a]	0.07[b]	0.14[a]	0.12[a]	0.15[a]	0.031
C-17:1	0.16	0.11	0.19	0.15	0.21	0.038
C-18:0	10.70	11.61	10.60	12.24	11.55	0.68
C-18:1 *n-9*	40.86[a]	45.34[b]	46.89[b]	41.18[a]	41.66[a]	2.74
C-18:1*n-7*	5.56	4.58	4.79	4.53	4.95	0.41
C-18:2 *n-6*	8.66[a]	2.94[b]	7.79[a]	5.91[a]	4.14[ab]	2.40
C-18:3*n-6*	0.06	0.03	0.08	0.04	0.03	0.02
C-18:3*n-3*	0.32[a]	0.17[b]	0.43[a]	0.22[b]	0.12[b]	0.12
CLA	0.05	0.06	0.09	0.07	0.06	0.015
C-20:0	0.14	0.17	0.14	0.15	0.18	0.018
C-20:1	0.50	0.43	0,73	0.57	0.66	0.12
C-20:2	0.13[a]	0.06[a]	0.30[b]	0.17[a]	0.12[a]	0.09
C-20:3 *n-6*	0.23[a]	0.06[b]	0.21[a]	0.09[b]	0.08[b]	0.08
C-20:4*n-6*	1.97[a]	0.38[b]	1.24[a]	0.69[b]	0.59[b]	0.64
C-20:5 *n-3*	0.09	0.03	0.05	0.03	0.02	0.028
C-22:4 *n-6*	0.21[a]	0.06[b]	0.26[a]	0.12[ab]	0.07[b]	0.087
C-22:5 *n-3*	0.29[a]	0.06[b]	0.26[a]	0.10[b]	0.07[b]	0.11
C-22:6 *n-3*	0.09[a]	0.01[b]	0.01[b]	0.04[b]	0.03[b]	0.03
PUFA *n-3*	0.79[a]	0.27[b]	0.75[a]	0.39[b]	0.24[b]	0.26
PUFA *n-6*	10.92[a]	3.47[b]	9.58[a]	6.85[ab]	4.91[b]	3.11
PUFA *n6/n3*	13.82[a]	12.85[a]	12.77[a]	17.56[ab]	20.46[b]	3.39

a,b,c – Mean values in the same columns designated by the different letters differ significantly at: a, b - P≤0.05
SEM- standard deviation

In table 3 there are presented meat pH, cooking loss and CIELab colour parameters.

Table 3. The quality characteristics of loin of analysed fatteners breeds

Meat quality parameters	Breed of fatteners					SEM
	Złotnicka Spotted (ZS)	Złotnicka White (ZW)	Pulawska (Pul)	Mangalitza (Ma)	Moravka (Mo)	
pH_{24}	5.80	5.72	5.54	5.55	5,64	0.32
cooking loss %	29,32[a]	22.76[b]	26.95[ab]	25.76[ab]	24.68[ab]	2,82
colour parameters						
L*	46.43[a]	49.54[ab]	55,45[a]	51,35[ab]	52.94[ab]	4.32
a*	8.20[a]	14.23[b]	13.82[b]	13,27[b]	2.74[c]	0.84
b*	2.95[a]	3.34[a]	6.32[b]	7.12[b]	10.15[c]	1.74

a,b,c – Mean values in the same columns designated by the different letters differ significantly at: a, b, c - P≤0.05
SEM- standard deviation

The pH of pork loin of Złotnicka Spotted was higher than pH of other analysed breeds but the difference was not statistically significant. Also the loin meat of Złotnicka Spotted was characterised by the highest cooking losses.

The meat of pigs of the Złotnicka Spotted breed was darker (statistically non-significantly lower value of the lightness parameter L*) in comparison with the Złotnicka White, Pulawska Mangalitza and Moravka pigs. Variations in the fibre type composition may affect meat colour. The right colour of meat can be conditioned by the ferrous oxymioglobin (oxyMb) - *Philips et al. (2001)*, which is directly connected with the percentage and size of muscle fibre types *(Warriss et al., 1990).* Colour parameters (L*, and a* values) of the *m. longissimus dorsi* of ZS pigs corresponded to the red meat parameters of other native pig breeds *(Serra et al., 1998; Fortina et al., 2005).* The colour of meat and the fat content may also be a significant determinant in the evaluation of pork quality *(Faustman and Cassens, 1990).* The meats of Mangalitsa and Moravka had statistically higher value of b* parameter. that was caused by a high share of corn in feeding mixture of those pigs' breeds.

Both native autochthonous breeds, beside gene preservation and expanding of the population (especially Moravka and Resavka), are very suitable for outdoor rearing, for organic livestock production and manufacturing of traditional pork meat products *(Petrović et al., 2010)*. The same can be stated for Polish native breeds - Złotnicka White, Złotnicka Spotted, Pulawska

Because the meat of those Polish breeds is used for manufacturing of traditional products, demanded by the consumer, the breeds need to be preserved and protected. The practical protection of native breeds depends on their market position, i.e. the quality of after slaughter raw material, the quality of traditional and regional products possible to obtain and on proper promotion and marketing.

To integrate the breeding of Pulawska pig with home livestock production Policy the Regional Union of Breeders and Users of Pulawska pig breed - „PUŁAWIAK", was established at 14th July 2005. Whereas the culmination of marketing activities was, at 27th of May 2009, placement of „Pulawska breed pig" on the governmental list leaded by minister of The Ministry of Agriculture and Rural Development *(Szulc & Skrzypczak, 2015).*

The meat of Pulawska breed pigs was appreciated by homeland consumers and at foreign markets – the lots of heavy fatteners of that breed were bought by Spanish swine producers for long ripened hams production. In 2010 there was established cooperation with the Auchan markets network which placed as delicatessen product the meat of Pulawska breed. Also meat and the products obtained of Zlotnicka pigs were placed on home governmental Traditional Products List. The products are among others: Great Poland Voivodship pig meat of Złotnicka breed, Nowy Tomyśl sausage, roasted haunch of Zlotnicka White pig and White sausage in the glass pot. The staff of Department of Breeding and Production of Swine of Poznan University of Life Sciences in cooperation with Great Poland Voivodship regional government are the organizers of "Złotnicka Premium" competitions which aim to enhance meat sector firms and producers to produce high quality meat products. Also there is a campaign "Regional swine meat – appreciate taste of tradition" to enlarge the knowledge concerning the native traditional pig breeds among restaurants' owners and also enlargement of knowledge concerning valuable sensory characteristics and possibilities of usage of culinary meat of Puławska, Złotnicka White and Spotted breeds. Similar breeding and marketing policies in Hungary, Croatia, Romania and Serbia caused that Mangalitsa pigs and the meat products produced of that species meat have become the most recognizable trademark. The specific example is the Hungarian market which offers the wide game of products made of Mangalitsa.

Conclusion

Meat from analysed pigs had proper chemical composition, favourable n3/n6 fatty acids profile, low drip loss, proper pH. This study confirmed good meat quality and its usefulness for production of traditional products.

Kvalitet mesa autohtonih rasa svinja

Władysław Migdał, Čedomir Radović, Vladimir Živković, Emilia Gwiazda, Łukasz Migdał, Anna Migdał, Maria Walczycka, Ewelina Węsierska, Marzena Zając, Joanna Tkaczewska, Piotr Kulawik, Katarzyna Krępa-Stefanik

Rezime

Proizvodnja tradicionalnih proizvoda od mesa, koje karakterišu vrlo dobre organoleptičke osobine, zahteva specifičnu sirovinu tj. meso od svinja težine na klanju od 120 kg, intramuskularnim sadržajem masti većim od 3% i uzgajanih u ekstenzivnom sistemu. Neke od rasa koje mogu ispuniti ove zahteve su domaće rase kao što su mangulica, moravka, zlotnička šarena, zlotnička bela i pulavska svinja. Cilj ove studije bila je analiza sirovog mesa svinja rase mangulica, moravka, zlotnička šarena, zlotnička bela i pulavska - kvalitet mesa i njegove tehnološke vrednosti. Meso od analiziranih svinja imalo je adekvatan hemijski sastav, povoljan profil n3/n6 masnih kiselina, nizak kalo, odgovarajući pH. Sastav slabine puławske svinje, złotničke svinje, mangulice i moravke je sličan u sadržaju proteina, pepela i ugljenih hidrata. Sadržaj intramuslularne masti u slabinama ispitanih rasa varira od 3,0% (zlotnička bela) do 5,1% (moravka). U ovoj studiji potvrdjen je dobar kvalitet mesa i njegova korisnost za proizvodnju tradicionalnih proizvoda.

Ključne reči: svinje, zlotnička šarena, zlotnička bela, puławska, mangulica, moravka, meso, kvalitet

Acknowledgment

Project *“The uses and the conservation of farm animal genetic resources under sustainable development”* co-financed by the National Centre for Research and Development within the framework of the strategic R&D programme “Environment, agriculture and forestry” – BIOSTRATEG, contract number: BIOSTRATEG2/297267/14/NCBR/2016.
Authors would also like to acknowledge the core financing by the project TR 31081 of the Ministry of Education, Science and Technological Development, Republic of Serbia.

References

ANDRÉS. A.I., CAVA. R., MAYORAL. A.I., TEJEDA. J.F., MORCUENDE. D., RUIZ. J. (2001): Oxidative stability and fatty acid composition of pig muscles as affected by rearing system, crossbreeding and metabolic type of muscle fiber. Meat Sci. 59, 39-47.

BABICZ. M., KAMYK. P., STASZIAK. A., PASTWA. M. (2009): Opportunities to use Puławska pigs for heavy fattener production. Ann. Anim. Sci. 9:259-268.
BABICZ. M., KROPIWIEC. K., KASPRZAK. K., SKRZYPCZAK. E., HAŁABIS. M. (2013): Analysis of the quality pork obtained from carcasses fatteners of Polish Landrace and Pulawska breed. Ann. UMC-S, Lublin-Polonia, sec. EE, XXXI (4): 1–7.
BARYŁKO-PIKIELNA. N. (1975): Zarys analizy sensorycznej żywności. WNT, Warszawa.
BRITISH NUTRITION FOUNDATION. (1994): Unsaturated fatty acids. Nutritional and physiological significance. Report of the British Nutrition TASk Force. London: British Nutrition Foundation.
BUCZYŃSKI. J., SZULC. K., LUCIŃSKI. P., SZYNDLER-NĘDZA. M. (2009): Program ochrony zasobów genetycznych świń rasy złotnickiej pstrej. Instytut Zootechniki PIB Balice.
BUCZYŃSKI. J., SWULIŃSKA–KATULSKA. A., CHOJNACKA. R., SZULC. K. (2005): Assessment of heating quality of meat from Zlotnicka White and Zlotnicka Spotted pigs. Ann. Anim. Sci. Suppl. 1:7-11.
BUCZYŃSKI. J., ZABOROWSKI. T., SZULC. K. (1997): Fattening and slaughter performance of meat - type crossbred porkers with share of złotnicka spotted pig. Anim. Sci. Pap. Rep. 15:149–154.
CAMERON. N.D., OKSBJERG. N., HENCKEL. P., NUTE. G., BROWN. S., WOOD. J.D. (1998). Relationships between muscle fibre traits with meat and eating quality in pigs. BSAS Annual Meeting, 123.
ČANDEK-POTOKAR. M., ŽLENDER. B., KRAMAR. Z., ŠEGULA. B., FAZARINC. G., URŠIČ. M. (2003): Evaluation of Slovene local pig breed Krškopolje for carcass and meat quality. Czech J. Anim. Sci. 48:120-128.
CZARNIECKA-SKUBINA. E., PRZYBYLSKI. W., JAWORSKA. D., WACHOWICZ. I., URBAŃSKA. I., NIEMYJSKI. S. (2007): Charakterystyka jakości mięsa wieprzowego o zróżnicowanej zawartości tłuszczu śródmięśniowego. Żywność. Nauka. Technologia. Jakość, 6, 55, 285-294.
DASZKIEWICZ. T., BĄK. T., DENABURSKI. J. (2005): Quality of pork with a different intramuscular fat (IMF) content. Pol. J. Food Nutr. Sci., 14/55:31-36.
EGERSZEGI I., RÁTKY J., SOLTI L., BRÜSSOW K-P. (2003): Mangalica – an indigenous swine breed from Hungary (Review). Arch. Tierz., Dummerstorf, 46, 3, 245-256.
FAUSTMAN. C., CASSENS. R. G. (1990) : The biochemical basis for discolouration in fresh meat: a review. J. Muscle Foods. 1:21l-243.
FLOROWSKI. T., PISULA. A., ADAMCZAK. L., BUCZYŃSKI. J.T., ORZECHOWSKA. B. (2006): Technological parametres of meat of two Polish local breeds – Zlotnicka Spotted and Pulawska. Anim. Sci. Pap. Rep. 24:217-224.

FLOROWSKI. T., PISULA. A., FLOROWSKI. T., PISULA. A., KURELA. W., BUCZYŃSKI. J.T. (2005): Ocena przydatności przetwórczej mięsa świń rodzimej rasy złotnickiej pstrej. Mięso i Wędliny, 6:38-40 (In Polish, summary in English).
FOLCH. J., LEES. M., STANLEY. S.G.H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497-509.
FORTINA. R., BARBERA. R., LUSSIANA. C., MIMOSI. A., TASSONE. S., ROSSI. A. (2005): Performances and meat quality of two Italian pig breeds fed diets for commercial hybrids. Meat Sci. 71:713–718.
GRZEŚKOWIAK. E., BORYS. A., BORZUTA. K., BUCZYŃSKI. J.T., LISIAK. D. (2009): Slaughter value, meat quality and backfat fatty acid profile in Zlotnicka Spotted fatteners. Anim. Sci. Pap. Rep. 27:115-125.
KAPELAŃSKI. W., BUCZYŃSKI. J.T., BOCIAN. M. (2006): Slaughter value and meat quality in the Polish native złotnicka spotted pig. Anim. Sci. Pap. Rep. Suppl. 24:7-13.
KASPRZYK. A., BABICZ. M., KAMYK-KAMIEŃSKI. P., LECHOWSKI. (2013): Slaughter value and meat quality of Pulawska and Polish Landrace breeds fatteners. Annales Universitatis Mariae Curie-Skłodowska Lublin – Polonia, XXXI, 3, Sectio EE, 1-9.
PARK. B.Y., KIM. N.K., LEE. C.S., HWANG. I.H. (2007): Effect of fibre type on postmortem proteolysis in longissimus muscle of Landrace and Korean native black pigs. Meat Sci. 77:482-491.
PARUNOVIĆ. N., PETROVIĆ. M., MATEKALO-SVERAK. V., RADOJKOVIĆ. D., VRANIĆ. D., RADOVIĆ. Č. (2012a): Cholesterol and total fatty acid content in *m. longissimus dorsi* of Mangalitsa and Swedish Landrace. Acta Alimentaria 41 (2), 161-171.
PARUNOVIĆ. N., PETROVIĆ. M., MATEKALO-SVERAK. V., TRBOVIĆ. D., MIJATOVIĆ. M., RADOVIĆ. Č. (2012b): Fatty acid profile and cholesterol content of *m. longissimus* of free-range and conventionally reared Mangalitsa pigs. S. Afr. J. Anim. Sci. 42, 101-113.
PARUNOVIĆ. N., PETROVIĆ. M., MATEKALO-SVERAK. V., RADOVIĆ. Č., STANISIC. N. Carcass properties, chemical content and fatty acid composition of the *musculus longissimus* of different pig genotypes. S. Afr J Anim Sci 2013;43:123-36.
PETROVIĆ. M., MIJATOVIĆ. M., RADOJKOVIĆ. D., RADOVIĆ. Č., MARINKOV. G., STOJANOVIĆ. L.J. (2007): Genetic resources in pig breeding – Moravka. Biotechnology in Animal Husbandry, 23, 1-2, 1-12.
PETROVIĆ. M., MIJATOVIĆ. M., RADOVIĆ. Č., RADOJKOVIĆ. D., JOSIPOVIĆ. S. (2007): Genetic resources in pig breeding – carcass quality traits of

breeds Moravka and Mangalitsa. Biotechnology in Animal Husbandry, 23, 5-6, 421-428.

PETROVIĆ. M., MIJATOVIĆ. M., RADOVIĆ. Č., RADOJKOVIĆ. D., PARUNOVIĆ. N., STANIŠIĆ. N. (2009): Genetic resources in pig breeding – carcass and meat quality traits of Moravka and Mangalitsa breeds. 1st Conference of the Balkan Network for the Animal Reproduction Biotechnology, Sofia, 14.

PETROVIĆ. M., RADOVIĆ. Č., PARUNOVIĆ. N., MIJATOVIĆ. M., RADOJKOVIĆ. D., ALEKSIĆ. S., STANIŠIĆ. N., POPOVAC. M. (2010): Quality traits of carcass sides and meat of Moravka and Mangalitsa pig breeds. Biotechnol. Anim. Husb. 26 (1-2), 21-27.

PETROVIĆ. M., MIJATOVIĆ. M., RADOVIĆ. Č., RADOJKOVIĆ. D., PARUNOVIĆ. N., STANIŠIĆ. N. (2009): Genetic resources in pig breeding – carcass and meat quality traits of Moravka and Mangalitsa breeds, Proceedings of the 1st Conference of the Balkan Network for the Animal Reproduction Biotechnology, 18–19 February 2009, Sofia, Bulgaria, p. 14.

PETROVIĆ. M., RADOVIĆ. Č., PARUNOVIĆ. N., RADOJKOVIĆ. D., SAVIĆ. R. (2012): Composition of carcass sides and quality of meat from swallow-belly Mangalitsa reared in two systems. Biotech. Anim. Husbandry.28, 301-11.

PETROVIĆ. M., MIJATOVIĆ. M., RADOJKOVIĆ. D., RADOVIĆ. Č., MARINKOV. G., STOJANOVIĆ. LJ. (2007): Genetic resources in pig breeding – Moravka. Biotechnology in Animal Husbandry, 23, 1-2, 1-12.

PETROVIĆ. M., MIJATOVIĆ. M., RADOVIĆ. Č., RADOJKOVIĆ. D., JOSIPOVIĆ. S. (2007): Genetic resources in pig breeding – carcass quality traits of breeds Moravka and Mangalitsa. Biotechnology in Animal Husbandry, 23, 5-6, 421-428.

PETROVIĆ. M., MIJATOVIĆ. M., RADOVIĆ. Č., RADOJKOVIĆ. D., PARUNOVIĆ. N., STANIŠIĆ. N. (2009): Genetic resources in pig breeding – carcass and meat quality traits of Moravka and Mangalitsa breeds. 1st Conference of the Balkan Network for the Animal Reproduction Biotechnology, Sofia, 14.

PETROVIĆ. M., WAHNER. M., RADOVIĆ. C., RADOJKOVIĆ., PARUNOVIĆ. N., SAVIĆ. R., Brkic. N. (2014): Fatty acid profile of *m. longissimus dorsi* of Mangalitsa and Moravka pig breeds. *Arch Tierzucht.* 2014, 17, 1-12.

PHILIPS. A., FAUSTMAN. M., LYNCH., K., GOVONI. T., HOAGLAND. S., ZINN. S. (2001): Effect of dietary a-tocopherol supplementation on color and lipid stability in pork. Meat Sci. 58, 389-393.

PIÓRKOWSKA. K., TYRA. M., ROGÓŻ. M., ROPKA-MOLIK. K., OCZKOWICZ. M., RÓŻYCKI. M. (2010): Association of the melanocortin-4 receptor (MC4R) with feed intake, growth, fatness and carcass composition in pigs raised in Poland. Meat Sci. 85, 297-301.

POLISH STANDARD PN ISO 1442:2000- Mięso i przetwory mięsne. Oznaczanie zawartości wody. (In Polish).
POLISH STANDARD PN ISO 1444:2000- Mięso i przetwory mięsne. Oznaczanie zawartości tłuszczu wolnego. (In Polish).
POLISH STANDARD PN-75/A-04018 – Produkty rolno-spożywcze. Oznaczanie zawartości azotu metodą Kjeldahla i przeliczanie na białko. (In Polish).
POLISH STANDARD PN-ISO 936:2000. Mięso i przetwory mięsne. Oznaczanie popiołu całkowitego
PUGLIESE. C., CALAGAN. G., CHIOFALO. V., MORETII. V.M, MARGIOTTA. S., FRANCI. O., GANDINI. G. (2004): Comparison of the performance of Nero Siciliano pigs reared indoors and outdoors: 2. Joints composition, meat and fat traits. Meat Sci. 68, 523-528.
PUGLIESE. C., SIRTORI. F. (2012): Quality of meat and meat products produced from southern European pig breeds. Meat Sci. 90, 511–518.
RUIZ. J., CAVA. R., ANTEQUERA. T., MARTIN. L., VENTANAS. J., LÓPEZ-BOTE. C.J. (1998): Prediction of the feeding background of Iberian pigs using the fatty acid profile of subcutaneous, muscle and hepatic fat. Meat Sci. 49, 155-165.
SCHWŐRER. D., HOFER. A., LORENZ. D., REBSAMEN. A. (2000): Selection progress of intramuscular fat in Swiss pig production. Conference paper on Quality of meat and fat in pigs as affected by genetics and nutrition. EAAP Zurich, Switzerland, August 25, 1999, 69–72.
SERRA. X., GIL. F., PEREZ-ENCISO. M., OLIVER. M.A., VAZQUEZ. J.M., GISPERT. M., DIAZ. I., MORENO. F., LATORRE. R. NOGUERA. J.L. (1998): A comparison of carcass, meat quality and histochemical characteristics of Iberian (Guadyerbas line) and Landrace pigs. Livest. Prod. Sci. 56, 215-223.
SERRANO. M.P., VALENCIA. D.G., NIETO. M., LAZARO. R., MATEOS. G.G. (2008): Influence of sex and terminal sire line on performance and carcass and meat quality of Iberian pigs reared under intensive production systems. Meat Sci. 78, 420-428.
STATSOFT, Inc. (2010): STATISTICA (data analysis software system), version 9.1. www.statsoft.com
SZULC. K., BORZUTA. K., LISIAK. D., BUCZYŃSKI. J.T., STRZELECKI. J., GRZEŚKOWIAK. E., MAGDA. F., LISIAK. B. (2011): Influence of cross-breeding of native breed sows of Zlotnicka spotted with boars of Duroc and polish large white (PLW) breeds on the slaughter value fatteners. Afr. J. Biotechnol. 10, 72,16402-16405.
SZULC. K., LISIAK. D., GRZEŚKOWIAK. E., NOWACZEWSKI. S. (2012): The influence of cross-breeding Zlotnicka Spotted native breed sows with boars of Duroc (D) and Polish Large White (PLW) breeds on meat quality. Afr. J. Biotechnol. 11, 19, 4471-4477.

SZULC. K., SKRZYPCZAK. E. (2015): Jakość mięsa polskich rodzimych ras świń. Wiadomości Zootechniczne, R. LIII (2015), 1, 48–57

ŚWITOŃSKI. M., STACHOWIAK. M., CIEŚLAK. J., BARTZ. M., GRZEŚ. M. (2010): Genetics of fat tissue accumulation in pigs: a comparative approach. J. Appl. Genetics. 51, 153-168.

TYRA. M., MITKA. I. (2015): Rola tłuszczu śródmięśniowego (IMF) w kształtowaniu parametrów jakościowych (sensorycznych) mięsa. Wiadomości Zootechniczne, R. LIII (2015), 4, 50–56.

TYRA. M., ŻAK. G. (2010): Characteristics of the Polish nucleus population of pigs terms of intramuscular fat (IMF) content of *m. longissimus dorsi*. Ann. Anim. Sci. 10, 3, 241–248,

WARRISS. P.D., BROWN. S.N., ADAMS. S.J.M., LOWE. D.B. (1990): Variation in heam pigment concentration and colour in meat from British pigs. Meat Sci. 28, 321-329.

WOOD. J.D., ENSER. M., FISHER. A.V., NUTE. G.R., RICHARDSON. R.I., SHEARD. P.R. (1999): Manipulting meat quality and composition. Proc. Nutr. Soc. 58, 363-370.

WOOD. J.D., ENSER. M., FISHER. A.V., NUTE. G.R., SHEARD. P.R., RICHARDSON. R.I., HUGHHES. S.I., WHITTINGTON. F.N. (2008): Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78, 343-358.

SENSORY COMPARISON OF MEAT AND FAT FROM ENTIRE MALE, SURGICALLY CASTRATED AND FEMALE PIGS DEPENDING ON SEX OF CONSUMERS AND PORK PREFERENCE

Ivan Bahelka[1], Ondřej Bučko[2], Emília Hanusová[1], Martina Gondeková[1]

[1]National Agricultural and Food Centre - Research Institute for Animal Production, Lužianky, Slovakia
[2]Slovak Agricultural University, Nitra, Slovakia
Corresponding author: bahelka@vuzv.sk
Original scientific paper

Abstract: The aim of this study was to compare sensory characteristics of meat and fat from entire males, surgical castrates and female pigs using consumer test depending on sex and pork preferences. Samples of loin and backfat over the loin were removed 24 h after slaughtering. A totally, 102 consumers evaluated odour, flavour, tenderness and juiciness of meat as well as odour and flavour of fat using 5-point scale. Meat and fat from entire males were evaluated as less acceptable compared to that from castrates and gilts. Women were more critical especially in evaluation of fat from entire males. Consumers eating pork very often ranked boar meat and fat more negatively than those consuming pork occasionally.

Key words: pigs, entire males, sensory characteristics, consumer test, pork preference

Introduction

Boar taint is an unpleasant odour that influences pork quality and it is due to the two main compounds – steroid androstenone synthesized in the testis of maturing young boars, and skatole, the product of anaerobic degradation of the aminoacid tryptophan in the hind gut.

Human sensitivity to detect these two compounds of boar taint is very different. First of all, sensitivity to androstenone varies greatly between different world regions. British *(Kempster et al., 1986)* and Irish *(Cowan and Joseph, 1981)* consumers are less sensitive to boar taint than Dutch and Swedish *(Desmoulin et al., 1982).* Some consumers are not able to perceive androstenone at all even in high concentrations. In a world wide study *(Gilbert and Wysocki, 1987)*, consumers not able to detect androstenone ranged between 24.1 and 25.5 % for men and 15.8

and 17.2 % for women in continental Europe, Asia and Australasia, whereas in USA and UK it was 37.2 and 30.0 % for men and 29.5 and 20.9 % for women. In Germany, the percentage of insensitive people was 70 for men and 66 for women, in Spain 60 and 48, respectively *(Weiler et al., 2000)*. In Norway, 53.6 % of women and 73.7 % of men have been classified as insensitive to androstenone *(Lunde et al., 2009)*. The sensitivity to androstenone is also influenced by age. Older people are less sensitive than younger *(Matthews et al., 2000)*. In contrast to androstenone, skatole is detected by 99 % of consumers and regarded as unpleasant *(Weiler et al., 1997).*

The objective of this study was to sensorially characterize meat from entire males compared to that from surgical castrates and gilts and to find the effect of age and pork preference in slovak consumers.

Material and Methods

Forty-two pigs, entire males (EM), surgical castrates (SC) and gilts (G), each of 14, was randomly selected for the experiment. Pigs were crosses of Landrace sows and YxL boars. From seven litters was always selected 6 sibs (2 EM, 2 SC and 2 G). They were housed in test station at 22-26 kg live weight because of acclimation to new space and feed. Pigs were housed in pairs in pen according to gender. After reaching the average slaughter weight of 105 kg, pigs were slaughtered at the experimental slaughter house of the Research Institute for Animal Production (RIAP) situated approximately 200 m from the test stable. During the experiment, two pigs (1 EM and 1 SC) were excluded because of health reasons.

Pork muscles and fat samples from entire males, surgical castrates and female pigs were obtained 24 hours after slaughter. Samples – loins and backfat over the loin – were removed from the right carcass side over the last thoracic vertebra. Each sample was marked, individually packed in microtene bag and stored frozen (-20 °C) until consumer test. One part of each fat sample was analysed for androstenone and skatole concentrations in authorised private laboratory according to methods described in studies of *Ampuero Kragten et al. (2011)* and *Bekaert et al. (2012).*

Frozen meat and fat samples were thawed for two hours at room tempearture (23 °C). Fat samples were cut into 20 x 20 mm cubes and then heated at 80 °C in microwave oven for one minute. Meat samples were cut into 50 x 10 mm slides and heated on the contact grill at 180 °C for 4 minutes. After the heat treatment, meat and fat samples were served separately to the respondents.

A total of 102 consumers, both men and women, were used to evaluate meat and fat samples of entire males, castrates and females. One part of respondents were the employees of the RIAP Nitra and the second one was obtained using field workers. Each consumer evaluated four meat and four fat samples **(**1 from castrate, 1 from gilt and 2 from entire males). First, consumers evaluated odour, then flavour, and finally tenderness and juiciness of meat samples and odour and flavour of fat samples, respectively. They used 5-point scale where 1 = the worst and 5 = the best quality. Before the test, consumers were asked to complete a questionnaire where they answered the question: “How often do you eat pork?” – a) very often (2-3 times a week), b) occasionally (once a week), c) very rarely (once a month), d) never.

Statistical analysis was done using statistical programme SAS 9.2 (2009). We used analysis of variance to study the influence of sex and the pork preference on sensory traits of entire males, castrates and gilts. Statistical significances of effects were tested using Fischer´s F-test.

Results and Discussion

When comparing regardless gender of the consumers, meat from entire males had significantly worse evaluated odour (P<0.001) compared to other two sexes and flavour (P<0.05) than castrates (Table 1). Similar results were observed in fat of entire males. Odour and flavour obtained lower score compared to castrates and gilts (P<0.01). On the other hand, differences in tenderness and juiciness between pork from entire males and castrates and females were not significant. This is in agreement with other studies *(Nold et al., 1997; Souza and Mullan, 2003; Font and Furnols et al., 2009)* reported drier, tougher and lower overall acceptability of pork from entire males compared to surgically, immunologically castrated pigs or gilts. Also, odour and flavour were lower in entire males than other two sexes. In contrast, some studies reported higher tenderness, juiciness and
better overall acceptability in pork from boars than in gilts *(Wood et al., 1993; Blanchard et al., 1999).*

Men in our study evaluated odour of meat from entire males significantly worse than that of barrows (P<0.01) and gilts (P<0.05) (Table 2). Other meat parameters as well as fat scores of entire males were lower than castrates and females, however differences were not significant. Within a group of women, sensory characteristics of meat from entire males were lower compared to castrates and females but not significant (Table 3). In contrast, fat odour and flavour from entire males were significantly worse evaluated than those of castrates and females (P<0.01, P<0.05).

Consumers in our study answered the question in the questionnaire were only two groups: a) those who eat pork very often or b) occasionally. It is an evidence of popularity of pork consumption in Slovakia. Consumers eating pork occasionally evaluated meat and pork from entire males worse compared to castrates and females but only difference in fat flavour was significantly worse (P<0.05) (Table 4). In contrast, much more critical were consumers eating pork very often (Table 5). Score for meat odour of entire males was significantly lower (P<0.0001) than that of barrows and gilts. Similarly, fat odour from entire males had lower score compared to barrows (P<0.05) and gilts (P<0.01). Also, difference between meat flavour of entire males and castrates was significant (P<0.05) whereas fat flavour from entire males and females was at the level of significance (P=0.06).

Conclusion

Generally, consumers perceived meat and fat from entire males as less acceptable than that from surgically castrated and female pigs. Gender of the consumers had significant effect on meat odour and both meat odour and flavour. As expected, women were more critical than men, especially in fat characteristics of entire males. Consumers with strong pork preference evaluated meat and fat from entire males less favourable compared to those eating pork occasionally.

Table 1. Consumer´s sensory characteristics of pork and fat regardless gender and pork preference

	Castrates	Entire males	Females
Pork			
Odour	3.97[a]	3.17[b]	3.97[a]
Flavour	3.84[a]	3.37[b]	3.79
Tenderness	3.79	3.34	3.76
Juiciness	3.76	3.31	3.48
Fat			
Odour	3.32[a]	2.29[b]	3.34[a]
flavour	3.24[a]	2.49[b]	3.41[a]

[a,b]P<0.05 min.

Table 2. Sensory characteristics of pork and fat within men

	Castrates	Entire males	Females
Pork			
Odour	3.90[a]	2.90[b]	3.82[a]
Flavour	3.75	3.38	3.64
Tenderness	3.70	3.19	3.27
Juiciness	3.65	3.19	3.00
Fat			
Odour	3.20	2.43	3.45
flavour	3.10	2.62	3.27

[a,b]P<0.05 min.

Table 3. Sensory characteristics of pork and fat within women

	Castrates	Entire males	Females
Pork			
Odour	4.06	3.50	4.06
Flavour	3.94	3.36	3.89
Tenderness	3.89	3.57	4.06
Juiciness	3.89	3.50	3.78
Fat			
Odour	3.44[a]	2.07[b]	3.28[a]
flavour	3.39[a]	2.29[b]	3.50[a]

[a,b]P<0.05 min.

Table 4. Sensory characteristics of pork and fat by consumers eating pork occasionally

	Castrates	Entire males	Females
Pork			
Odour	3.67	3.83	3.85
Flavour	3.75	3.58	3.85
Tenderness	3.58	3.25	3.92
Juiciness	3.67	3.33	3.92
Fat			
Odour	3.33	2.33	3.00
flavour	3.33[a]	2.17[b]	3.46[a]

[a,b]P<0.05 min.

Table 5. Sensory characteristics of pork and fat by consumers eating pork very often

	Castrates	Entire males	Females
Pork			
Odour	4.17[a]	2.76[b]	4.13[a]
Flavour	4.00[a]	3.33[b]	3.80
Tenderness	3.88	3.38	3.53
Juiciness	3.79[a]	3.29	3.00[b]
Fat			
Odour	3.21[a]	2.19[b]	3.60[a]
flavour	3.13	2.62*	3.40*

[a,b]P<0.05 min. *P=0.06

Senzorno upoređivanje mesa i masnog tkiva nekastriranih i hirurški kastriranih muških, i ženskih svinja u zavisnosti od pola potrošača i preferencije u pogledu svinjetine

Ivan Bahelka, Ondřej Bučko, Emília Hanusová, Martina Gondeková

Rezime

Cilj ove studije bio je upoređivanje senzornih karakteristika mesa i masti od nekastriranih, hirurški kastriranih i ženskih svinja, koristeći test potrošača u zavisnosti od njihovog pola i preferenc u pogledu svinjetine. Uzorci slabine i masnog tkiva slabine uklonjeni su 24 časa nakon klanja. Ukupno 102 potrošača procenjivalo je miris, ukus, mekoću i sočnost mesa, kao i miris i ukus masti pomoću šeme od 5 tačaka. Meso i masno tkivo muških grla ocenjeno je manje prihvatljivim u poređenju sa mesom kastrata i ženskih svinja. Žene su bila kritičnije naročito u proceni masnog tkiva nekastriranih mužjaka. Potrošači koji često jedu svinjetinu su rangirali meso i masno tkivo nerastova negativnije u poređenju sa onima koji povremeno konzumiraju svinjetinu.

Ključne reči: svinje, nekastrirani mužjaci, senzorne karakteristike, test potrošača, preference

References

AMPUERO KRAGTEN S., VERKUYLEN B., DAHLMANS H., HORTÓS M., GARCÍA-REGUEIRO J.A., DAHL E., ANDRESEN Ø, FEITSMA H., MATHUR P.K., HARLIZIUS H. (2011): Inter-laboratory comparison of methods to measure androstenone in pork fat. Animal, 5, 10, 1634-1642.

BEKAERT K.M., VANDEN BUSSCHE J., FRANÇOIS S., TUYTTENS F.A.M., DE BRABANDER H.F., VANDENDRIESSCHE F., VANHAECKE L. (2012): A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds. Journal of Chromatography A, 1239, 49-55.

BLANCHARD P.J., ELLIS M., WARKUP C.C., CHADWICK J.P., WILLIS M.B. (1999): The influence of sex (boars and gilts) on growth, carcass and pork eating quality characteristics. Animal Science, 68, 487-493.

COWAN C.A. and JOSEPH R.L. (1981): Production and quality of boar and castrate bacon. 2. Consumer and panel response to bacon and fat samples. Irisj Journal of Food Science and Technology, 5, 105-116.

DESMOULIN B., BONNEAU M., FROUIN A., BIDARD J.P. (1982): Consumer testing of pork and processed meat from boars: the influence of fat androstenone level. Livestock Production Science, 9, 707-715.

D´SOUZA D.N., MULLAN B.P. (2003): The effect of genotype and castration method on the eating quality characteristics of pork from male pigs. Animal Science, 77, 67-72.

FONT I FURNOLS M., GONZÁLEZ J., GISPERT M., OLIVER M.A., HORTÓS M., PÉREZ J., SUÁREZ P., GUERRERO L. (2009): Sensory characterization of meat from pigs vaccinated against gonadotropin releasing factor compared to meat from surgically castrated, entire male and female pigs. Meat Science, 83, 438-442

GILBERT A.N. and WYSOCKI C.J. (1987): The smell survey results. National Geographic, 172, 514-525.

KEMPSTER A.J., DILWORTH A.W., EVANS D.G., FISCHER K.D. (1986): The effects of fat thickness and sex on pig meat quality with special reference to problems associated with overleanness. Butcher and consumer panel results. Animal Production, 43, 517-533.

LUNDE K., SKUTERUD E., NILSEN A., EGELANDSDAL B. (2009): A new method for differentiating the androstenone sensitivity among consumers. Food Quality and Preference, 20, 304-311.

MATTHEWS K.R., HOMER D.B., PUNTER P., BEAGUE M.-P., GISPERT M., KEMPSTER A.J., AGERHEM H., CLAUDI-MAGNUSSEN C., FISCHER K., SIRET F., LEASK H., FONT I FURNOLS M., BONNEAU M. (2000): An international study on the importance of androstenone and skatole for boar taint: III. Consumer survey in seven European countries. Meat Science, 54, 271-283.

NOLD R.A., ROMANS J.R., COSTELLO W.J., HENSON J.A., LIBAL G.W. (1997): Sensory characteristics and carcass traits of boars, barrows, and gilts fed high- or adequate-protein diets and slaughtered at 100 or 110 kilograms. Journal of Animal Science, 75, 2641-2651.

SAS Institute Inc. (2009). SAS/STAT® 9.2 User's Guide, Second Edition, Cary, NC USA.

WEILER U., FISCHER K., KEMMER H., DOBROWOLSKI A., CLAUS R. (1997): Influence of androstenone sensitivity on consumer reactions to boar meat. Bonneau et al. (Eds.) Boar taint in entire male pigs (pp. 147-151). EAAP Publication No. 92.

WEILER U., FONT I FURNOLS M., FISCHER K., KEMMER H., OLIVER M.A., GISPERT M., DOBROWOLSKI A., CLAUS R. (2000): Influence of differences in sensitivity of Spanish and German consumers to perceive androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations Meat Science, 54, 297-304.

WOOD J.D., NUTE G.R., FURSEY G.A.J., CUTHBERTSON A. (1993): Effects of final cooking temperature on the eating quality of pork loin steaks. Animal Production, 56, 422 (abstr.).

GENETIC PARAMETERS FOR GROWTH AND CARCASS TRAITS OF PEKIN DUCKS

Martin Wähner[1], Heinz Pingel[2], Ralf Fischer[3], Romi Wehlitz[3]

[1]Anhalt University of Applied Sciences Bernburg, Germany
[2]Martin-Luther-University Halle-Wittenberg, Germany
[3]Saxon State Office for Environment, Agriculture and Geology, Köllitsch, Germany
Corresponding author: martin.waehner@hs-anhalt.de
Original scientific paper

Abstract. In Beijing duck breeding process a remarkable progress was observed. In front of this background the current performance potentials must be recognized and measured at Beijing ducks again. New investigations have to document the current situation and the progress of breed in Beijing ducks for following traits: growth, feed conversion and carcass quality. Aim of the investigation was: documentation of current level of performance traits in growth and carcass traits; calculation of h2-for elected traits of growth and carcass quality;based on genetic and phenotypic correlation approaches should be worked out for continued effective breeding and selection process in Beijing ducks. The average body weight at the age of six weeks of 3.72 kg with $h^2 = 0.47$ characterizes a heavy strain of Peking ducks. There is a high feed efficiency from 15 to 42 days of age with 0.45. The heritability for feed efficiency (FE) is $h^2 = 0.36$. The breast muscle thickness (BMT) amounts to 1.98 cm with $h^2 = 0.56$. The same heritability was estimated for breast muscle percentage. The most important results of estimation of genetic correlations are: Body weight at six weeks to FE: 0.09, to breast muscle percentage: 0.39, to leg muscle percentage: -0.03. Genetic correlations of FE to BMT and to breast muscle percentage are low with 0.04 and 0.17, respectable, but it is high to leg muscle percentage (rg = 0.66). The genetic correlations of breast muscle thickness to breast muscle percentage and to leg muscle percentage amount to 0.78 and -0.15. Generally, there is a negative tendency for correlation of breast and leg muscle percentage (rg = -0.14). There is a high genetic correlation between FE and skin, it is rg= -0.63. There is an agreement with low genetic correlation between FE and breast muscle percentage (rg = 0.17) as well as high genetic correlation between FE and amount of skin (rg = -0.63).

The heritability coefficients in this study agree with results of *Rouvier (1999)* and *Cheng et al (2003*). With regard to genetic and phenotypic correlations between growth (body weight) and feed conversion ratio *Klemm (1995*) has found similar results.

Keywords: Pekin ducks, growth, carcass, feed efficiency, genetic parameters

Introduction

In last years in Pekin duck breeding process a remarkable progress was observed. Compared to results in past especially the daily gain, carcass quality and feed conversion have been improved significantly. In front of this background the current performance potentials must be recognized and measured at Pekin ducks again. Compared to earlier studies new investigations have to document the current situation and the progress of breed in Pekin ducks for following traits: growth, feed conversion and carcass quality.

Aim of investigation

- Documentation of current level of performance traits in growth and carcass traits
- Calculation of $h^2$for elected traits of growth and carcass quality
- Based on genetic and phenotypic correlation approaches should be worked out for continued effective breeding and selection process in Pekin ducks

Material and Methods

The trail has been happened in Chinese Pekin duck farm in the north of China. From a heavy strain of Pekin ducks 1376 pedigreed birds could be used for calculation of heritability and phenotypic and genetic correlation. The family structure contains full- and half-sibs. The ducklings were kept from the age of 15 to 42 days in single boxes (2.00 by 0.70 m) with individual feeding of standard duck grower pellets (fig. 1). Beside of growth it could be measured feed efficiency and breast muscle thickness. At the age of six weeks all ducks were slaughtered and the carcass composition measured (breast muscle with and without skin as well as legs with and without skin).

Fig. 1: Single box for individualtest of growth and feed consumption of Pekin ducks

The genetic parameters were estimated by software packages PEST *(Groeneveld et al., 1990)* and *VCE-6 (Groeneveld et al. 2008).* The following mixed linear model was used:

$$\mathbf{y_{ijk} = YxSxL_i + animal_j + e_{ijk}}$$

with: y_{ijk} = trait observation
$YxSxL_i$ = combined fixed effect (year x sex x line), n = 8
$animal_j$ = random animal effect (with pedigree matrix)
e_{ijk} = random residual effect

Following traits were selected:

- Body weight aged 14 and 42 days (BW14, BW42)
- Gain aged between 15^{th} and 42^{nd} live day (Gain)
- Feed efficency (FE, kg gain per kg food intake)
- Breast muscle thickness (BMT)
- Carcassweight (CW)
- Slaughteryield(CW/BW)
- Breast muscle without skin (BM)
- Leg muscle without skin (LM)

- Skin Breast + Leg (Skin)
- Ratio breast muscle with skin to Carcass weight (BM + Skin/CW)
- Ratio breast muscle without skin to Carcass weight (BM -Skin/CW)
- Ratio leg with skin to Carcass weight (LM + Skin/CW)
- Ratio leg without skin to Carcass weight (LM - Skin/CW)

Results and Discussion

The average body weight at 2 and 6 weeks was 0.59 and 3.72 kg, respectively.Table 1 contains averages and standard variations and heritability-coefficients (h^2).

Table 1. Results of growth and carcass traits in Pekin ducks

Traits		**Mean**	**s**	**s%**	**h^2**
Body weight aged 14days (BW14)	g	587.7	59.8	10.2	0.62
Body weight aged 42 days (BW42)	g	3,722.9	268.3	7.21	0.47
Gain	g	3,135.2	281.4	8.98	0.39
Feed efficiency (FE)kg gain/kg food intake		0.45	0.033	7.3	0.36
Breast muscle thickness (BMT)	cm	1.98	0.09	4.5	0.55
Carcass weight (CW)	g	2,484.9	193.3	7.8	0.48
Slaughter yield(CW/BW)	%	66.75	2.7	4.05	0.34
Breast muscle (BM)	g	424.1	64.1	15.1	0.58
Leg muscle(LM)	g	411.0	39.4	9.6	0.42
Skin (Breast + Leg)	g	245.6	37.4	15.2	0.18
BM + Skin/CW	%	22.0	1.9	8.6	0.51
BM - Skin/CW	%	17.07	1.8	10.6	0.56
LM + Skin/CW	%	16.54	1.7	10.6	0.30
LM - Skin/CW	%	12.0	1.0	8.3	0.32

The average body weightat the age of six weeks of 3.72 kg with $h^2 = 0.47$ characterizes a heavy strain of Pekin ducks. There is a high feed efficiency from 15 to 42 days of age with 0.45. The heritability for feed efficiency is $h^2 = 0.36$. The breast muscle thickness amounts to 1.98 cm with $h^2 = 0.55$. The same heritability was estimated for breast muscle percentage. It is interesting to find thatthe average value of breast muscle thickness with 1.98 cm has a low variation (s% = 4.5) but a relatively high heritability ($h^2 = 0.55$).
For comparison table 2 contains h^2-coefficients with corresponding values from literature.

Table 2: Heritability coefficients (h²) for main traits of ducks and geese
(Pingel et al. 1984, Rouvier 1999, Cheng et al. 2003)

Traits	h^2-coefficient
Body weight (BW)	0.30 – 0.60
Breast muscle thickness (BMT)	0.30 – 0.40
Percentage of breast meat (BM/CW)	0.30 – 0.40
Feed conversion ratio (FCR)	0.20 – 0.40

While the h^2-coefficients for body weight and feed conversion ratio are in the same level like literature, higher values were estimated for breast muscle thickness and percentage of breast meat.The h^2-value for feed efficiency for the time from 15 to 42 days of age with 0.36 agrees with values of literature.

Table 3contains genetic and phenotypic correlations between the examined traits.The standard error of genetic correlations varied dependent on the respective trait between 0.05 and 0.17. The standard errors of heritability lay between 0.05 and 0.06.

Table 3: Genetic and phenotypic parameters of growth and carcass traits of Pekin ducks
(correlations: phenotypic ($r_{p;}$ below) and genetic ($r_{g,}$ above))

$r_p r_g$	BW14	BW42	Gain	FE	BMT	CW	CW/BW	BM	LM	Skin	BM + Skin/CW	BM - Skin/CW	LM + Skin/CW	LM - Skin/CW
BW14		0.57	0.33	-0.37	0.40	0.55	0.19	0.44	0.19	0.38	0.30	0.26	-0.35	-0.33
BW42	0.33		0.97	0.09	0.47	0.96	0.27	0.71	0.72	0.62	0.41	0.39	-0.06	-0.03
Gain	0.11	0.86		0.23	0.41	0.93	0.25	0.66	0.77	0.57	0.37	0.36	0.05	0.09
FE	-0.20	0.29	0.35		0.04	0.06	-0.14	0.15	0.50	-0.53	-0.01	0.17	0.43	0.66
BMT	0.19	0.31	0.28	0.10		0.51	0.37	0.78	0.29	0.18	0.77	0.78	-0.25	-0.15
CW	0.35	0.89	0.85	0.23	0.37		0.51	0.79	0.72	0.67	0.52	0.49	-0.07	-0.05
CW/BW	0.12	0.13	0.10	-0.17	0.27	0.54		0.61	0.29	0.42	0.60	0.56	-0.14	-0.14
BM	0.28	0.64	0.60	0.26	0.50	0.76	0.54		0.52	0.27	0.91	0.92	-0.30	-0.11
LM	0.13	0.55	0.55	0.34	0.18	0.61	0.29	0.48		0.22	0.18	0.29	0.57	0.66
Skin	0.19	0.46	0.44	-0.06	0.12	0.51	0.21	0.24	-0.02		0.14	-0.02	-0.12	-0.38
BM + Skin/CW	0.21	0.42	0.39	0.15	0.57	0.55	0.49	0.85	0.20	0.36		0.97	-0.53	-0.31
BM - Skin/CW	0.19	0.37	0.35	0.28	0.57	0.49	0.50	0.89	0.33	-0.01	0.91		-0.40	-0.14
LM + Skin/CW	-0.15	-0.09	-0.06	0.13	-0.19	-0.12	-0.10	-0.19	0.52	0.25	-0.27	-0.28		0.89
LM - Skin/CW	-0.15	-0.10	-0.07	0.20	-0.11	-0.12	-0.08	-0.07	0.69	-0.46	-0.22	0.00	0.74	

The most important results of the estimations of genetic correlations are following:

- Body weight at six weeks to feed efficency: 0.09, to breast muscle percentage: 0.39,to leg muscle percentage: -0.03.
- Genetic correlations of feed efficency to breast muscle thickness and to breast muscle percentage are low with 0.04 and 0.17, respectively, but it is high to leg muscle percentage (r_g = 0.66).
- The genetic correlations of breast muscle thicknessto breast muscle percentage and to leg muscle percentage amount to 0.78 and -0.15. Generally, there is a negative tendency for the correlation of breast and leg muscle percentage (r_g = -0.14).
- As shown in table 3 the genetic correlation between breast muscle thickness and the percentage of breast muscle to carcass with 0.78 is high enough for practical selection.
- There is also a high genetic correlation between feed efficiency and amount of skin, it is r_g= -0.63.
- There is an agreement with low genetic correlation between feed efficency and breast muscle percentage (r_g = 0.17) as well as high genetic correlation between feed efficency and amount of skin (r_g = -0.53).

The heritability coefficients in this study agree with results of *Rouvier (1999)* and Cheng et al. (2003). With regard to genetic and phenotypic correlations between growth (body weight) and feed conversion ratio *Klemm (1995)* has found similar results (tab. 4).

Table 4: Phenotypic (r_p) and genetic (r_g) correlations and heritability (diagonal) of body weight (BW) and feed conversion ratio (FCR) (*Klemm,1995*)

$r_p r_g$	BW (21st d)	BW (49th d)	BW-Gain	Feed Consumption	FCR
BW (21st d)	*0.40*	+ 0.58	+ 0.35	+ 0.35	+ 0.29
BW (49th d)	+ 0.57	*0.47*	+ 0.83	+ 0.70	- 0.28
BW-Gain	+ 0.03	+ 0.85	*0.50*	+ 0.63	- 0.55
Feed cons.	+ 0.21	+ 0.65	+ 0.64	*0.58*	+ 0.25
FCR	+ 0.34	- 0,27	- 0.54	+ 0.59	*0.52*

High feed efficiency is a presupposition for optimum biological efficiency and sustainability in duck production. The late start of breast muscle growth is a limit for the reduction of slaughter age and an indirect improvement of feed efficiency. Therefore, the main objective of successful duck breeding for meat production is beside of the percentage of breast muscle the improvement of feed efficiency.

Conclusions

Present investigation has documented the current situation of breed in Beijing ducks for following phenotypical and genetical traits: growth, feed conversion and carcass quality. Calculation of h2 for elected traits of growth and carcass quality.

The average body weight at the age of six weeks of 3.72 kg with $h^2 = 0.47$ characterizes a heavy strain of Peking ducks. There is a high feed efficiency from 15 to 42 days of age with 0.45. The heritability for feed efficiency (FE) is $h^2 = 0.36$. The breast muscle thickness (BMT) amounts to 1.98 cm with $h^2 = 0.56$. The same heritability was estimated for breast muscle percentage. The most important results of estimation of genetic correlations are:

Body weight at six weeks to FE: 0.09, to breast muscle percentage: 0.39, to leg muscle percentage: -0.03.

Genetic correlations of FE to BMT and to breast muscle percentage are low with 0.04 and 0.17, respectable, but it is high to leg muscle percentage (rg = 0.66).

The genetic correlations of breast muscle thickness to breast muscle percentage and to leg muscle percentage amount to 0.78 and -0.15.

Generally, there is a negative tendency for correlation of breast and leg muscle percentage (rg = -0.14).

There is a high genetic correlation between FE and skin, it is rg= -0.63.

There is an agreement with low genetic correlation between FE and breast muscle percentage (rg = 0.17) as well as high genetic correlation between FE and amount of skin (rg = -0.63).

Genetski paprametri za osobine porasta i trupa pekinških patki

Martin Wähner, Heinz Pingel, Ralf Fischer, Romi Wehlitz

Rezime

U Pekingu u je zabeležen izuzetan napredak u odjoju patki. Trenutni potencijali performansi bi trebalo da budu iznova prepoznati i izmereni. Nove studije bi treblo da dokumentuju trenutnu situaciju i napredak rase za sledeće osobine: rast, konverzija hrane i kvalitet trupa. Cilj istraživanja je bio: dokumentacija trenutnog nivoa proizvodnih osobina porasta i osobina trupa; izračunavanje h^2-za izabrane osobine rasta i kvaliteta trupa; na osnovu genetičkih i fenotipskih korelacionih pristupa izraditi pristupe i strategiju za nastavak efikasnog procesa uzgoja i selekcije pekinških pataka. Prosečna telesna težina u starosti od šest nedelja od 3,72 kg sa h^2 = 0,47 karakteriše teši soj pekinških pataka. Postoji visoka efikasnost korišćenja hrane od 15 do 42 dana od 0,45. Heritabilnost za efikasnost ishrane (FE) je h^2 = 0,36. Debljina mišića grudi (BMT) iznosi 1,98 cm sa h^2 = 0,56. Ista heritabilnost procenjena je za procenat mišića grudi. Najvažniji rezultati procene genetskih korelacija su: telesna težina šest nedelja prema FE: 0,09, procenat mišića grudi: 0,39, prema procenta mišića nogu: -0,03. Genetske korelacije FE prema BMT i procenta mišića grudi su niske sa 0,04 i 0,17, respektivno, ali je visok prema procentu mišića nogu (rg = 0,66). Genetske korelacije za debljinu mišića grudi prema procentu mišića grudi i procentu mišića nogu iznose 0,78 i -0,15. Generalno, postoji negativna tendencija korelacije procenta mišića grudi i nogu (rg = -0.14). Postoji velika genetička korelacija između FE i kože, i to rg = -0,63. Postoji saglasnost sa niskom genetičkom korelacijom između FE i procenta mišića grudi (rg = 0,17), kao i visokom genetskom korelacijom između FE i količine kože (rg = -0,63).

Koeficijenti heritabilnosti u ovoj studiji slažu se sa rezultatima *Rouvier (1999)* i *Cheng et al (2003).* Što se tiče genetičkih i fenotipskih korelacija između rasta (telesne težine) i konverzije hrane, *Klemm (1995)* je našao slične rezultate.

Ključne reči: pekinške patke, porast, trup, efikasnost korišćenja hrane, genetski parametri

References

CHENG, Y.S., ROUVIER, R., Hu, Y.H., TAI, J.J.L. and TAI, C. (2003): Breeding and genetics of waterfowl. WPSJ 59, 509-519.

KLEMM, R. (1995): Selection on feed conversion ratio in ducks. Proc. 10th Europe. Symp. on waterfowl. Univ. Halle, 433-438.
PINGEL, H, KLEMM, R., WOLF, A. (1984): Selection for feed conversation rate of ducks and the effect on carcass composition. Proc. of XVII World Poultry Congress, Helsinki, 110-111.
ROUVIER, R. (1999): Genetics and physiology of waterfowl. Proceedings of the 1st World Waterfowl Conference, Taichung, Taiwan, pp. 1-18.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

CARCASS AND MEAT QUALITY OF MALE LAYER-TYPE CHICKENS AT DIFFERENT AGE

Teodora Popova[1], Evgeni Petkov[1] and Maya Ignatova[1,2]

[1]Institute of Animal Science, 2232 Kostinbrod, Bulgaria,
[2]Agricultural Academy, 1373 Sofia, Bulgaria
Corresponding author: Teodora Popova, tlpopova@yahoo.com
Original scientific paper

Abstract: An experiment was carried out with a total of 150 male layer-type chickens of Lohmann Brown Classic, in order to study their carcass and meat quality characteristics at different age. The chickens were reared on litter and at the age of 5 and 12 weeks, 20 chickens were slaughtered, and subjected to carcass analysis, as well as to analysis of the physicochemical traits of breast and thigh meat. Significantly higher dressing percentage and reduced content of by-products ($P<0.001$) were observed in the chickens at older age of slaughter. Increasing the age led to higher values of pH24 ($P<0.001$) and better water holding capacity ($P<0.001$) in breast and thighs. Lipid and ash content decreased while protein increased in the meat of the chickens slaughtered at 12 weeks of age.

Keywords: male layer-type chickens, carcass, meat quality, slaughter age

Introduction

Globally the production and consumption of chicken meat has been rapidly increasing in the recent years. The reason for this growth is the higher demands of the consumers for healthier meat with high dietetic qualities, as well as its cheaper price. With its lower fat content chicken meat is often preferred to pork and beef.

The intensified chicken meat production is associated mostly with fast growing meat-type strains. So far, only a small premium niche market prefers slower growing broilers from free range or organic farming, while currently there is no demand for meat of male layer-type poultry (*Damme and Ristic, 2003*). Usually, male layer-type chickens have not been considered for meat production by the majority of the breeders and farmers, due to their higher feed consumption per body weight unit and a worse slaughter value when compared to broiler chickens (*Murawska et al., 2004*). Recent research (*Líchovniková et al., 2009*) however, show that this type of birds responds entirely to all the requirements of the slow-growing broilers, at the same time are well adapted to the alternative rearing systems and their meat quality is higher compared to that of fast-growing birds. In some Asian countries, such as Thailand the male layer type chickens are also used for meat production (*Soisontes, 2015*).

The effect of age at slaughter in terms of poultry product quality has not been well established. According to *Young et al.* (*2001*) and *Baéza et al*. (*2012*), increasing the slaughtering age is associated with increased yield of carcass as well as meatier parts. On the other hand, according to *Poole et al.* (*1999*), tenderness decreases in older birds. The aim of this study was to compare the carcass and meat quality in male layer-type chickens at different age.

Material and methods

Animals and diets

The experiment was carried out in the poultry farm of the Institute of Animal Science- Kostinbrod with male layer-type chickens of Lohmann Brown Classic. The one day-old chickens (n=150) were placed into a deep litter facility with an area 7.3 m^2 and stocking density of 10 kg/m^2. All the birds were fed *ad libitum* starter (protein content 20.26%, ME 2728.90 kcal/kg), grower (protein content 18.51%, ME 2825.14 kcal/kg) and finisher (protein content 19.13%, ME 2835.01kcal/kg) diets.

Water was provided *ad libitum* with a nipple waterer. The lighting regime was 15 h of light and 9 h of darkness, and the temperature ranged between 20 and 24 °C. During rearing, individual body weight of the chickens and their feed intake was recorded every week and the number of dead birds every day.

Slaughtering and sampling

At the age of 5 and 12 weeks, 20 chickens were selected for slaughter. The average body weight was 0.358 ± 0.022 kg and 1.657±0.132 kg, respectively for the birds at 5 and 12 weeks. The chickens were stunned, manually decapitated and bled. After plucking, the carcasses were eviscerated, feet removed, and the neck, liver, gizzard, heart and spleen were weighed. Their content was calculated as a percentage of the live weight. Hot carcass weight was registered and dressing percentage was calculated. The carcasses were then placed into chilling room, kept at 4 °C for 24 hours and weighed. pH 24 was measured on breast and thighs of the chickens using HI 2211 pH/OPR meter (Hanna Instruments). Breast and thigh muscles of each chicken were separated, minced with meat grinder, and samples for measuring of water-holding capacity (WHC) were taken while the rest of the muscles were vacuum packed and stored at -20 °C until analysis.

Analysis of physicochemical composition

Water-holding capacity (WHC) was determined according to the method of *Grau and Ham* (*1952*). Analysis of the chemical composition of meat including determination of lipid, protein, ash and moisture content was performed according to the *AOAC methods* (*2004*).

Statistical analysis

Data were statistically analysed by t-test using JMP v.7 software package, at levels of significance: * P < 0.05; **P < 0.01; ***P < 0.001. The results are presented as Mean±SEM.

Results and discussion

Carcass analysis

Dressing percentage is an essential quantitative index for meat. In this study the older chickens displayed higher (P<0.001) dressing percentage than the birds slaughtered at 5 weeks of age (Fig. 1).

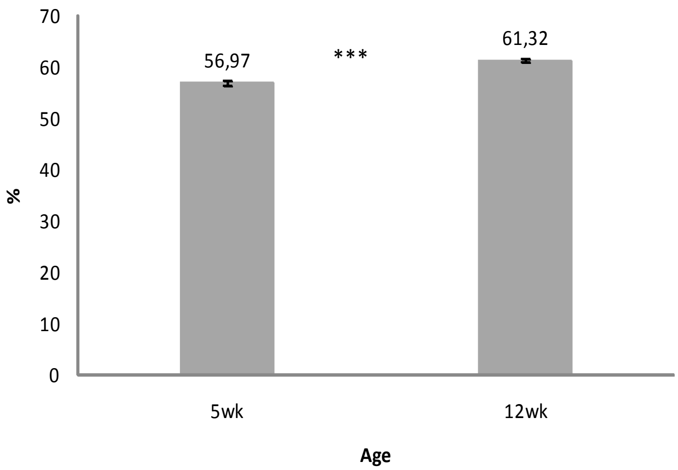


Figure 1. Dressing percentage in male layer-type chickens, slaughtered at different age

Similar to us, *Marcu et al.* (*2014*) reported increased dressing percentage in older chickens. According to *Coban et al.* (*2014*), slaughter age affected the dressing percentage in broilers. These authors observed higher values of this parameter in broiler chickens slaughtered at 56 days than in those at the age of 42 days. On the other hand, *Faria et al.* (*2010*) reported no difference in the dressing percentage between ages in male broiler chickens while in female it increased with age, depending however on the genetic strain. The dressing percentage reported in this study is lower when comparing to data obtained for broilers. *Damme and Rustic* (*2003*) showed similar values (64.2 %) in layer male chickens.

The content of the edible by-products (giblets and neck) presented on Figure 2, was significantly higher in the chickens at the age of 5 weeks (P<0.001). According to *Faria et al.* (2010), the yield of giblets tended to decrease with increasing the age of slaughter, however it depended on the sex and genotype of the chickens. The higher yield of these edible parts in the younger birds corresponded to the lower dressing percentage, compared to the older ones (P<0.001).

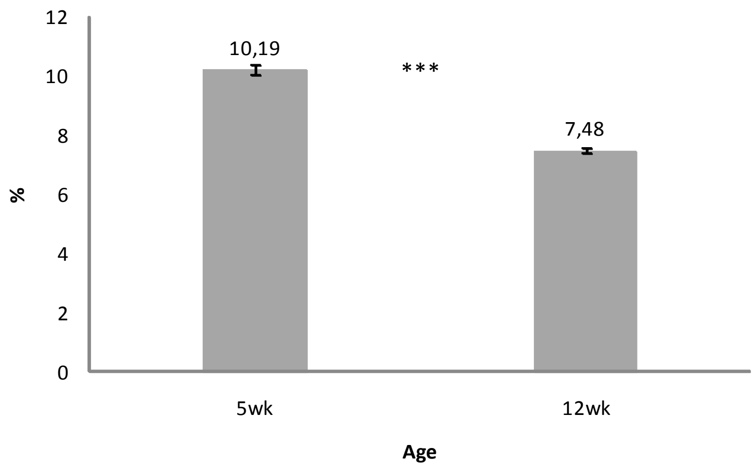


Figure 2. Content of edible by-products in male layer-type chickens, slaughtered at different age

Physicochemical composition of meat

The rate of pH decline *post mortem* is an important event into the conversion of muscle into meat and ultimate pH has been used as a predictor of chicken meat quality. These values are determined by the glycogen reserves in the muscle at the time of slaughter (*Bendall, 1973*). The ultimate pH differed significantly between ages in breast and thigh muscle (P<0.001) displaying higher values in the chickens slaughtered at 12 weeks of age (Table 1).

This is in agreement with the results of *Ponte et al.* (*2008*). *Eleroğlu et al.* (*2013*) also reported consistently higher pH values in slow-growing chickens slaughtered at older age (14 weeks). *Baéza et al.* (*2012*) observed lowest ultimate pH values in chickens slaughtered at the age of 5 weeks, associated with substantially increased lactate and glycolytic potential at this age compared to later stages. On the other hand, *Abdullah et al.* (*2010*) observed decreased values of pH24 *post mortem* in chickens slaughtered at later age, while *DalBosco et al.* (*2014*) did not observe dependence of pH on the slaughter age. The values of pH24 in the breast were within the range of 5.49-5.67, while in thighs they were higher- around 6.00 and even above in the chickens at the older age. Such values in thighs are in line with the reported by *Souza et al.* (*2011*) and *Mikulski et al.* (*2011*). In breast of male layer-type chicken, *Líchovnicková et al*. (*2009*) reported slightly higher results than ours (5.77), while *Fanatico et al*. (*2007*) measured pH24 values of 5.60 in slow-growing chickens, reared indoors. Higher pH24 in the older chickens in this experiment was associated with better WHC ($P<0.001$) in both muscles. Elevated pH values lead to decreased denaturation of the proteins and hence the better capacity of muscle to retain water. The increased WHC in the chickens with advancing the age coincides with the results of *Baéza et al.* (*2012*), who reported decreased percentage of drip loss in older birds. Our results are in line with *Abdullah et al*. (*2010*), who observed higher WHC in the chickens slaughtered at older age, however these authors associated it with increased lipid content in the older birds. The water holding capacity is important trait determining the quality of meat and its possibility for further processing into meat products. The decreased WHC is associated with poor sensory characteristics in meat.

Table 1. Physicochemical traits of the breast and thigh in male layer-type chickens, slaughtered at different age

Item	Age		Significance
	5 weeks	12 weeks	
Breast			
pH24	5.49±0.02	5.67±0.01	***
WHC,%	32.98±0.85	28.39±0.85	***
Lipid,%	0.61±0.04	0.44±0.08	*
Protein,%	21.54±0.15	22.98±0.09	***
Moisture,%	74.69±0.10	73.46±0.09	***
Ash,%	1.16±0.01	1.12±0.01	*
Thigh			
pH24	5.90±0.03	6.24±0.02	***
WHC,%	27.68±0.88	21.90±0.65	***
Lipid,%	3.85±0.15	2.47±0.13	***
Protein,%	18.11±0.10	18.77±0.09	***
Moisture,%	74.98±0.18	75.70±0.11	***
Ash,%	1.13±0.01	1.11±0.01	*

The chemical composition of meat showed differences between the ages of slaughter. The lipid content decreased significantly between 5 and 12 weeks of age in breast ($P<0.05$) and thighs ($P<0.001$), while protein increased in both muscles with advancing of the age ($P<0.001$) but more substantially in breast. The increase of protein content in the older birds has been reported in the studies of *Touraille et al*. (*1981*), *Grey et al.* (*1983*), *Zanusso* (*2002*), however *Abdullah et al.* (*2010*) observed decrease in the protein content in breast between 32 and 42 days of age. On the other hand, the results of the lipid content changes at different age have been inconsistent. Similar to us, *Touraille et al*. (*1981*) observed decrease in the lipids in breast in chickens after 8 weeks of age, while *Grey et al.* (*1983*) and *Zanusso* (*2002*) reported increased lipid content in both breast and legs between 6 and 22 weeks of age. *Zelenka et al*. (*2001*) found significant increase in the lipid content of breast in slow-growing chickens, depending on their age.

Moisture content in the muscles also displayed significant differences between ages. In breast moisture decreased in the older chickens ($P<0.001$), while in thighs we observed the opposite. Ash content differed significantly between ages ($P<0.05$) showing decreased content in the older chickens. According to *Prändl et al.* (*1994*), the contents of the ashes in the muscle increase in animals with advancing the age, however in our study we failed to observe this. Our results are in agreement with those of *Grey et al.* (*1983*) while *Abdulla et al.* (*2010*) reported no change in ash in chicken meat resulting from the age at slaughter.

Conclusions

Increasing of the age of slaughter led to significantly higher dressing percentage in the male layer-type chickens at the expense of reduced percentage of neck and giblets. Older chickens displayed higher pH 24 and better water-holding capacity of their breast and thighs. Furthermore, lipid and ash content decreased while protein increased in the chickens, slaughtered at 12 weeks of age.

Kvalitet trupa i mesa muških pilića lakog tipa različitog uzrasta

Teodora Popova, Evgeni Petkov, Maya Ignatova

Rezime

Eksperiment je obavljen sa ukupno 150 muših pilića lakog tipa Lohmann Brown Classic, kako bi se proučavale njihove osobine kvaliteta trupa i mesa u različitim uzrastima. Pilići su odgajani na prostirci, a u starosti od 5 i 12 nedelja uzrasta, 20 pilića je zaklano i podvrgnuto analizi trupa, kao i analizi fizičko-hemijskih osobina mesa grudi i karabataka. Značajno veći randman i smanjeni sadržaj nusproizvoda (P<0,001) su primećeni kod starijih pilića na klanju. Povećanje starosti dovelo je do veće vrednosti pH24 (P<0,001) i bolje sposobnosti zadržavanja vode (P <0,001) u mesu grudi i karabataka. Sadržaj lipida i pepela se smanjio dok je protein povećan u mesu pilića zaklanih u 12 nedelja života.

Ključne reči: muški pilići lakog tipa, trup, kvalitet mesa, starost pri klanju

References

ABDULLAH Y. A., MUWALLA M.M., MAHARMEH H.O., MATARNEH S.K., ABU ISHMAIS, M.A. (2010): Effects of strain on performance, and age at slaughter and duration of post-chilling aging on meat quality traits of broiler. Asian-Australasian Journal of Animal Science, 23, 1645 – 1656.

AOAC (2004): Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA.

BAÉZA E., ARNOULD C., JLALI M., CHARTRIN P., GIGAUD, V., MERCERAND F., DURAND C., MÉTEAU K., LE BIHAN-DUVAL E., BERRI, C. (2012): Influence of increasing slaughter age of chickens on meat quality, welfare, and technical and economic results. Journal of Animal Science, 90, 2003–2013.

BENDALL J. R. (1973): Post-mortem changes in muscle. In: The structure and function of muscle Ed. Bourne G.H., Academic Press, New York, 243-309.

COBAN O., LACIN E., AKSU M.I., KARA A., SABUNCUOGLU N. (2014): The impact of slaughter age on performance, carcass traits, properties of cut-up pieces of carcasses, and muscle development in broiler chickens. European Poultry Science, 78:

DAL BOSCO A., MUGNAI C., AMATO M.G., PIOTTOLI L., CARTONI, A., CASTELLINI C. (2014): Effect of slaughtering age in different commercial chicken genotypes according to the organic system: 1. Welfare, carcass and meat traits. Italian Journal of Animal Science, 13, 467- 472.

DAMME K., RISTIC M. (2003): Fattening performance, meat yield and economic aspects of meat and layer type hybrids. World Poultry Science Journal, 59, 50-53.

ELEROĞLU H., YILDIRIM A., IŞIKLI N.D., ŞEKEROĞLU A., DUMAN, M. (2013): Comparison of meat quality and fatty acid profile in slow-growing chicken genotypes fed diets supplemented with Origanum vulgare or Melissa officinalis leaves under the organic system. Italian Journal of Animal Science, 12, 395-403.

FANATICO A.C., PILLAI P. B., EMMERT J. L., OWENS C. M. (2007): Meat quality of slow- and fast-growing chicken genotypes fed low- nutrient or standard diets and raised indoors or with outdoor access. Poultry Science, 86, 2245–225.

FARIA P.B., BRESSAN M.C., SOUZA X.R., DE ROSSATO L.V., BOTEGA L.M.G., DA GAMA L.T. (2010): Carcass and parts yield of broilers reared under a semi-extensive system. Brazilian Journal of Poultry Science, 12, 153 – 159.

GRAU R., HAMM R. (1952): Eine einfache Methode zur Bestimmung der Wasserbindung in Fleisch. Fleischwirschaft, 4, 295-297.

GREY T.C., ROBINSON D., JONES J.M., STOCK S.W., THOMAS N.L. (1983): Effect of age and sex on the composition of muscle and skin from a commercial broiler strain. British Poultry Science, 24, 219-231.

JMP Version 7, SAS Institute Inc. Cary, NC.

LICHOVNÍKOVÁ M., JANDÁSEK J., JŮZL M., DRAČKOVÁ E. (2009): The meat quality of layer males from free range in comparison with fast growing chickens. Czech Journal of Animal Science, 54, 490–497.

MARCU, A., ȘTEF L., DUMITRESCU G., CIOCHINĂ L. P., DRONCA D., PEȚ I., BAUL S., MARCU A. (2014): Influence of nutrition, sex and slaughter age on the carcass characteristics at broiler chicken Ross-308. Scientific Papers Animal Science and Biotechnologies, 47, 271-278.

MARCU A., VACARU-OPRIŞ I., MARCU A., DĂNĂILĂ L., DRONCA D., KELCIOV B. (2013): The influence of genotype and sex on carcass characteristics at broiler chickens, Scientific Papers USAMV Iasi, Animal Science, Iaşi 59, 16-21.

MIKULSKI D., CELEJ J., JANKOWSKI J., MAJEWSKA T., MIKULSKA M. (2011): Growth performance, carcass traits and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access, Asian-Australasian Journal of Animal Science, 24, 1407 – 1416.

MURAWSKA D., BOCHNO R., MICHALIK D., JANISZEWSKA M. (2005): Age-related changes in the carcass tissue composition and distributionof meat and fat with skin in carcasses of laying-type cockerels. Archiv Geflügelkunde 69, S. 135–139.

PONTE P.I.P., ROSADO C.M.C., CRESPO J.P., CRESPO D.G., MOURAO J.L., CHAVEIRO-SOARES M.A., BRAS, J.L.A., MENDES I., GAMA L.T., PRATES

J.A.M., FERREIRA L.M.A., FONTES C.M.G.A. (2008): Pasture intake improves the performance and meat sensory attributes of free-range broilers**.** Poultry Science 87, 71–79.
POOLE G. H., LYON C.E., BUHR R.J., YOUNG L.L., ALLEY A., HESS J.B., BILIGLI S.F., NORTHCUTT J.K. (1999): Evaluation of age, gender, strain, and diet on the cooking yield and shear values of broiler breast fillets. Journal of Applied Poultry Research, 8, 170-176.
PRÄNDL, O., FISCHER A., SCHMIDHOFER T., SINELL H.J. (1994): Tecnología e hygiene de la carne. Zaragoza: Acribia.
SOISONTES S. (2015): An alternative use of one day-old male layer chicks: the case of Thailand. Available at: http://www.wing-vechta.de/pdf_files/themen-englisch/male-layer-chicks_for-poultry-international.pdf [Accessed 04.12.2015].
SOUZA X. R., FARIA P. B., BRESSAN M.C. (2011): Proximate composition and meat quality of broilers reared under different production systems. Brazilian Journal of Poultry Science, 13, 15-20.
TOURAILLE C., RICARD F.H., KOPP J., VALIN C., LECLERCQ B. (1981): Qualité du poulet. 2. Evolution en fonction de l'âge des caractéristiques physico-chimiques et organoleptiques de la viande. Archiv Geflügelkunde, 45, 97–104.
YOUNG L. L., NORTHCUTT J. K., BUHR R. J., LYON C.E., WARE G.O. (2001): Effects of age, sex, and duration of postmortem aging on percentage yield of parts from broiler chicken carcasses. Poultry Science, 80, 376–379.
ZANUSSO J. (2002): Engraissement, structure des muscles et qualité de la viande de volailles: exemple du gavage chez le canard de Barbarie (*Cairina moschata*) et de la castration chez le poulet (*Gallus domesticus*). PhD Dissertation, Toulouse INP, France.
ZELENKA J., FAJMONOVÁ E., KOMPRDA T. (2001): Retention of protein and fat in the meat of fast and slow-growing chickens fattened to higher age. Czech Journal Animal Science, 46, 533–538.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE EFFECT OF GARLIC ON PRODUCTION INDICATORS AND THE SHARE OF INTERNAL ORGANS IN BROILER CHICKENS

Veselin Petričević[1], Miloš Lukić[1], Zdenka Škrbić[1], Maja Petričević[1], Snežana Bogosavljević-Bošković[2], Vladimir Dosković[2], Simeon Rakonjac[2]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agronomy, University of Kragujevac, Čačak, Republic of Serbia
Corresponding author: veselin5@live.com
Original scientific paper

Abstract: The aim of this study was to examine the effect of the addition of different concentrations of garlic in nutrition of broiler chickens on the fattening results and the share of internal edible organs. The trial was performed on 800 chickens divided into 4 groups with 4 repetitions per group. The chickens were fed ad libitum and the composition of the mixture only differed in the quantity of added garlic powder. In the control group (K) no garlic was added. Chickens of group (I) consumed mixtures with the addition of 0.2% garlic powder, in group (II) 0.4% was added, while 0.6% of garlic was added to the mixture consumed by chickens in the group (III). At the beginning (day 1) and at the end of the experimental period (day 42) weight of chickens was measured. Average daily gain, mortality, food conversion ratio and EPEF were determined. At the end of the trial, by random sample method, 12 chickens (6 male and 6 female) were sacrificed in each group in order to determine the share of edible internal organs. The obtained results have shown that the use of garlic in mixtures influences higher body weight and average daily gain as well as more favourable mortality. The food conversion ratio was statistically significantly better ($p<0.01$) in chickens of II and III group compared to the control group. Statistically significantly lower ($p<0.01$) value of EPEF was established in the control group compared to II and III group. Heart, liver and stomach shares were not significantly affected by the tested factor.

Key words: broiler chickens, nutrition, garlic, production indicators

Introduction

Consumer demands for high quality poultry products can be ensured by the use of new and appropriate health safe food systems. In the modern production of chicken meat, the goal is to reduce the use of antibiotics in food because of the more frequent occurrence of resistant strains of bacteria. An alternative to antibiotics is the use of probiotics (*Perić et al., 2010*), and various parts of plants such as garlic (*Puvača et al., 2013*), mint (*Al-Kassie, 2010*), rosemary (*Al-Kassie et al., 2011*) and many others. By using herbal additives in the nutrition of the poultry, the development of the digestive tract and immunity is stimulated in a natural way, while reducing the use of drugs. A group of phytogenic additives consists of substances originating from medicinal and spice plants. Phytogenic additives influence the improvement of food consumption and conversion and the gain of broiler chickens (*Stanachev et al., 2011*). Considering the results obtained so far, it is considered that phyto-additives have significant potential in nutrition, but it is necessary to choose the right combination and dose. Some researchers say that the addition of phytogenic additives to feed mixtures for chickens does not have a significant effect on food intake, consumption, and conversion *(Issa and Omar, 2012).*

Useful effects of garlic have been known since ancient times due to antioxidant and antimicrobial properties *(Konjufka et al. 1997*). Positive effects on the animal body can be attributed to bioactive components. Garlic contains organosulfuric compounds (dialyl sulfide and dialyl trisulfide) (*Kumar and Berwal, 1998).* Allicin is a potentially active component of garlic that provides a distinctive smell and aroma (*Rahmatnejad and Roshanfekr*, *2009*). Garlic is known as a remedy for the prevention and treatment of various heart diseases, as well as metabolic and diabetic diseases.

The aim of this research was to study the effect of the garlic supplement in the nutrition of broiler chickens on the fattening results and the share of edible internal organs.

Materials and methods

The experiment for this research was carried out during the month of April and May 2016, at the Institute of Animal Husbandry. As the experimental material, 800 daily broiler chickens of Ross 308 were selected. Chickens were divided into 4 groups and were placed in 16 boxes, so there were 4 repetitions per treatment, and the impact of possible differences in the environment was minimized. During the research broilers were fed with three mixtures, the composition of which is given in Table 1. The diet was ad libitum, the composition of the mixture only differed in the amount of added white garlic powder. In the control group (K) no garlic was added. Chickens of group (I) consumed mixtures with added 0.2% garlic powder, in group (II) 0.4% was added, while 0.6% of garlic was added in group (III).

Body weight of chicken was measured at the beginning (day 1), and at the end of the experiment (day 42). On the basis of differences in weight of chickens, average daily gain was calculated. Food consumption control included measuring the amounts of complete mixtures given to broiler chickens as well as the food remains for each box. Based on data on food consumption and chicken gain, food conversion ratio was calculated. Based on data on body weight, food conversion ratio and mortality, the European Production Efficiency Factor (EPEF) was calculated according to the formula:

$$\text{EPEF} = \frac{\text{Body weight (kg)} \times \text{Vitality (\%)} \times 100}{\text{Duration of fattening (days)} \times \text{Feed conversion (kg/kg)}}$$

At the end of the trial, by method of total random selection, 6 male and 6 female chickens were selected, measured and slaughtered after 12 hours of starvation. After slaughter, measurements of the weight of the heart, liver and stomach were performed. The obtained masses were placed in relation to the pre-slaughter weight of the chickens. In this way, the shares of these internal organs were obtained.

Table 1. The raw material and chemical composition of the broiler diet during trial

Components, %	Starter	Grower	Finisher
	(0-10 days)	(11-24 days)	(25-42 days)
Maize	50.0	53.0	56.0
Wheat bran	3.0	3.0	4.0
Soybean cake	25.0	20.0	14.0
Soybean grit	12.0	17.0	20.0
EKOFISH*	6.0	3.0	-
Soybean oil	-	-	2.0
Calcium carbonate	1.4	1.4	1.3
Mono calcium phosphate	1.2	1.2	1.1
Sodium chloride	0.2	0.2	0.3
MINAZEL PLUS**	0.2	0.2	0.3
Premix	1.0	1.0	1.0
Σ	100	100	100
Chemical composition			
ME. MJ/kg	12.7	13.0	13.4
Crude protein, %	23.3	21.0	18.7
Moisture, %	11.2	11.3	11.8
Crude fat, %	7.5	7.8	9.5
Crude fibre, %	4.0	4.3	4.3
Calcium. %	0.9	0.85	0.8
Total phosphorus, %	0.7	0.7	0.6
Sodium, %	0.2	0.2	0.2

Statistical processing of the obtained data was performed using the software package "STATISTICA". Variance analysis and F-test were used to determine the presence of statistically significant differences. The Tuckey test served to determine the statistical significance of the differences between individual mean values.

Results and discussion

Table 2 shows the production traits of chickens. Chickens fed mixtures with added garlic powder had higher body weight compared to chickens of the control group, but the established differences were not statistically significant. The highest mortality was found in the control group (3.5%), while the percentage of mortality in the groups that consumed garlic was lower. The average daily gain of chickens does not significantly differ under the influence of the garlic supplement. The lowest average gain was recorded in chickens of the control group and the highest in III group of chickens. The food conversion ratio differed statistically significantly ($p<0.01$) under the influence of the garlic supplement. With the reduction in the share of garlic in mixtures, food conversion was worse. Chickens of II and III groups (1.80 and 1.79 kg) had statistically significantly better food conversion ratio compared to chickens of the control group (1.93 kg). Better feed conversion ratio of chickens fed mixture containing garlic can be attributed to antimicrobial activities, which influence the better utilization of nutrients in the digestive tract of chickens. EPEF, as the most comprehensive production indicator, was under statistically significant impact ($p<0.01$) of the examined factor. With the increase in the proportion of garlic in the mixtures, the EPEF gradually increased. Statistically significantly lower EPEF was found in the control group (238.82) compared to groups II and III (271.76 and 275.82).

Table 2. Production traits

Production parameters	Groups				p-value
	K	I	II	III	
Body weight, g	2005.8±403.9	2035.9±355.4	2079.1±324.6	2080.3±344.8	0.113
Mortality, %	3.50	0.50	1.00	0.50	
Average gain, g	46.82±9.16	47.54±8.26	48.57±7.93	48.59±7.21	0.132
Feed conversion, kg	$1.93^{a}±0.03$	$1.85^{ab}±0.03$	$1.80^{b}±0.02$	$1.79^{b}±0.03$	$p<0.01$
EPEF	$238.82^{b}±14.28$	$261.25^{ab}±9.07$	$271.76^{a}±8.48$	$275.82^{a}±5.23$	$p<0.01$

* a-b The average values in each column without common marks are significantly different at the level of 1%

Results of the share of internal organs of broiler chickens are shown in Table 3. The shares of heart, liver and stomach were not statistically significantly affected by the examined factor.

Table 3. The shares of internal organs

Shares, %	Groups				p-value
	K	I	II	III	
Heart	0.52±0.07	0.51±0.09	0.53±0.07	0.53±0.04	0.811
Liver	1.75±0.21	1.75±0.36	1.75±0.25	1.66±0.22	0.159
Stomach	1.97±0.24	1.86±0.14	1.95±0.19	1.99±0.22	0.349

The share of hearts ranged from 0.51% in the I group to 0.53% in the II and III groups. The smallest share of liver (1.66%) was found in group III, while in other groups the same value for this trait was determined (1.75%). The highest share of stomachs was in chickens of the group III (1.99%) and the lowest in chickens of the group I (1.86%).

Similar to our results, *Fayed et al. (2011)* state better production results (final weight, mortality and food conversion) of chickens fed garlic, however, the authors have not established differences in the weight of edible offal (heart, liver, and stomach). Investigations of *Puvača et al. (2015)* show that the addition of garlic in the amount of 0.5 kg/t and 1 kg/t has positive effects on the production characteristics of broiler chickens. *Fadlalla et al. (2010)* show that the addition of garlic in the diet of fattening chickens significantly improves the conversion of food. Unlike them *Ashayerizadeh et al. (2009)* has found no significant differences in food conversion. *Milošević et al. (2013)* have found significantly higher liver and heart shares in broiler chickens fed with mixtures in which the share of garlic was 1.5 and 3%. *Petričević et al. (2013),* similar to the results obtained in this study, have not revealed significant differences in the share of edible chicken offal fed with mixtures with a different share of raw soybean grain.

Conclusions

Based on the results of the study of the effect of the addition of garlic powder on the production results and the share of the edible internal organs of broiler chickens, we can conclude that:

- The use of garlic in mixtures affects both body weight and average daily gain, as well as more favourable mortality. Statistically significantly better ($p<0.01$) food conversion and a significantly higher EPEF values were found in groups consuming mixtures with an addition of 0.4 and 0.6% of garlic compared to the control group.

- The negative effects of using garlic on the share of edible ingredients have not been determined.
- On the basis of the established production characteristics, positive effects of the use of garlic can be concluded.

Efekat upotrebe belog luka na proizvodne pokazatelje i udeo unutrašnjih organa brojlerskih pilića

Veselin Petričević, Miloš Lukić, Zdenka Škrbić, Maja Petričević, Snežana Bogosavljević-Bošković, Vladimir Dosković, Simeon Rakonjac

Rezime

Cilj ovih istraživanja bio je da se ispita uticaj dodatka različite koncentracije belog luka u ishrani brojlerskih pilića na rezultate tova i udele unutrašnjih jestivih organa.
Ogled je izveden na 800 pilića koji su podeljeni u 4 grupe sa 4 ponavljanja po grupi. Pilići su hranjeni po volji a sastav smeša se jedino razlikovao u količini dodatog belog luka u prahu. U kontrolnoj grupi (K) nije dodavan beli luk. Pilići (I) grupe su konzumirali smeše sa dodatkom 0.2% belog luka u prahu, u grupi (II) dodato je 0.4% dok je u grupi (III) dodato 0.6% belog luka.Na početku (1. dana) i na kraju oglednog perioda (42. dana) izvršeno je merenje telesnih masa pilića. Utvrđen je prosečni dnevnii prirast, mortalitet, konverzija hrane i EPEF. Na kraju ogleda metodom slučajnog uzorka iz svake grupe žrtvovano je po 12 pilića (6 muških i 6 ženskih) u cilju utvrđivanja udela jestivih unutrašnjih organa.
Dobijeni rezultati su pokazali da korišćenje belog luka u smešama utiče na veće telesne mase i prosečne dnevne priraste kao i na povovniji mortalitet. Konverzija hrane je bila statistički značajno bolja ($p<0.01$) kod pilića II i III grupe u odnosu na kontrolnu grupu. Statistički značajno manja ($p<0.01$) vrednost EPEF utvrđena je u kontrolnoj grupi u odnosu na II i III grupu Udeli srca, jetre i želudca nisu bili pod značajnim uticajem ispitivanog faktora.

Ključne reči: brojleri, ishrana, beli luk, proizvodni parametri

Acknowledgments

The research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR-31033.

References

AL-KASSIE G. A. M. (2010): The role of peppermint (*Mentha piperita*) on performance in broiler diets. Agriculture and biology journal of North America. 1(5), 1009-1013.

AL-KASSIE G. A. M., AL-JALEEL R. A. A, MOHSEEN A. M. (2011): The effect of a mixture of anise and rosemary on broiler performance. Agriculture and biology journal of North America. 2(9), 1279-1282.

ASHAYERIZADEH O., DASTAR B., SHAMS S. M., ASHAYERIZADEH A., RAHMATNEJAD E., HOSSAINI S. M. R. (2009): Use of garlic (*Allium sativum*), black cumin seeds (*Nigella sativa* L.) and wild mint (*Mentha longifolia*) in broiler chicken diets. Journal of Animal and Veterinary Advances 8, 1860-1863.

FADLALLA I. M. T., MOHAMMED B. H., BAKHIET A. O. (2010): Effect of feeding garlic on the performance and immunity of broilers. Asian Journal of Poultry Science 4, 182-189.

FAYED R. H., RAZEK A. H. A., JEHAN M. O. (2011): Effect of dietary garlic supplementation on performance, carcass traits and meat quality in broiler chickens. Animal hygiene and sustainable livestock production Proceedings of the 15 International Congress of the International Society for Animal Hygiene, Vienna, Austria, 3-7 July 2011, 1, 471-474.

ISSA K. J., OMAR J. M. A. (2012): Effect of garlic powder on performance and lipid profile of broilers. Open Journal of Animal Sciences 2, 62-68.

KONJUFKA V., PESTI G., BAKALLI R. (1997): Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poultry Science 76: 1264-1271.

KUMAR M, BERWAL J. S. (1998): Sensitivity of food pathogenes to garlic (Allium Sativum). J. Appl. Microbiol., 84, 213-215.

MILOŠEVIĆ, N., STANAĆEV V., PERIĆ L., ĐUKIĆ STOJČIĆ M., VELJIĆ M. (2013): Effects of different levels of garlic powder in the diet on production parameters and slaughter traits of broiler chickens. European Poultry Science 77, 254-259.

PERIĆ, L., MILOŠEVIĆ N., ŽIKIĆ D., BJEDOV S., CVETKOVIĆ D., MARKOV S., MOHNL M., STEINER T. (2010): Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. Archiv fur Tierzucht-Archives of animal breeding 53, 350-359.

PETRIČEVIĆ V.. JOKIĆ Ž.. LUKIĆ M.. ŠKRBIĆ Z.. PAVLOVSKI Z.. VITOROVIĆ D.. PETRIČEVIĆ M. (2013): Effect of use of raw soybean in the final mixtures for broiler chickens on body weight. relative weight of the pancreas and the edible inner organs. Proceedings of the 10th International Syposium "Modern Trends in Livestock Production" Belgrade. Republic of Serbia 2-4 October 2013. (CD of Proceedings. ISBN: 987-86-8243169-5 p 902-910).

PUVAČA N., KOSTADINOVIĆ LJ., LJUBOJEVIĆ D., LUKAČ D., LEVIĆ J., POPOVIĆ S., NOVAKOV N., VIDOVIĆ B., ĐURAGIĆ O. (2015): Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. European Poultry Science 79, 1-13.

PUVAČA, N., STANAĆEV V., GLAMOČIĆ D., LEVIĆ J., PERIĆ L., STANAĆEV V., MILIĆ D. (2013): Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal 69, 27-34.

RAHMATNEJAD E., ROSHANFEKR H. (2009): Evaluation the effect of several non-antibiotic additives on growth performance of broiler chickens. Journal of Animal and Veterinary Advances 8, 1757-1760.

STANAĆEV V., GLAMOČIĆ D., MILOŠEVIĆ N., PUVAČA N., STANAĆEV V., PLAVŠA N. (2011): Effect of garlic (*Allium sativum* L.) in fattening chicks nutrition. African Journal of Agricultural Research 6, 943-948.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE MOST COMMON VIRAL INFECTIONS THAT CAN CAUSE SWINE REPRODUCTIVE PROBLEMS

Diana Lupulović, Jasna-Prodanov Radulović, Tamaš Petrović, Gospava Lazić, Jelena Apić, Milena Samojlović, Sava Lazić

Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia
Corresponding author: Diana Lupulović, diana@niv.ns.ac.rs
Original scientific paper

Abstract: Many viruses are responsible for the reproductive failure in sows and gilts, especially in countries with intensive pig production. The most important viral infectious agents are Aujeszky´s disease virus (ADV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV) and porcine circovirus type 2 (PCV2). The presence of these viruses can cause fetal death, abortions, mummified and stillborn piglets, increased number of neonatal deaths or return to oestrus. Contaminated boar semen can also serve as a source of infection on the farm. The objective of this study was to analyze the presence of ADV, PRRSV, PPV and PCV2 in the tissue of aborted fetuses and placenta of sows and PRRSV in boar semen for the five-year period (2012-2017). The specimens originated from Vojvodina Province, Serbia. In total, 150 samples of aborted fetuses were submitted to the lab and 445 different analyses were performed in 150 samples (80 virus isolations of ADV, 78 virus isolations of PPV, 52 PCR analyzes of ADV, 41 PCR analyzes of PPV, 133 RT-PCR analyzes of PRRSV and 61 PCR analyzes of PCV2. PRRSV was detected in 4.51% (6/133) of tested specimens, while 1.64% (1/61) samples were positive for PCV-2. Neither ADV nor PPV were isolated on cell culture tissue or identified by PCR. During the same period, 16 samples of boar semen were examined on the presence of PRRSV and no evidence of this viral pathogen was identified. The number of detected positive cases is probably underestimated due to several factors: absence of data for the abortions in backyard pigs, lower sensitivity of classical virological methods comparing to molecular techniques, low quality of analyzed samples and only sporadic testing of boar semen for the artificial insemination. The authors of this article concluded that more intensive laboratory analyses should be conducted with the aim to identify and control the viral pathogens in pig production systems.

Key words: pigs, reproductive problems, viruses, Vojvodina Province

Introduction

Reproductive failure in pigs can significantly increase the production costs and may reduce the profit in pig industry. Fertility problems are displayed mainly through abortions of pregnant saws and premature farrowing, but also through embryonic or fetal deaths with the resorption, mummification and maceration of fetuses. However, the absence of any pathological findings is not rear and often are present only disorders in reproductive parameters (pregnancy rate, farrowing rate, back in oestrus after insemination, etc.) *(Pozzi and Alborali, 2012).*The causes of swine abortions can be divided in two major groups: agents that affect reproduction by local and systematic infections and factors that are responsible for hormonal disbalance and physiological disorders *(Salogni et al., 2016).* The first category encompasses the large number of viruses and bacteria that are involved in fetal deaths or placental infections. The most important viral infectious agents are: *Aujeszky´s disease virus* (ADV), *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Porcine parvovirus* (PPV) and *Porcine circovirus type 2* (PCV2), but also *Classical swine fever virus* (CSFV), *Porcine enteroviruses* (PEVs) and *Encephalomyocarditis virus* (EMCV). The most prevalent bacteria are: *Escherihia coli, Streptococcus sp., Staphylococcus sp., Eryipelothrix rhusiopathiae, Leptospira sp.* and *Brucella suis* (*Salogni et al., 2016).* Viruses may infect embryos and fetuses via blood and placenta, but contaminated boar semen can also serve as a source of infection on the farm. In general, viral agents usually cause stillbirths and mummifications, while the consequences of bacterial infections are resorption and maceration of fetuses. The second group of factors includes different toxins, systematic diseases in sows, environmental factors, nutrition, etc. *(Givens and Marley, 2008; Maldonaldo et al., 2005).*

The objective of this study was to analyze the presence of ADV, PRRSV, PPV and PCV2 in the tissue samples of aborted fetuses and placenta of sows and PRRSV in boar semen for the five-year period (2012-2017). In addition, current epidemiological situation and the prevalence of these pathogens in the region will be discussed and compared to the situation in Serbia.

Aujeszky disease (AD)

Aladar Aujeszky described for the first time new disease of animals in Hungary in 1902, which was named after him - *Aujeszky disease (Aujeszky, 1902).* Nowadays it is also known as *Pseudorabies*, because of some clinical features that are similar to rabies.

Aujeszky disease virus (ADV), or Suid herpesvirus-1 (SHV-1), is a member of the subfamily *Alphaherpesvirinae* within the family *Herpseviridae*. The mature virion has a double-stranded DNA genome *(Pomeranz et al., 2005; OIE, 2016)*. Pigs are the natural host of the disease and can survive the infection, but can remain latently infected and long-term shedder of the virus. The majority of other mammals (except humans and teiless primates) are susceptible to infection. The clinical signs of Aujeszky disease depend on the age of the animals: in suckling piglets are present neurological disorders with hyperthermia and mortality rate of up to 100%; pigs older than two months develop mostly respiratory signs with pneumonia and hyperthermia but usually survive the infection. The virus can cross the placenta and in pregnant saws abortions are often the only sign of the disease while infected boars can spread ADV through semen. Other animals cannot survive the infection and die after 2-3 days with intensive pruritus and scratching parts of the body *(Pejsak and Truszczynski, 2006; OIE, 2016).*

Control of Aujeszky disease is conducted by the use of attenuated and inactivated vaccines. But, vaccination of animals reduces only clinical signs of the disease and animals remain reservoir of virus and spread ADV among the herd. Because of that, many countries implemented national eradication programs to eliminate the disease from pig population. Eradication programs are based on the intensive vaccination with marker vaccines and application of various biosecurity measures *(Pensaert at al., 2004).* The status of ADV-free countries acquired: The United States of America, New Zealand, Canada and the majority of EU members *(Pejsak and Truszczynski, 2006)*. However, AD is still endemic worldwide and is present in Spain, Greece, Japan, China, Portugal, Poland, Romania, etc.*(Maldonaldo et al., 2005; Papageorgiou et al., 2011; Sun et al., 2016; OIE WAHIS, 2016).* Wild boars represent reservoir of ADV in nature and so far Spain and Germany reported outbreaks of AD in feral pigs in 2002 and 2010, respectively *(Meier et al., 2015; Gortazar et al, 2002; Schultze et al., 2010).*

The outbreaks of AD have been recorded in suckling piglets and domestic carnivores in Vojvodina province *(Pušić et al., 2006; Lazić et al., 2014).* Aujeszky virus was detected for the first time in 2015 in the wild boar population in Serbia *(Milicevic at al., 2016)*. There is neither wide national vaccination program nor eradication strategy for AD established in Serbia.

Porcine reproductive and respiratory syndrome (PRRS)

Porcine reproductive and respiratory syndrome (PRRS) has enormous economical influence on the swine industry. The first clinical case was reported in 1987 in North Carolina (USA), but the aetiological agent of the disease remained unknown *(Loula T., 1991).* The similar outbreaks were registered in Germany and spread rapidly through Europe in 1990-1992. The researchers from the Netherlands were identified porcine reproductive-respiratory syndrome virus (PRRSV) in 1991

(Wensvoort et al., 1991). The disease is now present worldwide, with the exception of Australia, New Zealand, Finland, Norway, Sweden and Switzerland. There are present two major strains: genotype1 (Lelystad virus), which is predominant in Europe and genotype 2 that is prevalent in North America (VR-2332 isolate as represenative).

The PRRSV is RNA virus belonging to the family *Arteriviridae,* genus *Arterivirus.* Epidemic outbreaks of PRRS involve abortions (usually in the third-trimester of the gravity), stillbirths, weakborn and mummified piglets, etc. Clinical signs in sows and gilts are often absent or atypical with the loss of appetite, fever and lethargy *(Mengeling et al., 2000).* In weaned pigs are described reduced growth, rapid breathing and elevated mortality due to complication of secondary bacterial infections. However, the intensity of clinical signs vary depending on the strain of the virus, variation in pathogenicity, immune status of the herd but also of the age of animal and presence of co-infections with bacterial agents *(Cho et Dee, 2006; OIE, 2008).* Despite these facts, the clinical picture of PRRS dramatically changed in 1996 in North America and the outbreak of disease was characterized with abortions in all stages of gestation and fever and high mortality in saws and gilts. This emergency was called “atypical” or “acute” PRRS *(Mengeling et al., 2000).*

The routes of PRRSV transmission are mainly via direct contact between infected and naïve animals and through infected boar semen. Horizontal transmission of PRRSV from mother to fetus is also previously described and is responsible for reproductive problems *(Cho and Dee, 2006; Yaeger et al., 1993, Karniychuk and Nauwynck, 2013).* The control of PRRS infection involves the use of attenuated (modified live vaccines, MLV) or inactivated (IV) vaccines *(Pileri and Mateu, 2016).*

It is assumed that PRRS was introduced for the first time in Serbia in 2001. The clinical picture encompasses massive respiratory disorders on 2 pig farms with high mortality in pigs. Following these outbreaks, more extensive research was conducted and tissue samples (lung, lymphonodes) from 42 PRRS suspicious pigs were analyzed by RT-PCR from 2008-2010. Out of 42 samples, PRRS RNA was detected in 21 specimens (21/42, 50%). Phylogenetic analyzes revealed that Serbian PRRS isolates belong to genotype-1 and form different clusters in pan-European subtype 1 *(Petrović et al., 2011).*

Porcine Parvovirus (PPV)

Porcine parvovirus (PPV), recently named *Ungulate protoparvovirus 1 (classical porcine parvovirus, PPV-1),* is associated with reproductive disorders in the early period of gestation, involving the increased number of fetal deaths and mummified fetuses. However, in gilts and saws the only clinical signs often are only abortions and delayed returns to oestrus, while acutely infected boars can spread PPV via semen *(Mengeling et al., 2000; Guerin and Pozzi, 2005).*

Porcine parvovirus was described for the first time in 1966 as a causative agent of abortions and neonatal losses in the pig herds in England and Scotland *(Cartwright et al., 1969).* In 1981 van *Leengoed et al. (1983)* reported the increased number of mummified piglets in litters in a commercial swine farm and PPV was isolated as an aetiological agent of the infection. It was recognized that PPV is responsible for the wide range of reproductive problems in pigs, which are summarized in the acronym SMEDI *(stillbirth, mummification, embryonic death and infertility) (Truyen and Streck, 2012).* Co-infections of porcine parvovirus (PPV) and porcine circovirus 2 (PCV) are quite often identified in samples of stillborn piglets *(Pescador et al., 2007; Woods et al., 2009; Sun et al., 2015).*

The genome of PPV is single-stranded DNA, belonging to the family *Parvoviridae*, subfamily *Parvovirinae*. Thanks to the modern molecular techniques, several novel PPVs, from the moment of discovery of PPV-1 have been described in pigs so far (from PPV-2 to newly discovered PPV-7). The pathogenicity of all these PPV types is still unknown *(Streck et al., 2015).*

Porcine parvovirus is endemic in most swine herds. Gilts develop active immunity after the contact with the infected animals and in that case, PPV rarely cause reproductive problems. Only pregnant gilts immunologicaly naïve for PPV, when are introduced into the already infected herd, are exposed to risk from acute infection and abortions *(Mengeling et al., 2000).* For protection against PPV-induced reproductive disorders vaccination with inactivated vaccines is recommended. Reproductive failure in wild boars associated to PPV is described in acute outbreak of abortions in China *(Zhang et al., 2010).*

Porcine parvovirus infection was introduced into Serbia during 1970´ and nowadays is present as a persistent inaparent infection. From 1998 to 2002 a couple of studies were conducted with the established seroprevalence of 70-77% *(Došen et al., 2002).*

Porcine Circovirus 2 (PCV 2)

Porcine circovirus 2 is DNA virus that belongs to the family *Circoviridae,* genus *Circovirus*.

Porcine circovirus 2 (PCV2) was first described in specific-pathogen-free pigs in 1991 in Canada with the clinical picture of wasting and jaundice *(Clark, 1996)*. In general, PCV 2 is linked to pre- and post-natal complications. Pre-natal manifestations involved reproductive problems, while post-natal disorders include post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC) and exudative epidermitis *(Chae, 2005; Segales, 2012).* There are reports of PCV 2 induced late term abortions, stillbirths and mummified fetuses *(Brunborg et al., 2007). Madson et al. (2009)* demonstrated that PCV 2 could be present in semen of infected boars, but the amount of virus is not sufficient to cause reproductive problems.

First detection of PCV 2 in Serbia was reported in 2003. DNA PCV2 was identified by PCR method in tissue samples of diseased pigs that were submitted to the laboratory with suspicious on classical swine fever disease *(Toplak et al., 2003).* Furthermore, genetic characterization and phylogenetic analyzes were conducted. The obtained result indicated that the predominant genotype in Serbia is PCV2b, which is closely related to the previously described genotypes in Europe *(Toplak et al., 2012).*

Materials and Methods

A total number of 150 aborted pig fetuses and placentas, as well as 16 imported semen samples of boars were submitted to the Scientific veterinary institute “Novi Sad” during a five-year period (2012 – 2017). The submitted fetuses and placentas were analyzed on the presence of ADV, PRRSV, PPV and PCV2, while boar semen were examined on the presence of PRRSV. All reproductive failure cases originated from the pig farms with forrow-to finish production system on the territory of Vojvodina Province (Serbia).

After a necropsy, tissue specimens of lung, kidney, spleen, liver and lymfonodes or whole carcasses of aborted fetuses, as well as parts of placentas (if available) were collected. All organs were pooled (one poll per animal), homogenized (10% w/v in normal saline solution) and further analyzed or frozen on -80°C until testing. Diluted boar semen samples were submitted in fresh condition, in the amount of 2 ml and immediately analyzed within the same day for the presence of PRRSV.

In general, 445 different analyzes were performed in 150 samples (80 virus isolations of ADV, 78 virus isolations of PPV, 52 PCR analyzes of ADV, 41 PCR analyzes of PPV, 133 RT-PCR analyzes of PRRSV and 61 PCR analyzes of PCV2). Virus isolations of ADV and PPV were carried out in porcine kidney cell lines (PK-15) and daily observed for the presence of cytopathogenic effect, according to OIE protocol for isolation of ADV (2012) and previously described method by *Allan et al. (1998)* for isolation of PPV. For the extraction of RNA and DNA commercial kit for molecular diagnostics“QIAamp *cador* Pathogen Mini kit” (Qiagen, Germany) was used, according to manufacturer´s instruction.

Examination of the presence of ADV DNA, PPV DNA and PCV DNA were conducted by real-time polymerase chain reaction (real-time PCR), using commercial kit "*TaqMan Universal PCR Master kit*" (Applied Byosystems) and based on previously publish methodology, respectively *(Wenjun et al., 2008; Cuiping et al., 2010; Olvera et al., 2004)*. For detection of PRRSV RNA was used method of conventional gel-based reverse transcription - polymerase chain reaction (RT-PCR) with the commercial kit "OneStep RT-PCR kit" (Qiagen, Germany), in accordance to laboratory protocol described by *Donadeu et al. (1999).*

Results

Our results showed that only PRRSV and PCV2 were detected among analyzed tissue samples from aborted fetuses and placentas for the period 2012-2017 (Table 1). More specific, PRRSV RNA was found in 4.51% (6/133) of pooled samples and PCV2 DNA in 1.61% (1/61) specimens. The highest number of positive PRRS cases, 27.27% (3/11) was identified in 2013, followed by 11.11% (2/18) of cases in 2015 and 1.85% (1/54) in 2016. PCV2 DNA was found only in 1.75% (1/57) analyzed samples in 2016. From 2013 until mid-2015 for detection of ADV and PPV in pathological samples was used virus isolation that has been replaced with up-to date molecular method of detection. No evidence of ADV and PPV was registered during the period of 5 years, with both laboratory techniques. The simultaneous presence of two or more viral agents has not been identified in any of the tested samples, in fact, no viral co-infections were found (data not shown in Table 1).

Table 1.ADV, PPV, PRRSV and PCV2 in pig fetuses and placentas

Year	Isolation of ADV	PCR ADV	Isolation of PPV	PCR PPV	RT-PCR PRRSV	PCR PCV2
	Positive/tested (%)	Positive/tested (%)	Positive/tested (%)	Positive/tested (%)	Positive/tested (%)	Positive/tested (%)
2012	0/1 (0%)	No data	1/0 (0%)	No data	0/3 (0%)	-
2013	0/18 (0%)	No data	17/0 (0%)	No data	3/11 (27.27%)	0/1 (0%)
2014	0/46 (0%)	No data	47/0 (0%)	No data	0/47 (0%)	-
2015	0/15 (0%)	0/3 (0%)	13/0 (0%)	1/0 (0%)	2/18 (11.11%)	0/3 (0%)
2016	No data	0/49 (0%)	No data	40/0 (0%)	1/54 (1.85%)	1/57 (1.75%)
Total	**0/80 (0%)**	**0/52 (0%)**	**0/78 (0%)**	**0/41 (0%)**	**6/133 (4.51%)**	**1/61 (1.64%)**

Sixteen imported boar semen was checked for the presence of PRRSV by conventional gel-based RT-PCR and no one sample was found to be positive.

Discussion

According to the *Program of animal health protection measures*, which is prescribed each year by Veterinary Directorate of Republic of Serbia, diagnostic examination of all abortions are notifable to the competent veterinary organization and veterinary inspection. First investigations were undertaken in 2007, but regular submitting of samples started in 2012. From 2012 until now, the program changed from year to year and different analyzes and methods were used. From the beginning of 2017 program is finally well defined and encompasses investigation of the following virological agents: ADV, PRRSV, PCV2 and PPV with the addition of classical swine fever virus (CSFV). Diagnosis of viruses in pathological material is based on real-time PCR and RT-PCR.

The results in our study suggested that PRRSV and PCV2 are the most prevalent virological agents identified in aborted pig fetuses and placentas in the tested region and time interval. PRRSV was observed in 6 out of 133 tissue samples (4.51%) and PCV2 in 1 out of 61 specimens (1.64%). ADV and PPV were not found in the tested samples. Our finding is in accordance to the survey conducted in Spain where PRRSV and PCV2 were detected in 9% (9/100) and 1% (1/100) of analyzed samples, respectively, with no evidence of the presence of ADV and PPV *(Maldonaldo et al., 2005).* The research group of *Salogni et al. (2016)* also detected PCV2 and PRRSV in the majority of aborted fetuses in Italy.

Although only PRRSV and PCV2 were found in our tested samples, it is unlikely that these viruses are the only agents responsible for the reproductive failure. The presence of PCV2 usually weakens the immune system that further enhances the severity of bacterial infections. In the field conditions, PCV 2 rarely acts alone and usually requires the presence of other viruses, such as PPV or PRRSV *(Allan and Ellis 2000; Opriessnig and Halbur, 2007).* Nowadays, but also in the past, PRRS infection was clinically manifested in the form of respiratory disorders and no massive abortions connected to PRRSV were reported in Serbia *(Petrović et al., 2011).*

The absence of ADV in tested samples was expected, since Aujeszky disease is lately often detected in the category of piglets up to two weeks old and is not recognised as a causative agent of abortions. On large farms control of ADV previously was based on the vaccination with live attenuated vaccines, while pigs on small farms and backyard pigs weren´t vaccinated. Epidemiological situation has changed when the production of domestic live attenuated vaccine was stopped and were introduced imported marker vaccines. The use of these vaccines is irregular which led to the moderate immunization of sows and gilts without the occurance of abortions while piglets easily acquired infection after the expiring of low quality maternal immunity. This practice has the consequence of the several outbreaks of AD in pigs with the high loss in suckling piglets *(Pušić et al., 2011; Lazić et al., 2014).* It is also not surprising that we didn't diagnosed PPV in aborted fetuses, since the majority of farms implemented vaccination policy starting from 2001 *(Lupulović et al., 2008).* In the late 90´ abortions in pigs induced by PPV were registered sporadically, but nowadays reproductive failure caused by porcine parvovirus is of low incidence in Serbia.

In our survey viral agents were diagnosed in only 4.66% (7/150) of examined samples, however, we assumed that these results should be interpreted with caution. Several factors may affect on this result. For example, the number of submitted samples is probably underestimated, because many abortions aren´t regularly notified to the veterinary service, especially when happened in backyard pigs and small farms, which make 81.06% of all domestic pigs *(Prodanov-Radulović et al., 2017).* Quality of samples is also very important and has impact on the final outcome of the analysis. The sample of poor quality can cause a lack of virus isolation. On the other hand, molecular laboratory methods are more sensitive, less time consuming and can detect the viruses in lower quality samples *(Pestana et al., 2010).* This explains the higher number of PCV2 and PRRS positive results examined exclusively by real-time PCR and RT-PCR comparing to negative PPV and ADV results that were analyzed in the beginning by virus isolation test and later on by real-time PCR. It should be also noted that not all the samples were tested on the presence of ADV, PRRS, PPV and PCV2 and the decision of number of tests depended on the estimation of veterinary inspectors. When the program starts in 2012 just few samples were submitted for laboratory analysis and in the following years this number of samples raised, which also gives us incomplete picture of the causative agents of abortions for the five-year period of study.

Finally, our results confirm already reported finding that the high percentage of abortions can be the consequence of physiological problems in reproductive tract and often is not caused by the infectious agents *(Holler et al., 1994).* Also, bacterial infections more frequently induce abortions than viruses *(Givens and Marley, 2008).*

It has been previously demonstrated that boars can shed viruses in semen *(Maes et al., 2008).* In sixteen samples of imported boar semen wasn't detected the presence of PRRSV and this finding ensure the quality of semen for AI insemination.

Conclusion

In summary, our data indicates that only PRRSV and PCV2 are confirmed as the causal agents of the reproductive problems in pig produstions systems in Serbia during analysed time period, while no evidence of ADV and PPV was established. Viral agents were detected in 4.66 % (7/150) analyzed samples.
The number of detected positive cases is probably underestimated due to several factors: absence of data for the abortions in backyard pigs, lower sensitivity of classical virological methods comparing to molecular techniques, low quality of analyzed samples and only sporadic testing of boar semen for the artificial insemination. In addition, it is very important the adoption of Rulebooks for control and monitoring of AD, PRRS, PCV2 and PPV diseases. The authors of this article concluded that more intensive laboratory analyses should be conducted with the aim to identify and control the viral pathogens in pig production systems. These examinations will significantly improve the livestock production in Serbia and will give the solid base for necessary actions that should be done for the improvement of the health condition in pig industry.

Najčešće virusne infekcije koje izazivaju reproduktivne poremećaje kod svinja

Diana Lupulović, Jasna-Prodanov Radulović, Tamaš Petrović, Gospava Lazić, Jelena Apić, Milena Samojlović, Sava Lazić

Rezime

Mnogi virusi su odgovorni za pojavu reproduktivnih problema kod krmača i nazimica, posebno u zemljama sa intenzivnom proizvodnjom svinja. Najvažniji virusni infektivni agensi su: virus Aujeszkijeve bolesti (ADV), virus reproduktivnog i respiratornog sindroma svinja (PRRSV), parvovirus svinja (PPV) i cirkovirus svinja tip 2 (PCV2). Prisustvo ovih virusa može da izazove smrt fetusa, abortus, rađanje mumificirane i mrtvorođene prasadi, povećan broj neonatalnih uginuća ili povađanja. Kontaminirana sperma nerastova može biti izvor zaraze na farmi.

Cilj ovog istraživanja bio je da se analizira prisustvo ADV, PRRSV, PPV i PCV2 u tkivu pobačenih fetusa i placenti i PRRSV u uzorcima sperme nerastova u periodu od pet godina (2012-2017). Uzorci potiču sa teritorije Vojvodine (Srbija). U laboratoriju je ukupno dostavljeno 150 uzoraka pobačenih fetusa a izvršeno je 445 različitih analiza u 150 uzoraka (80 virusnih izolacija ADV, 78 izolacija PPV, 52 PCR analiza za ADV, 41 PCR analiza za PPV, 133 RT-PCR naliza za PRRSV i

61 PCR analiza za PCV2). PRRSV je detektovan u 4,51% (6/133) testiranih uzoraka, dok je 1,64% (1/61) uzoraka bilo pozitivno na PCV-2. Ni u jednom uzorku nije utvrđeno prisustvo ADV i PPV metodom izolacije na kulturi tkiva ili PCR tehnikom. U istom periodu pregledano je 16 uzoraka sperme nerastova na prisustvo PRRSV i ni u jednom uzorku nije identifikovan virus.
Broj otkrivenih pozitivnih slučajeva je verovatno manji zbog nekoliko faktora: nedostatak podataka za pobačaje u malim gazdinstvima, manja osetljivost klasičnih virusoloških metoda u odnosu na molekularne tehnike, loš kvalitet analiziranih uzoraka i neredovno testiranje semena nerastova koje se koristi za veštačku oplodnju. Autori ovog članka su zaključili da je potrebno sprovesti intenzivnije laboratorijske analize sa ciljem da se identifikuju i kontrolišu virusni patogeni u proizvodnom sistemu uzgoja svinja.

Ključne reči: svinje, reproduktivni problemi, virusi, AP Vojvodina

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant TR 31084.

References

ALLAN G. AND ELLIS J. (2000): Porcine circoviruses: a review. Journal of Veterinary Diagnostic Investigation 12, 3-14.
ALLAN G.M., MCNEILLY F., KENNEDY S., DAFT B,, CLARKE E.G., ELLIS J.A., HAINES D.M., MEEHAN B.M., ADAIR B.M. (1998): Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. Journal of Veterinary Diagnostic Investigation (10), 3–10.
AUJESZKY A. (1902): Ueber eine new Infektions krankheit bei Haustieren. Zentbl. Bakt. ParasitKde, I 32, 353-357.
BRUNBORG IM., JONASSEN CM., MOLDAL T., BRATBERG B., LIUM B., KOENEN F., SCHONHEIT J. (2007): Association of myocarditis with high viral load of porcine circovirus type 2 in several tissues in cases of fetal death and high mortality in piglets. A case study. Journal of Veterinary Diagnostic Investigation 19(4), 368-375.

CARTWRIGHT S.F., LUCAS M., HUCK R.A. (1969):A small haemagglutinating porcine DNA virus: Isolation and properties. Journal of Competitive Pathology (79), 371–377.
CHAE C. (2005): A review of porcine circovirus 2-associated syndromes and diseases. The Veterinary Journal, 169(3), 326-336.
CHO J. AND DEE S. (2006): Porcine Reproductive And Respiratory Syndrome Virus. Theriogenology 66, 655-662.
CLARK E.G. (1996): Post-weaning multisystemic wasting syndrome. Proceedings West-Canadian Association of Swine Practitioners, 19–20.
CUIPING S., CHAO Z., CHAOFAN Z., SHANGJIN C. (2010): Detection of porcine parvovirus using a taqman-based real-time pcr with primers and probe designed for the NS1 gene. Virology Journal, 7:353.
DONADEU M., ARIAS M., GOMEZ-TEJEDOR C., AGUERO M., ROMERO L.J., CHRISTIANSON W.T., SANCHEZ-VIZCAINO J.M. (1999): Using polymerase chain reaction to obtain PRRSV-free piglets from epidemically infected herds. Swine Health Production (7), 225–261.
DOŠEN R., GAGRČIN M., PRODANOV J., ORLIĆ D. (2002): Parvoviroza svinja. Veterinarski glasnik 56 (1-2), 9-13.
GIVENS M.D. AND MARLEY M.D. (2008): Infectious causes of embryonic and fetal mortality. Theriogenology (70), 270–285.
GORTAZAR C., VICENTE J., FIERRO Y., LEON L., CUBERO M., GONZALEZ M. (2002): NATURAL AUJESZKYS DISEASE IN A SPANISH WILD BOAR POPULATION. Annals of the New York Academy of Sciences, 969, 210-212.
GUERIN B. AND POZZI N. (2005): Viruses in boar semen: detection and clinical as well as epidemiological consequences regarding disease transmission by artificial insemination. Theriogenology (63), 556–572.
HOLLER L.D. (1994): Diagnosis of swine abortion. Swine Health and Production 2, 29–31.
KARNIYCHUK U., NAUWYNCK H. (2013): Pathogenesis and prevention of placental and transplacental porcine reproductive and respiratory syndrome virus infection. Veterinary Research 44 (95).
LAZIĆ S., LUPULOVIĆ D., LAZIĆ G., PRODANOV-RADULOVIĆ J., PUŠIĆ I., DOŠEN R., POLAČEK V., PETROVIĆ T. (2014): Potrebe i iskorenjivanja Aujeckijeve bolesti u Republici Srbiji. Proceedings of the Symposium XVI Epizootiology days of Serbia, 6-7 November, Zrenjanin, 17-22.
LOULA T. (1991): Mystery pig disease. Agri-Practice 12, 23-34.
LUPULOVIĆ D., PRODANOV J., LAZIĆ S., DOŠEN R. (2008): Rasprostranjenost parvovirusne infekcije svinja na farmama u Vjvodini i prednosti

vakcinacije. VI kongres medicinske mikrobiologije Mikromed 2008, Beograd, 11-14. jun 2008, 293-294.

MADSON D.M., RAMAMOORTHY S., KUSTER C., PAL N., MENG X.J., HALBUR P.G., OPRIESSNIG T. (2009): Infectivity of porcine circovirus type 2 DNA in semen from experimentally-infected boars. Veterinary Research, 40:10.

MAES D., NAUWYNCK H., RIJSSELAERE T., MATEUSEN B., VYT PH., DE KRUIF A., VAN SOOM A. (2008): AI transmitted diseases in swine: an overview. Theriogenology (70), 1337-1345.

MALDONADO J, SEGALÉS J, MARTÍNEZ-PUIG D, CALSAMIGLIA M, RIERA P, DOMINGO M, ARTIGAS C. (2005): Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain. Veterinary Journal, 169, 454-456.

MEIER R.K., RUIZ-FONS F., RYSER-DEGIORGIS MP. (2015): A picture of trends in Aujeskys disease virus exposure in wild boar in the Swiss and European contexts. BMC Veterinary Research, 11, 277.

MENGELING W.L., LAGER K.M., VORWALD A.C. (2000): The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. Animal Reproduction Science, 60-61, 199-210.

MILIĆEVIĆ V., RADOJIČIĆ S., VALČIĆ M., IVOVIĆ V., RADOSAVLJEVIĆ V. (2016): Evidence of Aujeszkys disease in wild boar in Serbia. BMC Veterinary Research, 12, 134.

OIE (2008): PRRS: the disease, its diagnosis, prevention and control. Report of the OIE *AD HOC* GROUP on Porcine reproductive respiratory syndrome, 9-11 June, Paris.

OIE TERRESTRIAL MANUAL (2016): Aujeszkys disease (Infection with Aujeszkys disease virus). Chapter 2.1.2., 97-113.

OIE WORLD ANIMAL HEALTH INFORMATION SYSTEM (WAHIS) (2016): Disease information, http://www.oie.int/wahis_/public/wahid.php/

OLVERA A., SIBILA M., CALSAMIGLIA M., SEGALÉS J., DOMINGO M. (2004): Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs. Journal of Virololgy Methods (117), 75-80.

OPRIESSNIG T., MENG X.J., HALBUR P.G. (2007): Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. Journal of Veterinary Diagnostic Investigation 19 (6), 591-615.

PAPAGEORGIOU K.V., BURRIEL A.R., FILIOUSSIS G:, CHRISTODOULOPOULOS G., NAUWYNCK H.J., KRITAS S.K. (2011):

Aujeszky's Disease (Pseudorabies). An old threat in current pig industry? Part II. Epidemiology, Immunity, Prevention and the current situation in Greece. Journal of the Helenic Veterinary Medical Society, 62(2), 125-131.

PEJSAK Z.K., TRUSZCZYNSKI M.J. (2006): Aujeszkys Disease (Pseudorabies). Diseases of Swine, 9th ed, Blackwell Publishing, 419-433.

PENSAERT M., LABARQUE G., FAVOREEL H., NAUWYNCK H. (2004): Aujeszky's disease vaccination and differentiation of vaccinated from infected pigs. Developments in biologicals (Basel), 119, 243-54.

PESCADOR C., BANDARRA P., CASTRO L., ANTONIASSI N., RAVAZZOLO AP., SONNE L., CRUZ C., DRIEMEIER D. (2007): Co-infection by porcine circovirus type 2 and porcine parvovirus in aborted fetuses and stillborn piglets in southern Brazil. Pesquisa Veterinária Brasileira 27(10), 425-429.

PESTANA E**.,** BELAK S**.,** DIALLO A., CROWTHERJ.R., VILJOEN G.J. (2010): Early, rapid and sensitive veterinary molecular diagnostics - real time PCR applications. Springer Netherlands.

PETROVIĆ T., MILIĆEVIĆ V., RADULOVIĆ-PRODANOV J., MAKSIMOVIĆ-ZORIĆ J., LUPULOVIĆ D., DOŠEN R., LAZIĆ S. (2011): Molecular detection and genetic analysis of Serbian PRRSV isolates. EuroPRRS2011 Symposium 'Understanding and combating PRRS in Europe' COST Action FA FA902, Novi Sad, 12th-14th October 2011, 50- 56.

PILERI E., MATEU E. (2016): Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. Veterinary Research 47, 108.

POMERANZ I.E., REYNOL S.A.E., HENGARTNER C.J. (2005): Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. Microbiology and Molecular Biology Reviews, 69, 462-500.

POZZI P.S. AND ALBORALI G.L. (2012): Reproductive diseases in sows (Sus scrofa domestica): A Review. Israel Journal of Veterinary Medicine 67 (1), 24-33.

PRODANOV-RADULOVIĆ J. (2017): COST Action: CA15116 Understanding and combating African Swine Fever in Europe (ASF-STOP) Meeting, Istituto Zooprofilattico Sperimentale Della Lombardia Ed Emilia Romagna (IZSLER), Brescia, Italy.

PUŠIĆ I., ĐURIČIĆ B., LAZIĆ S., DOŠEN R., PRODANOV J. (2006): Putevi prenošenja i širenja virusa Aujeskijeve bolesti na mesojede. Veterinarski glasnik 60 (5-6), 369-375.

PUŠIĆ I., PRODANOV-RADULOVIĆ J., DOŠEN R., STOJANOV I., STOJANOVIĆ I., STOJANOVIĆ D., PETROVIĆ T. (2011): Epizootical characteristics of Aujeskys disease in Vojvodina region and biosecurity concernes. Biotechnology in Animal Husbandry, 27(3), 875-882.

SALOGNI C., LAZZARO M., GIACOMINI E., GIOVANNINI S., ZANONI M., GIULIANI M., RUGGERI J., POZZI P., PASQUALI P., BONIOTTI M.B., ALBORALI L.G. (2016): Infectious agents identified in aborted swine fetuses in a high-density breeding area: a three-year study. Journal of Veterinary Diagnostic Investigation, 28(5), 550–554.

SCHULZE CHA., WOHLSEIN P., KUTZER P., MÜLLER T. (2010): Spontaneous Aujeszkys disease (pseudorabies) in European wild boar (Sus scrofa) in the federal state of Brandenburg, Germany. Berliner und Münchener Tierärztliche Wochenschrift, 123, 359-364.

SEGALÉS J. (2012): Porcine circovirus type 2 (PCV2) infections: Clinical signs, pathology and laboratory diagnosis. Virus Research 164, 10-19.

STRECK A.F., CANAL WAGECK C., TRUYEN U. (2015): Molecular epidemiology and evolution of porcine parvoviruses. Infection, Genetics and Evolution, 36, 300-306.

SUN J., HUANG L., WEI Y., WANG Y., CHEN D., DU W., WU H., LIU C. (2015): Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. Archives of Virology, 160, 1339-1344.

SUN Y., LUO Y., WANG CH., YUAN J., LI N., SONG K., QUI HJ. (2016): Control of swine pseudorabies in China: Opportunities and limitations. Veterinary Microbiology, 183, 119-124.

TOPLAK I., LAZIĆ S., LUPULOVIĆ D., PRODANOV-RADULOVIĆ J., BECSKEI Z., DOŠEN R., PETROVIĆ T. (2012): Study of the genetic variability of porcine circovirus type 2 detected in Serbia and Slovenia. Acta Veterinaria Hungarica 60(3), 409-420.

TOPLAK I., PETROVIĆ T., GROM J., HOSTNIK P., BARLIČ-MAGANJA D. (2003): First detection and molecular characterisation of porcine circovirus 2 (PCV 2) in Yugoslavia in tissues of pigs suspected for classical swine fever (CSF). Proceedings of the Symposium V Epizootiological Days, 2-5 April, Subotica, 314.

TRUYEN U. AND STRECK AF. (2012): Porcine parvovirus. In: Zimmerman J, Karriker L, Ramirez A, Schwartz K, Stevenson G (eds) Diseases of Swine, 10th Ed Oxfort: John Wiley & Sons Inc; 447-455.

VAN LEENGOED LA., VOS J., GRUYS E., RONDHUIS P., BRAND A. (1983): Porcine Parvovirus Infection: review and diagnosis in a sow herd with reproductive failure. The Veterinary Quarterly 5(3), 131-141.

WENJUN M.A, LAGER M.K., RICHT J.A., STOFFREGEN C.W., ZHOU F., YOON K.Y. (2008): Development of real-time polymerase chain reaction assays for rapid detection and differentiation of wild-type pseudorabies and gene-deleted vaccine viruses. Journal of Veterinary Diagnostic Investigation (20), 440–447.

WENSVOORT G., TERPSTRA C., POL JMA., TER LAAK EA., BLOEMRAAD M., DE KLUYVER EP. (1991): Mystery swine disease in the Netherlands: The isolation of Lelystad virus. Veterinary Quarterly 13, 121-130.

WOODS A., McDOWELL E., HOLTKAMP D., POGRANICHNIY R., GILLESPIE T. (2009). Reproductive failure associated with porcine parvovirus and possible porcine circovirus type 2 co-infection. Journal of Swine Health and Production 17(4), 210-216.

YAEGER MJ., PRIEVE T., COLLINSJ., CHRISTOPHER-HENNINGS J., NELSON E., BENFIELD D. (1993): Evidence for the transmission of porcine reproductive and respiratory syndrome (PRRS) virus in boar semen. Swine Health and Production (1),Number 5.

ZHANG CF., SONG CP., CHEN CM., CUI SJ., MIAO LF. (2010): Reproductive failure in wild boars associated to porcine parvovirus infection and in vivo and in vitro characterization of the casual isolate. Tropical Animal Health and Production 42, 1611-1613.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

WILD PIGS - THE SOURCE OF ZOONOTIC *STREPTOCOCCUS SUIS* STRAINS

Aleksandar Stanojković[1], Marija Gogić[1], Dušica Ostojić-Andrić[1], Maja Petričević[1], Jakov Nišavić[3], Ksenija Nešić[4], Aleksandra Stanojković-Sebić[2]

[1]Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[2]Institute of Soil Science, Belgrade, Republic of Serbia
[4]Scientific veterinary institute of Serbia, Republic of Serbia
[3]Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Republic of Serbia
Corresponding author, e-mail: izs.aleksandar@gmail.com
Short review article

Abstract: *Streptococcus suis* is a gram-positive facultative anaerobic coccoid bacterium that has the ability to synthesize capsule and secrete haemolysin. *Streptococcus suis* is primarily considered to be a domestic swine (Sus scrofa domestica) pathogen, but also can be frequently isolated from a wide range of mammalian species and also from birds. Transmission of *S. suis* among animals is considered to be mainly through the respiratory route. Different from infection in pigs, the main route of entry of *S. suis* in humans is thought to be through the contact with: cutaneous lesions (usually on the hands and arms), contaminated animals, carcasses or meat, although in some cases, no wound was detected. Also, bacteria may colonize the nasopharynx, as observed in swine and the gastrointestinal tract. It has been found that wild boar (*Sus scrofa*), which population has grown over few last decades in Europe, is indeed reservoir of the disease. This paper presents an overview of some cases in scientific literature where wild pigs were the source of infection for humans.

Key words: *Streptococcus suis*, wild pigs, source, zoonosis

Introduction

Streptococcus suis infection is probably one of the most important health issue in the swine industry worldwide. This bacteria in the most cases is the major cause of meningitis and septicemia in the porcine industry. However, other pathological conditions have also been described, such as arthritis, endocarditis, pneumonia, and sudden death.

Streptococcus suis is primarily considered to be a domestic swine (Sus scrofa domestica) pathogen, but also can be frequently isolated from a wide range of mammalian species and also from birds. These findings suggest the existence of complex epidemiological patterns of the infection, since other animal species might also be a source of swine infection *(Gottschalk et al, 2010b).* Human infections with S. suis have usually been considered sporadic except for Asian countries where this pathogen is one of the most important cause of meningitis *(Arends and Zanen, 1988).*

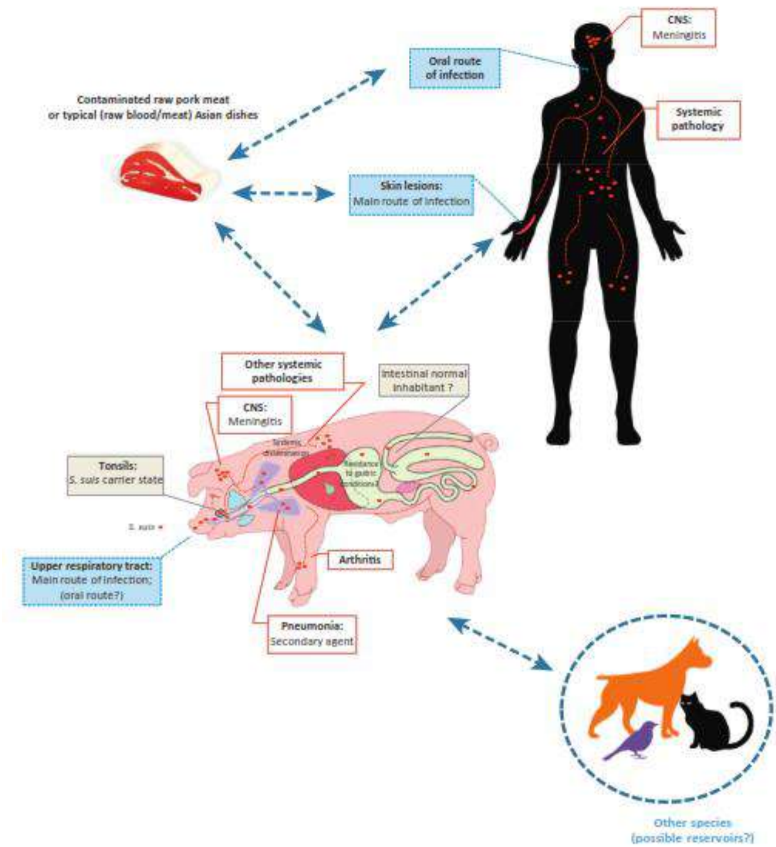
General characteristics of *Streptococcus suis* and transmission among pigs

Streptococcus suis is a gram-positive facultative anaerobic coccoid bacterium that has the ability to synthesize capsule and secrete haemolysin. Cell wall antigens of this pathogen are similar to those displayed by group D streptococci (*Stanojkovic et al., 2012),* but *S. suis* is not genetically associated with group D streptococci (*Kilpper-Balz. and Schleifer, 1987).*

Previously, *Streptococcus suis* had been classified into 35 serotypes (serotype 1/2, and 1–34) (*Higgins and Gottschalk, 1995*) and then reduced to 33 serotypes because serotypes 32 and 34 were determined to be Streptococcus orisratti, that can be often isolated from rats (*Hill et al., 2005*). More recently, serotypes 20, 22, 26 and 33 were removed from the *Streptococcus suis* taxonomy hypothesizing that this serotypes belong to some other bacterial species *(Tien et al., 2013, Okura et al., 2016).* Hence, it is currently considered that there are 29 true Streptococcus suis serotypes. According to literature, the only study of serotype distribution in Southern Europe was done in Serbia, where serotype 2 was the only serotype found in piglets with clinical symptoms of meningitis (*Stanojkovic et al., 2012*). Beside this, various *S. suis* serotypes were found in healthy animals (*Stanojković, 2012*).

Transmission of *S. suis* among animals is considered to be mainly through the respiratory route (*Stanojković et al, 2016*). Indeed, investigation of the presence of alpha hemolytic streptococci, enterococci and streptococci-like bacteria in tonsil and nose swabs of clinically healthy pigs showed that most species belonged to *S. suis* (64%) (*Stanojkovic et al., 2011*).

Different from infection in pigs, the main route of entry of *S. suis* in humans is thought to be through the contact with: cutaneous lesions (usually on the hands and arms), contaminated animals, carcasses or meat, although in some cases, no wound was detected; bacteria may colonize the nasopharynx, as observed in swine and the gastrointestinal tract, with the diarrhea as a prodromal symptom. The outbreak in China in 2005 caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health (*Stanojkovic et al., 2014).*



Reservoirs and the route of entry of Streptococcus suis (Segura et al., 2017)

Streptococcus suis and wild animals

Streptococcus suis is sporadically associated with infections in some mammalian and avian species (*Gottschalk et a., 2007).* It has been found that wild boar (*Sus scrofa*), which population has grown over ~~these~~ few last decades in Europe is indeed reservoir of the disease.

Baums et al. (2007) in Germany found that wild boar carry potentially zoonotic strains of *S. suis* serotype 2. These authors isolated *S. suis* from 92% of wild boar tonsil samples, and ninety-five percent of the cps2 strains were positive for mrp, sly and epf*. So they conclude that at least 10% of wild boars in northwestern Germany carry *S. suis* strains that are potentially virulent in humans.

According to Sánchez del Rey et al. (2014), wild boar are widely distributed throughout the Iberian Peninsula and can carry potentially virulent strains of *Streptococcus suis*. In their research samples were collected from 425 hunted wild boars between 2007 and 2010: 254 and 171 animals from North-east and Central regions of Spain, respectively, and encompassed mainly tonsils, nasal cavity or lung.

Bacteria was isolated from 39,1% of the wild boars examined and most frequently was isolated serotype 9 (12.5%), followed by serotype 1 (2.5%), while serotype 2 was rarely isolated (0.3%). Multilocus sequence typing (MLST) data of the strains from Iberian Peninsula suggest that these isolates are not related to the prevalent clonal complexes ST1, ST16, ST61 and ST87 typically associated with the infection of pigs or humans in Europe. Also, wild pigs can be a source of transmission of *S. suis* to domestic pigs that are farmed outdoors. Connection of infection of farmed outdoors mangalitsa pigs, which can carry zoonotic strains of S. suis serotype 2 and wild boars is yet to be determined (authors unpublished results). According to this paper, this indigenous pig breed can be a source of more than one serotype of S. suis, although these pigs were not in contact with either farmed pigs or wild pigs, or this contact could not be confirmed.

There are several cases of *S. suis* infection in hunters that have been reported so far *(Halaby et al., 2000; Rosenkranz et al., 2003; Dalsjo et al., 2014).* According to *Dalsjo et al. (2014)* in Sweden, more than 70 000 wild boars are shot annually, and the number of animals is steadily increasing. Authors describe the first a non-serotype 2 isolate transmitted from a wild boar to a human and is the third case of S. suis infection reported from Sweden. The patient was healthy 63-year-old smoker that had previously cut his finger during slaughtering of a wild boar 2 days before admission to the hospital. Although, nothing is known about the presence of the putatively virulent S. suis serotype 14 in wild boars, it seems that S. suis of this serotype has a high pathogenic potential. ~~and~~ Increasing numbers of wild boars in Sweden and the present case of severe infection described in that research calls for increased vigilance regarding wild boar slaughtering.

Halaby et al. (2000) reported a case where a 63-year-old previously healthy bank employee was referred to the hospital with loss of consciousness. After the patient had regained consciousness it is noted that he had shoot and butchered wild boar and that he suffered from cuts on face and hands while he was dragging dead animal through the blackberry bushes.

Similar to this case, *Rosenkranz et al. (2003)* reported that an 51-year-old man was admitted to the hospital with dizziness, headache, neck rigidity, nausea, and progressive loss of consciousness. The history of the patient's lifestyle and habits regarding porcine exposure revealed that, although he never had contact with domestic pigs, he was a recreational hunter and had shot and butchered a wild sow two days preceding the onset of clinical symptoms. The point of bacterial entry was gingival, since hunter was chewing piece of wood while butchering the animal.

Many cases of S. suis infection are often unidentified or misidentified as some other infections. This is the case with infection acquired from domestic pigs but similarly, we believe that cases where reservoir were wild pigs are often non-identified or misidentified.

Conclusion

Streptococcus suis infection should be regarded as an occupational risk for people working with pigs, either domestic or wild, or participate in a wild boar hunting and butchering. The number of reported cases where wild pigs were reservoir of the disease are relatively small. But exactly like when butchering domestic pigs, protective gloves should be worn when slaughtering wild boars and special attention should be taken regarding infectious symptoms if traumatic cuts are inflicted. Promptly antibiotic treatment should be initiated if symptoms occur in any way.

Divlje svinje su izvor zoonotskih sojeva *Streptococcus suis*

Aleksandar Stanojković, Marija Gogić, Dušica Ostojić-Andrić, Maja Petričević, Jakov Nišavić, Ksenija Nešić, Aleksandra Stanojković-Sebić

Rezime

Streptococcus suis je gram pozitivna, fakultativno anaerobna bakterija koj ima sposobnost sintetisanja kapsule i sekrecije hemolizina. *Streptococcus suis* se smatra patogenom domaće svinje (Sus domestica), ali se često može izolovati iz velikog broja sisara i ptica. Transmisija bakterije među životinjama je uglavnom respiratornim putem. Za razliku od svinja, glavna vrata infekcije kod ljudi su male posekotine na koži, najčešće u kontaktu sa zaraženim životinjama, kontaminiranim mesom i leševima, mada u nekim slučajevima se male posekotine na koži nisu mogle potvrditi. Takođe, slično kao i kod svinja, bakterija može da kolonizuje nazofarinks i gastrointestinalni trakt ljudi. Utvrđeno je da i divlja svinja (*Sus scrofa*) čija je populacija sve brojnija u Evropi, može da bude izvor infekcije za ljude. U oovom pregledu prikazujemo mali broj slučajeva u kojima su divlje svinje bile izvor bolesti za ljude.

Ključne reči: *Streptococcus suis*, divlje svinje, izvor, zoonoza

Acknowledgment

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

References

ARENDS J.P., ZANEN H.C. (1988): Meningitis caused by Streptococcus suis in humans. Rev. Infect. Dis. 10:131-137.

DALSJO A., NILSSON A., RAMUSSEN M. (2014): Complicated infection caused by Streptococcus suis serotype 14 transmitted from a wild boar. JMM Case Reports (2014) DOI 10.1099/jmmcr.0.002105.

GOTTSCHALK M., SEGURA M., XU J. (2007): Streptococcus suis infections in humans: the Chinese experience and the situation in North America. Anim Health Res Rev; 8: 29–45.

GOTTSCHALK M., XU J., LECOURS MP, GRENIER D., FITTIPALDI N., SEGURA M. (2010): *Streptococcus suis* infections in humans: what is the prognosis for Western countries? (Part I). Clin Microbiol News 89-96.

HALABY T., E. HOITSMA, R. HUPPERTS , L. SPANJAARD, K M. LUIRIN, J. JACOBS :(2000). Streptococcus suis meningitis, a poacher's risk. Eur J Clin microbiol Infect Dis 19 , 943–945.

HIGGINS R., GOTTSCHALK M., BOUDREAU M., LEBRUN A., HENRICHSEN J.(1995): Description of six new capsular types (29–34) of Streptococcus suis. J Vet Diagn Invest 7, 405–406.

HILL J. E., GOTTSCHALK M., BROUSSEAU R., HAREL J., HEMMINGSEN S. M., GOH S. H. (2005): Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that Streptococcus suis serotypes 32 and 34, isolated from pigs, are Streptococcus orisratti. Vet Microbiol 107, 63–69.

KILPPER-BALZ R., SCHLEIFER K.H (1987): *Streptococcus suis* sp. nov.; nom.rev. International Journal of Systematic Bacteriology 37, 160-162.

OKURA M. ET AL. (2016): Current taxonomical situation of Streptococcus suis. Pathogens 5, e45.
ROSENKRANZ M., H. A. ELSNER, H. J. STÜRENBURG, C. WEILLER, J. ROTHER, I. SOBOTTKA : (2003). Streptococcus suis meningitis and septicaemia contracted from a wildboar in Germany.J Neurol 250, 869–870.
SÁNCHEZ DEL REY V., FERNÁNDEZ-GARAYZÁBAL J.F., BRIONES V., IRISO A., DOMÍNGUEZ L., GOTTSCHALK M., VELA A.I. (2013): Genetic analysis of *Streptococcus suis* isolates from wild rabbits. Veterinary Microbiology 165, 483–486.
SÁNCHEZ DEL REY V., FERNÁNDEZ-GARAYZÁBAL J.F., MENTABERRE G., BRIONES V., LAVÍN S., DOMÍNGUEZ L., GOTTSCHALK M., VELA A.I. (2014). Characterisation of *Streptococcus suis* isolates from wild boars (*Sus scrofa*). The Veterinary Journal 200, 464–467.
SEGURA M., FITTIPALDI N., CALZAS C., GOTTSCHALK M. (2017): Critical Streptococcus suis Virulence Factors: Are They All Really Critical? Trends Microbiol. 2017 Jul;25(7):585-599. doi: 10.1016/j.tim.2017.02.005.
STANOJKOVIĆ A. (2012): Investigation of presence and serotype affiliation of Streptococcus suis species in samples originating from pigs. Doctoral dissertation.
STANOJKOVIĆ A., STANIŠIĆ N., GOGIĆ M., PIVIĆ R., OSTOJIĆ-ANDRIĆ D., MANDIĆ V., STANOJKOVIĆ-SEBIĆ A. (2016): Streptococcus suis, from pen to the food chain. Second International Symposium of Veterinary Medicine – ISVIM 2016, Belgrade.
STANOJKOVIĆ A., AŠANIN R., AŠANIN J., PALIĆ K., STANOJKOVIĆ A., ŽUTIĆ J. (2011): Investigation of presence of α haemolytic streptococci, enterococci and streptococci-like bacteria in different materials originating from pigs Vet. glasnik 65 (3-4) 203 – 213.
STANOJKOVIĆ A., AŠANIN R., MIŠIĆ D., AŠANIN J., STANOJKOVIĆ-SEBIĆ A. (2012): The presence and serological types of Streptococcus suis strains isolated from pigs originating from some farms in Serbia. Fresenius Environmental Bulletin, 21 (11a) 1-4.
STANOJKOVIĆ A., PETROVIĆ M. M., ŠKRBIĆ Z., MANDIĆ V., STANIŠIĆ N., GOGIĆ M., STANOJKOVIĆ-SEBIĆ A. (2014): Biochemical characteristics of *Streptococcus strains* isolated from healthy and deceased pigs. Biotechnology in animal husbandry, vol 30, 4, ISSN 2217-7140 strane
TIEN H. T., NISHIBORI T., NISHITANI Y., NOMOTO R., OSAWA R. (2013): Reappraisal of the taxonomy of Streptococcus suis serotypes 20, 22, 26, and 33 based on DNA–DNA homology and sodA and recN phylogenies. Vet Microbiol 162, 842–849.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE UTILIZATION OF PREBIOTICS AND PROBIOTICS TO MODULATE THE INTESTINAL MICROBIOTA OF DOGS

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

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

Abstract: The intestinal microbiota plays an important role in maintaining the state of health of the gastrointestinal tract and of the host. The use of specific dietary supplements such as prebiotics and probiotics may improve the composition and metabolism of the intestinal microbial population. Among prebiotics, fructo-oligosaccharides (FOS) seem to be the most effective in modulating canine intestinal microbiota and improving intestinal absorption of minerals but with little or no effect on canine immune system. Conversely, there is evidence of the fact that feeding mannan-oligosaccharides and probiotics may have a positive influence on the canine immune system. Furthermore, evidence exists that some positive effects of prebiotics in dogs might be enhanced if these are used in combination with specific probiotic strains, in the form of a synbiotic. Clinical effects of prebiotics and probiotics have been investigated in humans and animal models but little evidence exists that they may be helpful in canine diseases. Furthermore, in the past, studies on canine intestinal microbiota were conducted using traditional culture methods and only recently modern molecular identification methods have been used to investigate the effects of dietary substances on the canine intestinal ecosystem. This paper presents an overview of the scientific literature regarding the use of prebiotics and probiotics in dogs.

Key words: dog, Immune system, Intestinal microbiota, Prebiotics, Probiotics

Introduction

Recently, considerable interest has arisen toward the possibility to modulate the intestinal microbiota of dogs by using specific dietary supplements, such as prebiotics and probiotics. In fact, several studies have demonstrated that by positively modifying the intestinal microbiota, prebiotic substances (mainly of vegetable origin) and probiotics may improve the trophic and health conditions of the canine digestive system and, consequently, the animal's general state of wellbeing.

The effects of intestinal microbiota on the host's health

The gastrointestinal microbiota is a very complex ecosystem that consists of hundreds of bacterial species, some of which are potentially pathogenic, while others are considered good for the host *(Roberfroid et al., 1995)*. The beneficial microbes that live in the animal intestine exert several effects, including: 1) detoxification of some toxic substances introduced through the diet or newly formed as a result of metabolic processes of the body and of intestinal microbiota *(Tomomatsu, 1994)*; 2) "barrier effect" against the proliferation of potentially pathogenic bacteria and their adhesion to the intestinal mucosa, thanks to occupation of the attack sites of these microorganisms and production of selective antimicrobial substances *(Liévin-Le Moal and Servin, 2006)*; 3) uptake of ammonia and amine used as a source of nitrogen to support microbial protein synthesis, with a consequent reduction in the intestinal absorption of these undesirable substances *(Howard et al., 2000)*; 4) interaction with the host immune system *(Round and Mazmanian, 2009; Cerf-Bensussan and Gaboriau-Routhiau, 2010)*; 5) production of vitamins *(LeBlanc et al., 2012)*.

Definition, modes of action and effects of prebiotics

A prebiotic has been recently defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" (*Roberfroid*, *2007*). A prebiotic has to withstand digestion and reach the hindgut where it selectively stimulates the growth and/or metabolism of bacteria that are able to exert a positive influence on the host's health.

Prebiotics are mainly non-digestible oligosaccharides that can be obtained by extraction from vegetable raw materials (for example, inulin from chicory, artichokes, and wheat), by enzymatic synthesis or by partial hydrolysis of oligosaccharides and polysaccharides (for example, the hydrolysis of inulin to fructo-oligosaccharides (FOS)).

Several studies have shown that prebiotics can have a positive influence on the composition of canine intestinal microbiota. For example, feeding dogs with inulin or FOS resulted in higher fecal concentrations of bifidobacteria and lactobacilli *(Grieshop et al., 2004; Middelbos et al., 2007)* and lower presence of undesired bacteria such as *C. perfringens (Flickinger et al., 2003)* and *E. coli (Middelbos et al., 2007).* Prebiotics might reduce concentrations of ammonia in the animal intestine, as increased fermentation leads to higher amounts of nitrogen converted into bacterial protein *(Howard et al., 2000).* However, only in a few studies the administration of prebiotics to dogs resulted in lower concentrations of fecal ammonia *(Flickinger et al., 2003).* Bifidobacteria and lactobacilli are, in general, the bacterial populations that should be increased when prebiotics are used but their presence in the canine intestine is inconsistent *(Willard et al., 2000).* This fact, among others, may explain why results from the literature show some discrepancies and the effects of prebiotics on the canine intestinal ecosystem are quite variable.

Several authors reported that prebiotic consumption by dogs resulted in lower crude protein total tract apparent digestibility (*Flickinger et al., 2003; Hesta et al., 2003; Middelbos et al., 2007; Beloshapka et al., 2012).* In general, this finding is the consequence of the higher faecal nitrogen concentrations that are found when the faecal bacterial mass is increased by prebiotics *(Hesta et al., 2003; Karr-Lilienthal et al., 2004*). Another possible effect of prebiotics is an improved intestinal absorption of minerals, as observed in dogs in the studies by *Beynen et al. (2001 and 2002).*

At present, the effects of FOS and inulin on canine immunity seem to be negligible. However, the utilization of MOS seems to enhance immune system in dogs *(Lomax and Calder, 2009).* In this regard, MOS are often described as prebiotic non-digestible oligosaccharides despite the fact that they are not fermented by bacteria; instead, MOS act by binding and removing pathogens from the gastrointestinal tract and stimulating the immune system *(Spring et al., 2000).*

Definition, modes of action and effects of probiotics (and synbiotics)

Probiotics have been defined as dietary supplements consisting of live microorganisms that have a beneficial effect on the host by improving the composition of the intestinal microbiota (Fuller, 1989). The efficacy of a probiotic depends on several factors, including its metabolism, the ability to survive during the passage along the host's gastrointestinal tract, the dose of use and the duration of the treatment. In addition, it should be noted that the probiotics will be present in the intestine during their administration and will disappear a few days after the end of treatment.

It has been seen that the composition of the canine intestinal microbiota may be improved by feeding strains of *Lactobacillus* spp. *(Baillon et al., 2004; Biagi et al., 2007)*.

With regard to the effects on the immune system, in a study with growing dogs, *Benyacoub et al. (2003)* observed that the administration of a strain of *Enterococcus faecium* determined a more effective antibody response. In addition, it should be noted that in humans and laboratory animals it has been observed that some probiotic strains possess anticarcinogenic properties *(Donaldson, 2004)* and even the ability to prevent some forms of food allergies *(Del Giudice et al., 2010)*. A recent trial involving the utilization of a probiotic strain of *Lactobacillus sakei* Probio-65 administered for 2 months to dogs with atopic dermatitis significantly improved the clinical status of the dogs compared to a placebo group *(Kim et al., 2015)*.

A synbiotic consists of the association of one or more probiotic strains with prebiotic molecules *(Schrezenmeir and De Vrese, 2001)* that are selected among those that the probiotic strains can easily ferment. During a study by *Swanson et al. (2002)*, the administration to a group of adult dogs of a combination of a strain of *L. acidophilus* and FOS determined an increase in fecal concentrations of lactobacilli and bifidobacteria and a reduction of fecal ammonia. A particularly interesting aspect of this study consisted in the fact that the same beneficial effects were less evident when the probiotic strain and the FOS were used individually. Similar results confirming the possible synergistic effect arising from the association of probiotics and prebiotics, were observed in some in vitro studies *(Tzortzis et al., 2004; Ogué-Bon et al., 2011)*.

Roles of prebiotics and probiotics in canine disease

With regard to the possible use of probiotics in the clinical practice, there is some evidence that, in dogs, the utilization of probiotic products may be helpful in the treatment of acute gastroenteritis *(Kelley et al., 2009; Herstad et al., 2010)* and inflammatory bowel disease (IBD) *(Rossi et al., 2014)*.

With regard to the utilization of prebiotic molecules in the clinical practice, the most interesting aspect seems to be the reduction of circulating ammonia concentrations, particularly useful in the management of chronic hepatic and renal failure. Furthermore, because of the dose-dependent laxative effect of prebiotics, their use may be considered in patients predisposed to intestinal constipation or in which the defecation is painful. At present, there is no scientific evidence that, in dogs, prebiotics may reduce the incidence of intestinal bacterial infections or could be of help in the management of small intestine bacterial overgrowth.

Conclusions

Today, the study of the intestinal microbiota of humans and domestic animals is a topic of great interest. The ability to modulate the composition and metabolism of the microbiota already represents a possible way to prevent and treat certain diseases. Unfortunately, to date, the scientific literature regarding the use of probiotics and prebiotic molecules in dogs is still scarce so that, in these animals, many of the beneficial effects observed in humans as well as in farm and experimental animals have yet to be confirmed.

Korišćenje prebiotika i probiotika za modulaciju mikrobiota creva pasa

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

Rezime

Mikrobiota creva igra važnu ulogu u održavanju zdravstvenog stanja gastrointestinalnog trakta kao i samog i domaćina. Upotreba specifičnih dijetetskih suplemenata kao što su prebiotici i probiotici može poboljšati sastav i metabolizam mikrobijalne populacije creva. Od prebiotika, frukto-oligosaharidi (FOS) se čine najefikasnijim u modulaciji mikrobiote creva pasa i poboljšavaju absorpciju minerala u crevima, ali sa malo ili nimalo efekta na imunološki sistem pasa. Nasuprot tome, postoje dokazi o tome da hranjenje manan-oligosaharidima i probioticima može imati pozitivan uticaj na imunološki sistem pasa. Pored toga, postoji dokaz da neki pozitivni efekti prebiotika kod pasa mogu biti poboljšani ako se koriste u kombinaciji sa specifičnim probiotskim sojevima, u obliku sinbiotika. Klinički efekti prebiotika i probiotika istraženi su kod ljudi i životinja, ali postoji malo dokaza o tome da li mogu biti korisni kod bolesti pasa. Štaviše, u prošlosti su studije o mikrobioti creva pasa bile sprovedene korišćenjem tradicionalnih metoda kulture i tek nedavno su korišćene moderne metode molekularne identifikacije za ispitivanje efekata dijetetskih supstanci na crevni ekosistem pasa. Ovaj rad predstavlja pregled naučne literature o upotrebi prebiotika i probiotika kod pasa.

Ključne reči: pas, imuni sistem, mikrobiota creva, prebiotici, probiotici

References

BAILLON M.L.A., MARSHALL-JONES Z.V., BUTTERWICK R.F. (2004): Effects of probiotic *Lactobacillus acidophilus* strain DSM13241 in healthy adult dogs. American Journal of Veterinary Research, 65, 338-343.
BELOSHAPKA A.N., WOLFF A.K., SWANSON K.S. (2012): Effects of feeding polydextrose on faecal characteristics, microbiota and fermentative end products in healthy adult dogs. British Journal of Nutrition, 108, 638-644.
BENYACOUB J., CZARNECKI-MAULDEN G.L., CAVADINI C., SAUTHIER T., ANDERSON R.E., SCHIFFRIN E.J., VON DER WEID T. (2003): Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune functions in young dogs. Journal of Nutrition, 133, 1158-1162.
BEYNEN A.C., KAPPERT H.J., YU S. (2001): Dietary lactulose decreases apparent nitrogen absorption and increases apparent calcium and magnesium absorption in healthy dogs. Journal of Animal Physiology and Animal Nutrition, 85, 67-72.
BEYNEN A.C., BAAS J.C., HOEKEMEIJER P.E., KAPPERT H.J., BAKKER M.H., KOOPMAN J.P., LEMMENS A.G. (2002): Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. Journal of Animal Physiology and Animal Nutrition, 86, 298-305.
BIAGI G., CIPOLLINI I., POMPEI A., ZAGHINI G., MATTEUZZI D. (2007): Effect of a *Lactobacillus animalis* strain on composition and metabolism of the intestinal microflora in adult dogs. Veterinary Microbiology, 124, 160-165.
CERF-BENSUSSAN N., GABORIAU-ROUTHIAU V. (2010): The immune system and the gut microbiota: friends or foes? Nature Reviews Immunology, 10, 735-744.
DEL GIUDICE M.M., LEONARDI S., MAIELLO N., BRUNESE F.P. (2010): Food allergy and probiotics in childhood. Journal of Clinical Gastroenterology, 44, S22-S25.
DONALDSON M.S. (2004): Nutrition and cancer: A review of the evidence for an anti-cancer diet. Nutrition Journal, 3, 19.
FLICKINGER E.A., SCHREIJEN E.M., PATIL A.R., HUSSEIN H.S., GRIESHOP C.M., MERCHEN N.R., FAHEY JR. G.C. (2003): Nutrient digestibilities, microbial population and protein catabolites as affected by fructan supplementation of dog diets. Journal of Animal Science, 81, 2008-2018.
FULLER R. (1989): Probiotics in man and animals. Journal of Applied Bacteriology, 66, 365-378.
GRIESHOP C.M., FLICKINGER E.A., BRUCE K.J., PATIL A.R., CZARNECKI-MAULDEN G.L., FAHEY JR. G.C. (2004): Gastrointestinal and immunological

responses of senior dogs to chicory and mannan-oligosaccharides. Archives für Tierererernährung, 58, 483-493.
HERSTAD H.K., NESHEIM B.B., L'ABEE-LUND T., LARSEN S., SKANCKE E. (2010): Effects of a probiotic intervention in acute canine gastroenteritis - a controlled clinical trial. Journal of Small Animal Practice, 51, 34-38.
HESTA M., JANSSENS G.P.J., MILLET S., DE WILDE R. (2003): Prebiotics affect nutrient digestibility but not faecal ammonia in dogs fed increased dietary protein levels. British Journal of Nutrition, 90, 1007-1014.
HOWARD M.D., KERLEY M.S., SUNDVOLD G.D., REINHART G.A. (2000): Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and intestinal microflora populations. Nutrition Research, 20, 1473-1484.
KARR-LILIENTHAL L.K., GRIESHOP C.M., SPEARS J.K., PATIL A.R., CZARNECKI-MAULDEN G.L., MERCHEN N.R., FAHEY JR. G.C. (2004): Estimation of the proportion of bacterial nitrogen in canine feces using diaminopimelic acid as an internal bacterial marker. Journal of Animal Science, 82, 1707-1712.
KELLEY R.L., MINIKHIEM D., KIELY B., O'MAHONY L., O'SULLIVAN D., BOILEAU T., PARK J.S. (2009): Clinical benefits of probiotic canine-derived *Bifidobacterium animalis* strain AHC7 in dogs with acute idiopathic diarrhea. Veterinary Therapeutics, 10.
KIM H., RATHER I.A., KIM H., KIM S., KIM T., JANG J., SEO J., LIM J., PARK Y.H. (2015): A double-blind, placebo controlled-trial of a probiotic strain *Lactobacillus sakei* Probio-65 for the prevention of canine atopic dermatitis. Journal of Microbiology and Biotechnology, 25,1966-1969.
LEBLANC J.G., MILANI C., SAVOY DE GIORI G., SESMA F., VAN SINDEREN D., VENTURA M. (2012): Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Current Opinions in Biotechnology 2012, http://dx.doi.org/10.1016/j.copbio.2012.08.005.
LIÉVIN-LE MOAL V., SERVIN A.L. (2006): The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clinical Microbiology Reviews, 19, 315-337.
LOMAX A.R., CALDER P.C. (2009): Prebiotics, immune function, infection and inflammation: a review of the evidence. British Journal of Nutrition, 101, 633-658.
MIDDELBOS I.S., FASTINGER N.D., FAHEY JR. G.C. (2007): Evaluation of fermentable oligosaccharides in diets fed to dogs in comparison to fiber standards. Journal of Animal Science 85, 3033-3044.
OGUÉ-BON E., KHOO C., HOYLES L., MCCARTNEY A.L., GIBSON G.R., RASTALL R.A. (2011): In vitro fermentation of rice bran combined with

Lactobacillus acidophilus 14 150B or *Bifidobacterium longum* 05 by the canine faecal microbiota. FEMS Microbiology Ecology, 75, 365-376.
ROBERFROID M.B. (2007): Prebiotics: the concept revisited. Journal of Nutrition, 137, 830S-837S.
ROBERFROID M.B., BORNET F., BOULEY C., CUMMINGS J.H. (1995): Colonic microflora: nutrition and health. Nutrition Reviews, 53, 127-130.
ROSSI G., PENGO G., CALDIN M., PALUMBO PICCIONELLO A., STEINER J.M., COHEN N.D., et al. (2014): Comparison of microbiological, histological, and immunomodulatory parameters in response to treatment with either combination therapy with prednisone and metronidazole or probiotic VSL#3 strains in dogs with idiopathic inflammatory bowel disease. PLoS One, 9 e94699.
ROUND J.L., MAZMANIAN S.K. (2009): The gut microbiota shapes intestinal immune responses during health and disease. Nature Reviews Immunology, 9, 313-323.
SCHREZENMEIR J., DE VRESE M. (2001): Probiotics, prebiotics and synbiotics – approaching a definition. American Journal of Clinical Nutrition, 73, 361S-364S.
SPRING P., WENK C., DAWSON K.A., NEWMAN K.E. (2000): The effect of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poultry Science, 79, 205-211.
SWANSON K.S., GRIESHOP C.M., FLICKINGER E.A., BAUER L.L., CHOW J., WOLF B.W., GARLEB K.A., FAHEY JR. G.C. (2002): Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. Journal of Nutrition, 132, 3721-3731.
TOMOMATSU H. (1994): Health effects of oligosaccharides. Food Technology, 48, 61-65.
TZORTZIS G., BAILLON M.L., GIBSON G.R., RASTALL R.A. (2004): Modulation of anti-pathogenic activity in canine-derived *Lactobacillus* species by carbohydrate growth substrate. Journal of Applied Microbiology, 96, 552-559.
WILLARD M.D., SIMPSON R.B., COHEN N.D., CLANCY J.S. (2000): Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. American Journal of Veterinary Research 61, 820-825.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

DAIRY COWS HEALTH PARAMETERS IN DIFFERENT SEASON - AN WELFARE APPROACH

Dušica Ostojić Andrić[1], Slavča Hristov[2], Milan M. Petrović[1], Vlada Pantelić[1], Dragan Nikšić[1], Violeta Caro Petrović[1], Aleksandar Stanojković[1]

[1]Institute for Animal Husbandry, Auto put 16, 11080 Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia
Corresponding author: Dušica Ostojić Andrić, andricdusica.iah@gmail.com
Original scientific paper

Abstract: The aim of the research was to determine whether the frequency of certain injuries and diseases of cows as important parameters for ensuring their welfare varies considerably depending on the growing season (summer/winter). The research was conducted by Welfare Quality Assessment Protocol for Dairy Cows (2009) on total of 16 dairy farms (N=4.833 cows), wherein the provision of good health was descriptively categorized as unacceptable, acceptable, enhanced and excellent, according to calculated score (0-100 points).The air temperature of given location was recorded during each farm visit, so the average temperature of conducted assessment for specific season, served as additional indicator of dairy cows' thermal comfort. The results of the study showed that the effect of the season on the incidence of injuries and illness was not significant ($p > 0.05$), but certain disorders were more frequent in one of the seasons. In the summer season, mastitis (2.63%), diarrhea (2.31%), discharge from the eye and nose (6.24% and 1.69%) were more frequent, and in the winter season lameness (27.78%) , tachypnoea (0.13%), and vaginal discharge (1.73%). Relying on welfare standards, it can be concluded that the parameters tested do not represent an alarming risk for the welfare of raised animals with the exception of mastitis rates and ocular discharge in the summer period. However, the overall health of dairy cows in both seasons is considered acceptable (in average 40 from 100 points) indicating that only minimal requirements for ensuring well-being are met and that there is a need to improve the health situation. Given that related studies have shown a low level of protection of the comfort of the examined animals as well as its significant influence on the provision of thermal comfort and health of cows, the recommendations are directed primarily at ensuring appropriate conditions of keeping with adequate and continuous health control of the herd.

Key words: dairy cows, season, health, welfare, parameters, thermal comfort

Introduction

Cattle are known as species of domestic animals that are easily acclimatized and adapted to living conditions worldwide, of course with the provision of adequate nutrition, water supply, as well as simple but appropriate protection against excessive airflow, solar radiation and precipitation. However, many studies have shown that housing cows in unfavorable weather conditions negatively affects their health and therefore the welfare and productivity (*Shearer and Beede, 1990; West, 2003; Hristov et al., 2008, EFSA, 2009*).

Generally, it is considered that adult cows can much better cope with cold stress when sufficiently fed than with heat stress. This is especially true of cows in lactation due to their "stronger" metabolic profile (*Kadzere et al., 2002*). Weather conditions (temperature and air humidity) affect the survival and spread of microorganisms and thus the appearance of various infectious diseases of cattle depending on their specific and non-specific resistance as well as the climate in which they are reared (*Webster, 1981). Belić et al. (2010)* note that in the summer months, milk cows have an increase in rectal temperature with pronounced diurnal variations, tachypnoea, and decreased rumen contraction. In addition, changes in haematological parameters (reduction in the number of erythrocytes, leukocytes, hemoglobin and hematocrit) have been observed, indicating activated cooling mechanisms such as evaporation.

Early research on the health of dairy cows from the perspective of welfare provision in Serbia has shown that it can be rated as acceptable to enhanced, i.e., grades 3 to 4 on scale 1-4 (*Ostojić Andrić et al., 2016*). This estimation was somewhat better than those achieved on EU farms *(Welfare Quality Network, 2012)* probably derived as a result of also better estimated feed provision and lower milk yield e.g. lower selection pressure of cows in Serbia.

Speaking of health - weather relation in the assessment of the quality of dairy cows' welfare, we most often encounter the term provision of thermal comfort, which is commonly linked to temperature-humidity index-THI, as well as detection of thermoregulatory behavior *(Hristov et al., 2008)*. Since this may be complicated and still unreliable procedure *(Welfare Quality Consortium, 2009)*, we based this research on seasonal effect which generally represent aggregation of most important factors such are temperature, humidity, solar radiation, precipitation and air movement. The aim of the research was to determine whether the incidence of certain injuries and diseases of cows as important parameters for ensuring their welfare varies considerably from season to season.

Materials and Methods

The farms

The study was conducted on 16 selected Serbian commercial dairy farms (N=4833; Mean ± SEM: 301±71.6 lactating cows). The tie-stall farms-TSH (n=9) and loose housing systems-LHS (n=7) were selected according to management practices, farm size, veterinary records and availability of different information necessarily for assessment. Presence of the races were 80% and 20% for Domestic Simmental and Holstein Friesian cattle, respectively. All the tie-stall farms were closed, with solid flooring. The cows were kept on stalls with length between 140 cm and 240 cm, and width between 100 cm and 120 cm. Straw bedding was used in all of tie-stall farms (3 kg/head/day or less). The cows had access to outdoor loafing area in 4 tie-stall farms and pasture only on one farm (24 hours a day for 60 days a year). The farms with LHS were both closed and half opened, having cubicles (4 farms) or straw yards (3 farms) for the cows' rest. Straw bedding was used in the majority of the loose housing barns. The milking (automatic) was done in milking parlours, twice per day. In the warm season, the cows were pasturing on one farm for 12 hours a day for 210 days a year.

Information about weather condition in winter and summer season

In order to examine the impact of the season on selected parameters of quality of welfare, basic information on weather conditions during the winter and summer season in the year of research were also analyzed (2012). The summer season of 2012 was classified as extremely warm in the average seasonal temperature and many tropical days, while the winter season was on average cold with high precipitation *(RHMZ, 2012a; RHMZ, 2012b).*

Each farm was visited twice a year, in order to compare values of welfare parameters in two main, opposite seasons: winter and summer (cold and hot weather). During each farm visit, the air temperature of given location was noted *(RHMZ, 2012c).* This information, in further analysis calculated as average temperature of conducted assessment for specific season, served as additional indicator of dairy cows thermal comfort.

Welfare assessment and statistical analysis

In this study welfare assessment of the cows was done according to the Welfare Quality® Assessment Protocol for Cattle *(Welfare Quality Consortium, 2009)* in which detailed information about the methodology of assessment can be found. Protocol includes 4 four major welfare principles, 12 criteria and 29 measures that are used to obtained overall welfare assessment. This study however, was focused on principle providing of good health (PGH) assessed by evaluation of two corresponding criterions: absence of injuries (CAI) and absence of diseases (CAD). In order to obtain information about state of mentioned principles in two seasons, three trained assessors (experienced in cows' welfare assessment) evaluated the sampled cows on each farm.

Processing of data collected on the farms was carried out using the Welfare Quality® scoring system software program for the calculation of the scores for the welfare criteria and principles and for classifying the farms in a certain welfare category (not classified, acceptable, enhanced and excellent).

All statistical analyses were performed using Statistica for Windows version 8.0 (StatSoft, Inc. 2007, data analysis software system). Descriptive statistical indicators were determined (mean, standard error of the mean, standard deviation, minimal and maximal values) for the assessed measures, criteria and for the scores of the of selected welfare principles (good feeding and housing). The statistical significance of the seasonal effect on welfare (measures, criteria and principles of welfare) in the studied farms was determined by the t-test or the Mann-Whitney test, depending on the normal or abnormal distribution of the data, established with the Kolmogorov-Smirnov test. P values less than 0.05 were considered as significant.

Results and discussion

Within principle providing of good health (PGH), the assessment of the appropriate indicators listed in Table 1 the absence of injuries (CAI) and the absence of disease (CAD) were considered as criteria for assessing the quality of the welfare of dairy cows. It was found that the season did not exhibit a significant influence on any of the observed criteria as well as on the overall health state of the cows - PGH ($p>0.05$). However, within the CAI, a slightly higher incidence of laminitis in the winter season (38.6% versus 32.3%) was determined, which is consistent with the results of *Rowlands et al. (1983)* and can be explained by poor conditions of comfort in the winter season (*Ostojić Andrić et al., 2017*). Score of CAD was almost the same for both seasons, but diseases such as mastitis, lameness, diarrhea, nasal and eye irritation were more common in summer and accelerated breathing and vaginal discharge in the winter season.

Based on the recommendations of *Forkman and Keeling (2009)* on the frequency of these diseases from the aspect of well-being, it can be concluded that the health of dairy cows in both seasons is satisfactory and does not represent a factor of risk for welfare. The exception is the appearance of discharge from the eye in the summer season that exceeds the so-called alarm threshold of 6.0% and can be due to eye irritation due to increased ventilation of objects during warm summer months *(Radostits et al., 1999).*

The frequency of mastitis in the summer months has also been increased in relation to the winter period and slightly exceeds the 2.25% warning threshold stated by *Forkman and Keeling (2009*). Increased frequency of mastitis in summer months was also found in studies by other authors (*Hogan et al, 1989; Shearer and Beede, 1990)*, as a result of higher resistance to microorganisms under given conditions and adaptive changes in the physiological status of cows that increase the disposition to inflammation (*Webster, 1981)* .

Table 1. Effect of season on provision of good health

Season	Winter					Summer					*F*
Number of farms, N	n=16					n=16					
Average temperature range (C˚)	-4.2 to 7.2					19.3 to 27.4					
Principle, criteria and indicators	$\bar{x}$	*SD*	S^2	*Min*	*Max*	$\bar{x}$	*SD*	S^2	*Min*	*Max*	
Principle: Good health (PGH)	40.93	8.63	74.45	23.90	56.60	41.41	7.83	61.38	26.30	55.20	ns
1. Criterion: Absence of injuries (CAI)	50.16	15.58	242.87	21.00	81.10	52.98	14.44	208.39	21.90	81.10	ns
Not lame cows, %	61.42	18.53	343.47	20.60	90.00	67.70	16.58	274.98	34.00	88.60	ns
Lame cows, %	27.78	14.62	213.85	6.98	61.80	25.23	13.12	172.15	9.20	51.00	ns
Severely lame, %	9.93	6.84	46.78	0.50	20.30	11.97	20.40	416.12	0.00	86.55	ns
Cows with at least one part of skin without hair, no lesion, %	17.14	14.42	208.02	0.00	56.70	18.47	18.71	350.04	2.40	73.68	ns
Cows with at least one skin lesion, %	6.37	7.13	50.86	0.00	30.00	6.62	6.99	48.81	0.00	29.82	ns
Cows without skin lesion, %	92.66	8.88	78.82	70.00	100.00	87.69	22.61	511.43	6.90	100.00	ns

Season	Winter					Summer					F
Number of farms, N	n=16					n=16					
Average temperature range (C°)	-4.2 to 7.2					19.3 to 27.4					
Principle, criteria and indicators	$\bar{x}$	*SD*	S^2	*Min*	*Max*	$\bar{x}$	*SD*	S^2	*Min*	*Max*	
2. Criterion: Absence of diseases (CAD)	59.51	22.12	489.43	33.30	100.00	59.56	21.94	481.27	30.20	100.00	ns
Cows with nasal discharge, %	0.19	0.60	0.36	0.00	2.31	1.69	4.21	17.75	0.00	15.18	ns
Cows with hampered respiration, %	0.13	0.34	0.12	0.00	1.00	0.00	0.00	0.00	0.00	0.00	ns
Cows with ocular discharge, %	1.72	3.69	13.59	0.00	14.20	6.24	8.87	78.61	0.00	29.17	ns
Cows with diarrhea, %	1.70	2.53	6.38	0.00	7.78	2.31	2.40	5.76	0.00	8.16	ns
Cows with vulvar discharge, %	1.73	1.83	3.34	0.00	5.55	1.16	1.10	1.21	0.00	3.20	ns
Frequency of coughing per cow per 15 min	0.06	0.25	0.06	0.00	1.00	0.13	0.34	0.12	0.00	1.00	ns
Frequency of mastitis, %	1.96	0.98	0.96	0.70	4.74	2.63	0.96	0.93	1.35	5.26	ns

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

In considering the seasonal impact on the health parameters of cows, it is important to emphasize the importance of adequate nutrition and housing conditions to the possibility of providing thermal comfort, and consequently to the general health of cows, which is assessed as acceptable here, indicating the need for its improvement. According to linked study *(Ostojić Andrić et al., 2017),* examined cows during both seasons were provided only by minimal comfort conditions in housing, which consequently indicates the lack of resources to ensure their thermal comfort, especially in extreme weather conditions. The main risk factors originate from inadequate housing conditions, reflected in a condition of dirtiness of cows, which, in particular during the winter period, when they are exposed to low temperatures and high humidity, favours the formation of not only the skin but also systemic diseases.

Conclusion

The results of the study showed that the effect of the season on the incidence of injuries and illness was not significant, but certain disorders were more prevalent in one of the seasons. It was found that mastitis, diarrhea, nasal and nose discharges were more frequent in the summer season, and lameness, tachypnoea and vaginal discharge in the winter season. Enhanced ventilation of objects during the summer months is often cited as the cause of the occurrence of ocular discharge whose frequency exceeds the alarm threshold from the welfare point of view. The more frequent occurrence of mastitis during the summer months results from higher resistance of microorganisms in given conditions and adaptive changes in the physiological status of cows that increase the disposition to inflammation. On the other hand, a greater share of lame cows in the winter can be associated with poorer hygiene and comfort conditions during this time of the year.

In conclusion, it is important to emphasize that the established, acceptable assessment of the health of dairy cows (in average 40 from 100 points) in both seasons indicates that only minimal requirements for ensuring well-being are met and that there is a need to improve the health situation. Given that related studies have shown a low level of ensuring of the comfort of the examined animals as well as its significant influence on the provision of thermal comfort and health of cows, the recommendations are directed primarily at ensuring appropriate housing and rearing conditions with adequate and continuous health control of the herd.

Parametri zdravstvenog stanja mlečnih krava u različitim sezonama – sagledavano iz ugla dobrobiti

Dušica Ostojić Andrić, Slavča Hristov, Milan M. Petrović, Vlada Pantelić, Dragan Nikšić, Violeta Caro Petrović, Aleksandar Stanojković

Rezime

Cilj istraživanja je bio da se utvrdi da li se učestalost pojave određenih povreda i bolesti krava kao važnih parametara obezbeđenja njihove dobrobiti, značajno razlikuje u zavisnosti od sezone gajenja (leto/zima). Istraživanje je sprovedeno prema *Protokolu o proceni kvaliteta dobrobiti mlečnih krava (2009)* na ukupno 16 farmi za proizvodnju mlijeka (N = 4.833 krava), pri čemu je obezbeđivanje dobrog zdravlja deskriptivno kategorizirano kao neprihvatljivo, prihvatljivo, poboljšano i odlično, prema izračunatim ocenama (0-100 poena). Temperatura vazduha određene lokacije beležena je tokom svake posete farmi, tako da je prosečna temperatura za određenu sezonu služila kao dodatni indikator termičkog komfora mlečnih krava. Rezultati istraživanja pokazali su da uticaj sezone na učestalost pojave povreda i bolesti nije bio signifikantan (p>0,05), ali su određeni poremećaji bili zastupljeniji u jednoj od sezona. U letnjoj sezoni učestalije su se javljali mastitis (2,63%), dijareja (2,31%), iscedak iz oka i nosa (6,24% and 1,69%), a u zimskoj sezoni šepavost (27,78%), tahipnea (0,13%) i vaginalni iscedak (1,73%). Oslanjajući se na standarde dobrobiti, može se zaključiti da ispitivani parametri ne predstavljaju alarmantan rizik po dobrobit gajenih životinja sa izuzetkom mastitis rate and ocular discharge u letnjem periodu., Ipak, celokupno zdravlje mlečnih krava u obe sezone ocenjeno je kao acceptable (in average 40 from 100 points) što ukazuje da su zadovoljeni samo minimalni zahtevi u obezbeđenju dobrobiti i da postoji potreba za poboljšanjem zdravstvene situacije. Sa obzirom da su srodne studije pokazale nizak nivo obezbeđenja komfora ispitivanih grla kao i njegov značajan uticaj na obezbeđenje termalnog komfora i zdravlja krava, preporuke su usmerene pre svega na obezbeđenje odgovarajućih uslova držanja uz adekvatan i kontinuiran zdravstveni nadzor stada.

Ključne reči: mlečne krave, sezona, zdravlje, dobrobit, parametri, termalni komfor

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053

References

BELIĆ B., CINCOVIĆ M.R., STOJANOVIĆ D., KOVAČEVIĆ Z., MEDIĆ S., SIMIĆ V. (2010): Hematology parameters and physical response to heat stress in dairy cows, Contemporary agriculture, 59, (1-2), p. 161-166.
EFSA (2009): Scientific opinion on welfare of dairy cows in relation to metabolic and reproductive problems based on a risk assessment with special reference to the

impact of housing, feeding, management and genetic selection. The EFSA Journal, 1140, p. 1-75.

FORKMAN B., KEELING L.(2009): Assessment of Animal Welfare Measures for Dairy Cattle, Beef Bulls and Veal Calves. Welfare Quality Reports. Cardiff University. Sweden. p. 1-314.

HOGAN J.S., SMITH K.L., HOBLET K.H., SCHOENBERGER P.S., TODHUNTER D.A., HUESTON W.D., PRITCHARD D.E., BOWMAN G.L., HEIDER L.E., BROCKETT B.L., CONRAD H.R. (1989): Field Survey of Clinical Mastitis in Low Somatic Cell Count Herds Journal of Dairy Science, 72, 6, p. 1547-1556.

HRISTOV S., STANKOVIĆ B., ZLATANOVIĆ Z., JOKSIMOVIĆ TODOROVIĆ M., DAVIDOVIĆ V. (2008): Uslovi držanja, zdravlje i dobrobit muznih krava. Biotechnology in Animal Husbandry. Vol 24 (1-2),p. 25-36.

KADZERE C.T., MURPHY M.R., SILANIKOVE N., MALTZ E. (2002): Heat stress in lactating dairy cows: a review. Livestock Production Science, 77, 59–91.

OSTOJIĆ ANDRIĆ D., HRISTOV S., PETROVIĆ M.M., PANTELIĆ V., BOJKOVSKI J., NIKŠIĆ D., MIĆIĆ N. (2017): Dairy Cows' Welfare Quality In Different Seasons. International Symposium On Animal Science (ISAS) 2017, 5th - 10th June 2017, Herceg Novi, Montenegro. p.302-308

OSTOJIĆ ANDRIĆ DUŠICA, HRISTOV SLAVČA, PETROVIĆ M. MILAN, PANTELIĆ VLADA, NIKŠIĆ DRAGAN, STANOJKOVIĆ ALEKSANDAR, CARO PETROVIĆ VIOLETA (2016): Health And Welfare Of Dairy Cows In Serbia. Scientific Papers. Animal Science, Series D., Vol. LIX, University of Agronomic Sciences and Veterinary Medicine of Bucharest. p.233-239

RADOSTITS O.M., GAY C.C; BLOOD D.C., HINCHCLIFF K.W., ARUNDEL J.H. (1999): Veterinary medicine: A Textbook of the diseases of cattle, sheep, pigs, goats and horses, 9th ed. W.B.Saunders Company, London.

RHMZ (2012a): Climatological Analysis For Winter 2012 For Republic Of Serbia. Season's Bulletin. Republic Hydrometeorological Institute - Department Of National Centre For Climate Change. p.1-22.

RHMZ (2012b): Climatological Analysis For Summer 2012 For Republic Of Serbia. Season's Bulletin. Republic Hydrometeorological Institute - Department Of National Centre For Climate Change. p.1-22.

RHMZ (2012c): http://www.android.co.rs/strana---androidaplikacija---20---com-rhmz-aktuelnipodaci---Vreme-Srbija.html. retrived on January 2012.

ROWLANDS G.J., RUSSELL A.M., WILLIAMS L.A. (1983): Effects of season, herd size, management system and veterinary practice on the lameness incidence in dairy cattle. Veterinary Record, 113, p.441-445.

SHEARER J.K. and BEEDE D.K. (1990): Effects of high environmental temperature on production, reproduction, and health of dairy cattle. Agri-Pract, 11, 5, p. 6-18.

WEBSTER A.J.F. (1981): Weather and infectious disease in cattle. Veterinary Record, 108, p.183-187.

WELFARE QUALITY NETWORK (2012): Welfare Quality® scoring system. Retrieved August, 2012 from http://www1.clermont.inra.fr/wq/index.php?id=farms

WELFARE QUALITY® ASSESMENT PROTOCOL FOR CATTLE (2009): Welfare Quality Consortium, Lelystad, Netherlands, p. 180.

WEST J.W. (2003): Effects of Heat-Stress on Production in Dairy Cattle. J. Dairy Sci., 86, p. 2131–2144.

WQAP-WELFARE QUALITY® ASSESMENT PROTOCOL FOR CATTLE (2009): Welfare Quality Consortium, Lelystad, Netherlands, p. 180.

MICRONUTRIENTS AND HEAVY METALS OF *AGROSTIETUM CAPILLARIS* IN BALKAN COUNTRIES

Aleksandar Simić[1], Željko Dželetović[2], Gordana Andrejić[2], Mirjam Radulaški[1], Zorica Bijelić[3], Violeta Mandić[3]

[1]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
[2]Institute for Applied Nuclear Energy, University of Belgrade, Belgrade, Serbia
[3]Institute for Animal Husbandry, Autoput 16, Belgrade 11080, Serbia
Corresponding author: alsimic@agrif.bg.ac.rs
Original scientific paper

Abstract: Permanent grasslands play an important role in agriculture production in Bosnia and Herzegovina and Montenegro. They cover more than half of the agricultural land in Bosnia and Herzegovina and about 33% of Montenegro's territory. Extensively managed natural grasslands are predominant in both countries, and, in general, the productivity of these communities is extremely low. One of the economically most important phytocoenoses is *Agrostietum capillaris*. A vast majority of *Agrostietum capillaris* associations closely correlate to human activities and many grassland communities are now being threatened by rapid changes in agricultural practices, primarily land abandonment. We inspected heavy metals and plant-available micronutrients in soils beneath *Agrostietum capillaris* communities (five sites in Montenegro and two in Bosnia and Herzegovina). Although some elements exceeded maximum permissible amount for soil and water (Ni, Cr), the ability of plants collected from the *Agrostietum capillaris* communities to accumulate micronutrients and heavy metals was generally low.

Key words: *Agrostietum capillaris*, Bosnia, elements, Montenegro, soil

Introduction

Six macro-elements (N, K, Ca, Mg, P, and S) normally occur in plants at concentrations greater than 1,000 mg kg^{-1} level. The remaining micro-elements (B, Cl, Cu, Fe, Mn, Mo, and Zn) normally occur in plants at concentrations lower than 50 mg kg^{-1}. Trace amounts of other elements (e.g., Co, Na, Ni, and Si) may be beneficial for plants *(Mayland and Shewmaker, 2001)*. Other elements are required for animal nutrition (Cu, Co, I, Fe, Mn, Mo, Se and Zn). In large concentrations, many of the trace elements/metals may be toxic to plants and/or animals, or may affect the quality of foodstuffs for human consumption. These potentially toxic elements include As, B, Cd, Cu, F, Pb, Hg, Mo, Ni, Se and Zn. For adequate growth and development of grasses and legumes, the basic macroelements (N, P, K) as well as numerous microelements are necessary, especially B, Mo, Mg, Cu, Zn and Co *(Mijatović et al., 1977)*. The relationship between trace elements in plants and amounts absorbed and utilized by the animal is again complex and depends on factors such as selectivity in grazing, the degree of dependence of the animal on grass as a source of trace element dietary intake, digestibility of the diet and form and 'availability' of the ingested trace elements *(Thornton and Alloway, 1974)*.

Mountain soils have performed vital services on the ecosystem for a long time that help to ensure food security and nutrition to 900 million mountain people around the world and benefit billions more living downstream *(FAO, 2015)*. Agricultural land covers about 38% of Montenegro. Meadows and pasture cover 33.1% of the territory or 88.25% of agricultural land. Only 10% of the territory is below 200 m above sea level, 35% from 200 to 1 000 m, 40% from 1 000 to 1 500 m, while about 15% is above 1 500 m *(Radojičić, 2002)*. On the basis of the influence of agro-ecological factors, primarily the climate and soil, as well as the way and level of exploitation, Montenegro is provisionally divided into five production regions, where the Northern-mountain and Polimlje-Ibar regions are more suitable than others for fodder production and animal husbandry *(Dubljević, 2009)*. Different forms of flysch are present in the coastal area and on the southern slope of Durmitor mountain and surrounding massifs ('Durmitor flysch'). Natural grasslands, especially pasture, cover poorer land, unsuited to intensive exploitation. They are mostly on steep slopes, shallow soils and with many large stones. Meadows are on deeper soil, flatter and fertile, especially in river basins and plateaux of hilly-mountain areas.

Forty-five percent of agricultural land in Bosnia is hilly (300– 700 m), of medium quality and well suited to semi-intensive livestock production *(Alibegović-Grbić, 2009)*. Mountain areas (> 700 m) account for a further 35% of agricultural land but high altitude, steep slopes and lower soil fertility limit the use of this land to grazing in spring and summer.

Natural grasslands, meadows and pasture are the most important sources of roughage in Montenegro and Bosnia, especially in hilly-mountain areas where they provide the only feed for cattle. In winter, cattle are mainly fed hay collected from natural meadows and in summer, cattle graze the pastures and the meadows after mowing. Mountain pastures, which make up the majority of the natural grasslands, are of great importance for production of animal feed and protection against soil erosion on steep and rocky terrain. Better pastures at altitudes of 1 000 to 1 500 m on deeper and more fertile soil are used for both mowing and grazing, but shallow and eroded sites are only grazed. At higher altitudes, there is a zone of montane pastures which are less used due to inaccessibility and distance unless they are near larger summer settlements *(Dubljević, 2005; 2007)*. Although the mountain region can be seen as a unique area of mountain and high mountain pastures, there are substantial differences between localities. These are characterized by varied floristic composition and grass cover influenced by differences in climate, relief and soil. The best pastures are on flat, less rocky terrain with permanent mountain settlements and higher altitude summer settlements. Beside natural conditions, human activities have had a big influence on the floristic composition and productivity of these pastures, by manuring (moving sheepfold) and more intensive exploitation. These are the main source of animal feed for both summer and winter. The presence of legumes in the yield is very low, below 5%; it is somewhat higher on fertilized areas. The land base for agriculture is thus very limited in both quantity and quality. Excessive deforestation, inappropriate conversion of grassland to arable and uncontrolled cultivation of sloping terrain are degrading the land, even in the valleys and lowland regions.

Meadows of *Agrostietum capillaris* are of secondary anthropogenic origin, as they are the result of two anthropogenic factors: reduced area under forest on the one hand, and mowing, on the other hand. The association *Agrostietum capillaris* covers a huge area in the hilly region of the Balkan Peninsula *(Vučković et al., 2010)*. The association *Agrostietum capillaris* prospers on low-nutrient acidic soils of this region. On southeast part of Bosnia and northern part of Montenegro, this community is the dominant meadow type and it is widely distributed. In this area, it develops on quite different sites.

Therefore, our research was directed to ascertain the content of microelements in the specific soils of the mountains in B&H and Montenegro, covered by *Agrostietum capillaris* communities, as well as heavy metal content in the same soils in order to estimate element concentration in the produced forage, because deficiency or excess of dietary mineral elements may cause animal health concerns.

Material and methods

The experiments were carried out in 2016 in the mountainous region of Montenegro and Bosnia & Herzegovina. Pasture areas of the study sites are occupied by plant community *Agrostietum capillaris*. For the purpose of determining the concentrations of metals and microelements in soil and plants, samples were collected from *Agrostietum capillaris* communities in diverse ecological conditions from seven sites in two countries (Figure 1).

Based on morphological and basic physico-chemical soil characteristics after *IUSS (2014)* classification, two types of pasture soils were determined: Dystric cambisol (Dystric leptosol), and Eutric Cambisol (on limestone) ie. Mollic Leptosol. Brown acid soils (Dystric cambisols), covering an area of 395 000 ha, are the second major type of soil in Montenegro, and are especially characteristic of the north-east of Montenegro. Brown eutric soils (Eutric cambisol), cover an area of 118 000 ha, and occupy the lowest land of river basins (old river terraces), ravines and karst fields.

The study fields were mowed once in the time of inflorescences formation of the dominant grass – *Agrostis capillaris* (late June – early July). Nutritive status of the topsoil samples (0-10 cm) collected in summer in each study site was determined by followed methods: pH values in deionized water and 1M KCl (1:2.5 w v^{-1}); total organic C by dichromate redox titration method; total N by semi-micro Kjeldahl method; and available P and K by the AL-method *(Pansu and Gautheyrou, 2006)*. The total concentrations of micronutrients (Cu, Fe, Mn, Co and Zn) and heavy metals (Pb, Ni, Cd and Cr) in the samples of plant material were determined, according to procedure described by *Jones and Case (1990)*, by the decomposition of HNO_3 at 125°C. After cooling down by 30% H_2O_2 was added and digesting was continued until the digest was clear. In order to determine the content of available microelements in the soil, dried and sieved soil was mixed continuously for 2 h in 1 M ammonium acetate and 0.01 M EDTA mixture (pH 7), according to the Standard NF X 31-120.22. The concentrations of the microelements and heavy metals were determined by atomic absorption spectrophotometry (Shimadzu AA 7000).

The obtained results were processed by calculating average value and standard deviation for each sample.

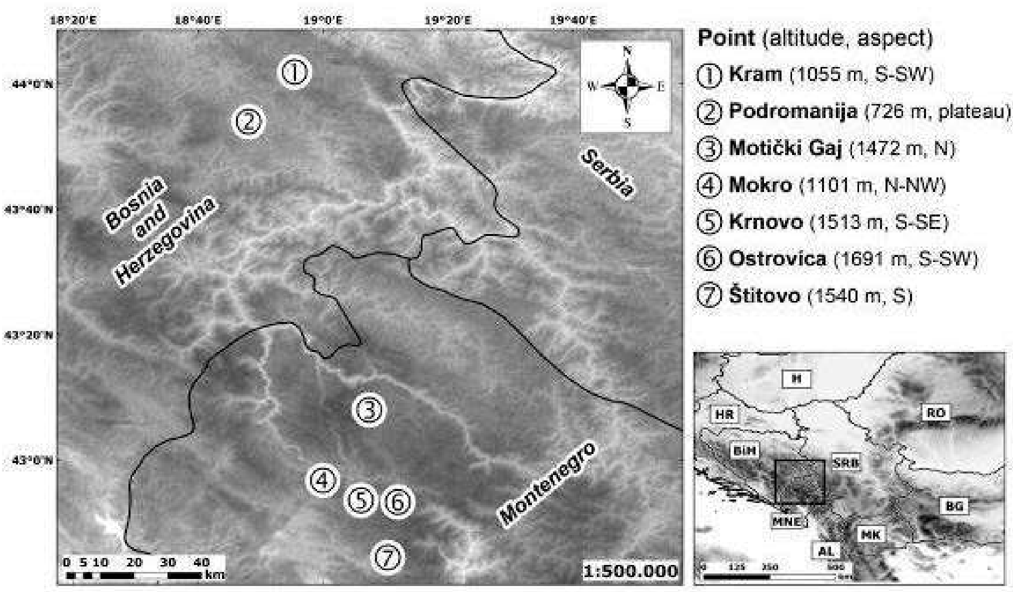


Figure 1. Association *Agrostietum capillaris* study locations in two countries.

Results and discussion

Environmental conditions

The basic agrochemical properties of the meadows are presented in Table 1. The soil reaction (pH in H_2O and 1M KCl) ranged from acid to very acid, while the contents of total organic C and N where high (*Džamić et al., 1996*).

Table 1. Chemical properties of the soil

Location	**pH**		**Total organic C %**	**OM %**	**Total N %**	**AL-P_2O_5 mg 100 g^{-1}**	**AL-K_2O mg 100 g^{-1}**
	in H_2O	**in 1M KCl**					
Podromanija	5.11	3.98	3.95	6.80	0.324	23.7	34.9
Han Kram	5.33	4.42	5.89	10.15	0.408	3.22	22.7
Motički Gaj	5.82	5.11	7.27	12.53	0.146	1.9	27.7
Mokro	5.40	4.16	4.92	8.48	0.313	1.01	21.9
Krnovo	5.52	4.52	5.27	9.08	0.445	1.01	21.2
Ostrovica	5.03	4.18	9.74	16.79	0.818	3.13	33.3
Štitovo	5.26	4.16	10.48	18.06	0.844	1.37	45.5

Relatively large amount of rainfall and low temperatures during the year, favour the slow pace of the process of mineralization of organic substances in these mountain soil, and which result in higher amounts C and N. The amount of precipitation is relatively high in central Balkan mountainous region, more than 1000 mm. Although the amount of precipitation has a great effect on the productivity of *Agrostietum capillaris* communities, especially that during vegetation, it is frequently not well disturbed. This happens because a rainy period is frequently followed by a marked fall of temperature, which retards growth and development of grasses, and hence reduces the productivity of the biomass (*Dubljević, 2007; Vučković et al., 2010*). Analysed soil substrate had low P content (except one location in Bosnia), and well-supplied with available K (*Džamić et al., 1996*).

Heavy metals content in plants

Heavy metal and microelement analyses were made of various plants shoots growing on soils with elevated Ni, covered by *Agrostietum capillaris* communities (Tables 2 and 3). According to Regulation of tolerant amount of hazardous and toxic materials in soil *(Official gazette of RS, 1994)*, in two tested soil samples Ni content surpassed the maximum permissible concentrations (Table 3).

Table 2. Total and available micronutrient content in topsoil (0-10 cm) and herbage in *Agrostietum capillaris* communities (mg kg^{-1} dry weight)

Available micronutrient concentrations in topsoil					
Location	Cu	Fe	Mn	Zn	Co
Podromanija	10.7±0.86	112±9.08	441±10.3	6.4±0.3	<0.01±0.0
Han Kram	2.2±0.11	98.5±7.23	107±1.9	4.0±0.2	0.098±0.0
Motički Gaj	1.8±0.16	55.2±2.78	76.5±5.1	4.7±0.8	<0.01±0.0
Mokro	1.5±0.06	88.3±2.48	29.8±1.1	3.3±0.1	0.095±0.10
Krnovo	4.2±0.13	63.7±3.43	120±2.9	3.1±2.0	<0.01±0.0
Ostrovica	4.7±0.03	118±11.02	241±4.2	13.2±5.0	0.31±0.07
Štitovo	1.9±0.14	118±1.87	88.7±3.9	8.4±1.1	0.115±0.0
Total micronutrient concentrations in topsoil					
Podromanija	23.9±1.22	24181±1692	3020±34	111±6	17.0±0.72
Han Kram	12.2±0.57	20243±717	962±20	53±0.0	7.49±0.38
Motički Gaj	17.8±0.33	22053±652	1417±212	93±2.0	8.89±0.22
Mokro	23.9±0.94	16180±1811	785±22	66±0.6	13.8±0.09
Krnovo	36.2±0.79	24444±1884	3063±77	128±11	13.0±0.48
Ostrovica	33.6±0.71	23820±2287	2292±54	152±3.3	12.8±0.51
Štitovo	15.7±2.30	23101±1484	915±12	158±3.4	6.86±0.08
MPL	100	-	-	300	-
Micronutrient concentrations in plants					
Podromanija	4.29±1.10	70.9±6.8	586±52.5	413±46.1	2.28±0.0
Han Kram	3.19±0.38	400±320	505±33.4	385±27.7	0.63±0.16
Motički Gaj	3.13±2.43	198±91.3	382±33.8	343±34.3	1.86±0.0
Mokro	8.35±1.20	126±24.6	110±16.2	402±60.2	<0.01±0.0
Krnovo	5.54±0.21	172±2.6	261±2.0	325±1.0	0.69±0.0
Ostrovica	6.20±0.74	520±70.2	288±19.1	293±10.6	0.48±0.0
Štitovo	5.70±2.21	86.9±42.8	171±33.5	303±19.0	0.68±0.0
NC*	3-15	50-250	15-100	15-150	0.05-0.5
TC**	20	(>500)	400	100-500	30-40
MTLF***	12-50	1250	-	2000	-

*Normal concentrations in plants according to*Chaney (1983)*; **TC - Toxic concentrations in plants according to*Kabata-Pendias (2010)*; ***MTLF - Maximum tolerant level for fodder in plants according to *NRC (2005)*; *Official Gazette of RS (2009)*

Some species are characterized by high Zn accumulation capability in roots, especially on the contaminated soil, and the greatest accumulation of Zn is reported in horsetail (*Equisetum arvense*) *(Kabata-Pendias, 2010)*. Zn becomes less available to plants as the pH increases *(Pavlović et al., 2004)*. The general values for the average total Zn contents in soils all over the world range between 60 and 89 mg kg^{-1} (*Kabata-Pendias, 2010*), and we measured total Zn content in meadows between 53-158 mg kg^{-1}. Mobility of Zn is closely associated with pH reaction *(Peganova and Edler, 2004),* although available Zn (table 2) was between

3 and 13 mg kg^{-1}, observed range of Zn concentration in plant tissues was between 293-413 mg kg^{-1}. All measured concentrations of Zn in herbaceous species from meadows were above usual concentrations for normal plant growth, but without detrimental effect on animal nutrition. The needs of animals in zinc range from 40-100 mg kg^{-1}. High levels of Zn can negative effects on organisms even if the organisms can tolerate very high concentrations of this element in nutrients and that to 1000-2000 mg kg^{-1} *(Đorđević et al., 2009)*.

Iron (Fe) can be accumulated in plants without any harmful effects *(Simić et al., 2014)*, so it is not uncommon that the contents of this element could be higher than the MPC. Slightly higher Fe and Mn accumulation in plant tissues (Han Kram, Podromanija, Ostrovica...), was below maximum tolerant concentrations for livestock food.

Table 3. Total and available heavy metal content of topsoil (0-10 cm) and herbage in *Agrostietum capillaris* communities (mg kg^{-1} dry weight)

Available heavy metals content in topsoil				
Location	Ni	Pb	Cr	Cd
Podromanija	2.9±0.14	10.9±0.9	<0.1±0	1.3±0.08
Han Kram	1.4±0.08	6.9±0.16	<0.1±0	0.1±0.01
Motički Gaj	0.6±0.00	7.5±0.57	<0.1±0	0.4±0.01
Mokro	3.1±0.04	3.5±0.22	<0.1±0	0.1±0.0
Krnovo	1.3±0.07	5.4±0.25	<0.1±0	0.2±0.0
Ostrovica	7.9±0.35	9.9±0.02	<0.1±0	0.6±0.0
Štitovo	2.0±0.02	19.2±0.7	<0.1±0	0.7±0.01
Total heavy metals content in topsoil				
Podromanija	31.8±1.9	26.1±4.8	25.4±1.1	2.18±0.02
Han Kram	17.5±0.1	15.2±0.3	23.0±0.1	0.17±0.00
Motički Gaj	22.3±0.1	30.8±2.1	1.62±0.0	1.41±0.05
Mokro	112±2.2	28.2±1.6	92.3±2.5	<0.05±0.0
Krnovo	50.7±3.8	11.9±0.6	27.4±4.2	0.45±0.00
Ostrovica	58.9±2.8	14.7±0.1	37.7±2.6	0.73±0.01
Štitovo	33.0±1.7	24.0±0.4	29.5±1.2	1.02±0.05
MPL§	50	100	100	3
Heavy metals concentrations in plants				
Podromanija	9.57±0.17	6.66±3.19	<0.1±0.0	0.31±0.0
Han Kram	7.22±0.61	11.1±3.56	<0.1±0.0	0.46±0.11
Motički Gaj	5.97±0.43	12.1±2.79	7.31±4.1	0.16±0.0
Mokro	9.53±1.15	6.17±1.92	<0.1±0.0	<0.05±0.0
Krnovo	3.79±0.29	1.26±1.18	<0.1±0.0	0.50±0.32
Ostrovica	7.08±0.22	4.62±2.09	<0.1±0.0	0.28±0.0
Štitovo	4.74±0.71	3.14±3.94	<0.1±0.0	0.69±0.0
NC*	0.1-5	1-5	<0.1-1	<0.1-1
TC**	30	20	2	10
MTLF***	50	40	-	1

§ MPL- maximum permissible levels of dangerous and hazardous matters according to *Official Gazette of RS, 1994*; *Normal concentrations in plants according to*Chaney (1983)*; **TC - Toxic concentrations in plants according to*Kabata-Pendias (2010)*; ***MTLF - Maximum tolerant level for fodder in plants according to *NRC (2005)*; *Official Gazette of RS (2009)*

Some plant species, mainly from families *Boraginaceae, Cruciferae, Myrtaceae, Leguminosae* and *Caryophyllaceae* are accumulators of Ni. In most cases, Ni is accumulated in the roots. All measured concentrations of Ni in plants from meadows were below the critical concentration for normal plant growth. On the other hand, Ni content in Montenegro on three locations, (Table 3) was higher than maximum permissible amount in soil, but available Ni concentrations were between 0.6-7.9 mg kg^{-1}. Concentrations of Ni in plants collected from all sites were below 10 mg kg^{-1}. The elements Ni and Cr, are presumed essential for ruminants, needs for Ni is from 60-70 mg kg^{-1} *(Miranda et al., 2009)*.

Although Pb occurs naturally in all plants, it has not been shown to play any essential role in their metabolism. The Pb concentration in forage crops is ranged from 2.1 (grasses) to 2.5 mg kg^{-1} (clovers) and Pb is considered as metal with the lowest biological accessibility and highest bioaccumulation in the roots *(Kabata-Pendias, 2010)*. It was not confirmed by results from investigated meadows, where some the examined plant tissues accumulated Pb content > 5 mg kg^{-1}, which could be explained by the vicinity of road and traffic-related air polution. The measured Cd content in soil was low and Cd was neither readily soluble nor easily phytoavailable.

The Cr concentration was <0.1 mg kg^{-1} in the soils and, consequently, the Cr concentration in plant tissue samples was low, except in location Motički gaj, where it was much higher than limit concentration (7.31 mg kg^{-1}), which could be potentially detrimental for the plant growth. Cr is an essential element for organisms as its important for normal metabolism of glucose. It is not a toxic element, and negative effects on the function of the organism halves at concentration greater than 50 mg kg^{-1} *(Đorđević et al., 2009)*.

Cu and Co are important micronutrients. Cu requirements for cattle are about twice as much for sheep, but the cobalt requirements for sheep are about twice as much for cattle *(Mayland and Shewmaker, 2001)*. Also, those authors reported several incidences of Cu toxicity in grazing sheep on recently manured pastures. Excess Co concentrations results in a decrease in appetite and animal growth. Rosters are highly tolerant to high Cu concentrations because there are broad boundaries between the levels of tolerance and toxicity. In examined species, the Cu and Co concentrations were found to be within the normal range.

Although some elements exceeded maximum permissible amount for soil and water (Ni, Cr), the ability of plants collected from the *Agrostietum capillaris* communities to accumulate micronutrients and heavy metals was generally low. It could be explained by the physiology of dominant plant species (grasses), which influences relatively low uptake and generally low accumulation of micronutrients, similar to the results reported by *Simić et al. (2015)* in grasses collected on ash deposit.

Conclusions

We assessed the micronutrients and heavy metal status of extensive meadows of *Agrostietum capillaris* in Bosnia and Montenegro, in relation to the abundance of those chemical elements of the soil. Iron and zinc were the most prevalent elements in plant tissues, in absolute values. Dominant species in *Agrostietum capillaris* are grasses and the level of all studied elements in the plant biomass collected from investigated meadows were within the allowed limits.

In general, it can be concluded that from the results of this study, the mineral element concentrations of analyzed herbage samples from all sites do not exceed maximal tolerance levels for fodder.

Mikrohraniva i teški metali zajednice *Agrostietum capillaris* u Balkanskim državama

Aleksandar Simić, Željko Dželetović, Gordana Andrejić, Mirjam Radulaški, Zorica *Bijelić, Violeta Mandić*

Rezime

Permanentni travnjaci igraju važnu ulogu u poljoprivrednoj proizvodnji Bosne i Hercegovine, kao i Crne Gore. Oni pokrivaju više od polovine poljoprivrednog zemljišta u Bosni i oko 33% crnogorske teritorije. Ekstenzivno korišćeni prirodni travnjaci dominiraju u obe države i generalno je produktivnost ovih biljnih zajednica ekstremno mala. Jedna od ekonomski najznačajnijih fitocenoza je *Agrostietum capillaris*. Najveći deo asocijacija *Agrostietum capillaris* je usko povezan sa ljudskim aktivnostima i mnoge zajednice su suočene sa ubrzanim promenama u poljoprivrednoj praksi, prvenstveno sa napuštanjem zemljišta. U sklopu toga je sprovedeno istraživanje sadržaja teških metala i biljkama pristupačnih mikrohraniva na zemljištima pod travnom zajednicom *Agrostietum capillaris* na pet lokacija u Crnoj Gori i dve lokacije u Bosni i Hercegovini.

Iako pojedini elementi (Ni, Cr) na pojedinim lokacijama prelaze granice maksimalno dopuštenih vrednosti za zemljište i vode, mogućnost biljaka zajednice *Agrostietum capillaris* da ih u značajnoj meri akumuliraju je generalno mala.

Ključne reči: *Agrostietum capillaris,* Bosna, Crna Gora, elementi, zemljište

References

ALIBEGOVIĆ-GRBIĆ, S. (2009): Bosnia and Herzegovina. Country Pasture/Forage Resource Profile, FAO, p. 23.
CHANEY R.L. (1983): Potential effects of waste constituents on the food chain. In: Parr, J.F., Marsh, P.B., Kla, J.M. (eds), Land Treatment of Hazardous Wastes, Noyes Data Corporation, Park Ridge, New Jersey (1983) 152-239.
DUBLJEVIĆ, R. (2005): Influence of fertilization on producing properties of degraded mountain grassland type of *Nardetum strictae*. Agroznanje, Banja Luka, B&H, 6, 4,31-38.
DUBLJEVIĆ R. (2007): Nitrogen fertilization influence on producing properties of meadow of type *Agrostidetum vulgaris* in hilly area of Polimlje. Zbornik radova, Institut za ratarstvo i povrtarstvo, Novi Sad, 44, 1, 361-367.
DUBLJEVIĆ R. (2009): Montenegro. Country Pasture/Forage Resource Profiles, FAO, p. 26.
FAO (2015): Understanding Mountain Soils: A contribution from mountain areas to the International Year of Soils 2015, by Romeo, R., Vita, A., Manuelli, S., Zanini, E., Freppaz, M. & Stanchi, S. Rome, Italy, p. 157.
DJORDJEVIĆ N., GRUBIĆ G., MAKEVIĆ M., JOKIĆ Ž. (2009): Ishrana domaćih životinja. Univerzitet u Beogradu, Poljoprivredni fakultet, 1022.
DŽAMIĆ, R. A., STEVANOVIĆ, D., JAKOVLJEVIĆ, M. (1996): Praktikum iz agrohemije. Poljoprivredni fakultet.
FAO (2015): Understanding Mountain Soils: A contribution from mountain areas to the International Year of Soils 2015, by Romeo, R., Vita, A., Manuelli, S., Zanini, E., Freppaz, M. & Stanchi, S. Rome, Italy
IUSS WORKING GROUP (2014): World reference base for soil resources 2014 international soil classification system for naming soils and creating legends for soil maps. FAO, Rome.
JONES J. B. JR., CASE V. W. (1990): Sampling, handling, and analyzing plant tissue samples. In: Soil testing and plant analysis, 3rd edition (Ed. R. L. Westerman), 389-427, SSSA, Madison.

KABATA-PENDIAS A. (2010): Trace Elements in Soils and Plants, Fourth Edition, p. 548.

MAYLAND H. F., SHEWMAKER G. E. (2001): Animal health problems caused by silicon and other mineral imbalances. Journal of range management, 441-446.

MIJATOVIĆ M., PANTOVIĆ M., GLINTIĆ M., PAVEŠIĆ-POPOVIĆ J., JAKOVLJEVIĆ M. (1977): Effect of microelements application on the production of sown grasslands on eroded soil in the mountains. In XIII International Grassland Congress: Leipzig, German Democratic Republic, 18-27 May, 1977, Ed. E. Wojahn and H. Thons. Berlin, Akademie-Verlag, 1117-1120.

MIRANDA M., BENEDITO J.L., BLANCO-PENEDO I., LÓPEZ-LAMAS C., MERINO A., LÓPEZ-ALONSO M. (2009): Metal accumulation in cattle raised in a serpentine-soil area: Relationship between metal concentrations in soil, forage and animal tissues. Journal of Trace Elements in Medicine and Biology, 23, 3, 231-238.

NATIONAL RESEARCH COUNCIL (NRC) (2005): Mineral Tolerance of Animals: Second Revised Edition, Washington, DC: The National Academies Press, 2005.

OFFICIAL GAZETTE OF RS (1994): Rule book of permissible concentrations of dangerous and hazardous materials in soil and in water for irrigation and methods for analysis, 23.

OFFICIAL GAZETTE OF RS (2009): Law on Food Safety, Rule book of animal food quality, 41/09.

PANSU M., GAUTHEYROU J. (2006): Mineralogical separation by selective dissolution. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods, Springer Berlin / Heidelberg, 167-219.

PAVLOVIĆ P., MITROVIĆ M., DJURDJEVIĆ L. (2004): An Ecophysiological Study of Plants Growing on the Fly Ash Deposits from the “Nikola Tesla-A” Thermal Power Station in Serbia. Environmental Management 33, 5, 654-663.

PEGANOVA S., EDLER K. (2004): Zinc. In: Elements and Their Compounds in the Environment, 2nd edn., eds. E. Merian, M. Anke, M. Ihnat, M. Stoeppler, 1203–1239, Wiley-VCH, Weinheim.

RADOJIČIĆ B. (2002): Geography of Montenegro, DANU, Podgorica, 191-254.

SIMIĆ A., DŽELETOVIĆ Ž., VUČKOVIĆ S., ĆUPINA B., MANDIĆ V., KRSTIĆ Đ. (2014): Trace elements concentrations in herbaceous plants from ash deposit of thermal power station. In: Soil 2014 - Planning and land use and landfills in terms of sustainable development and new remediation technologies (Proceedings of Integrated meeting, May 12-13, 2014., Zrenjanin, Serbia), Eds. Vrvić, M., Cokić, Z. and Tanasijević, Lj., Publisher: Association for the development and use soil and landfils, Belgrade, Serbia, 167-174.

SIMIĆ A., DŽELETOVIĆ Ž., VUČKOVIĆ S., SOKOLOVIĆ D., DELIĆ D., MANDIĆ V., ANĐELKOVIĆ B. (2015): Upotrebna vrednost i akumulacija teških metala u krmnim travama odgajenim na pepelištu termoelektrane. Hemijska industrija, 69, 5, 459-467.
THORNTON I., ALLOWAY B. J. (1974): Geochemical aspects of the soil-plant-animal relationship in the development of trace element deficiency and excess. Proceedings of the Nutrition Society, 33, 3, 257-266.
VUČKOVIĆ S., SIMIĆ A., ĆUPINA B., KRSTIĆ Đ., DURONIĆ G. (2010): Effect of mineral fertilization on yield of *Agrostidetum vulgaris* – type meadows in mountainous grasslands in Serbia. XII International Symposium on Forage Crops of Republic of Serbia, Biotechnology in Animal Husbandry, 26 (spec. issue), 389-394.
ZAK D.R., HOLMES W.E., MACDONALD N.W., PREGITZER K.S. (1999): Soil Temperature, Matric Potential, and the Kinetics of Microbial Respiration and Nitrogen Mineralization. Soil Science Society of America Journal, 63, 575-584.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EFFECT OF DIFFERENT NITROGEN FERTILIZATION LEVELS ON MAIZE FORAGE YIELD AND QUALITY

Violeta Mandić[1], Zorica Bijelić[1], Vesna Krnjaja[1], Dragana Ružić Muslić[1], Violeta Caro Petrović[1], Snežana Đorđević[2], Maja Petričević[1]

[1]Institute for Animal Husbandry, Department of Ecology and Animal Feed, Autoput 16, 11080, Belgrade, Republic of Serbia
[2]Agrounik d.o.o., Research and Development Centre, Beograd, Republic of Serbia
Corresponding author: violeta_randjelovic@yahoo.com
Original scientific paper

Abstract: The optimal nitrogen input is very important factor to achieve high crop yields at lower costs of production on farms. The aim of this research was to estimate the effects of different nitrogen levels (0, 60, 120 and 180 kg ha^{-1}) on forage yield and quality green mass in two maize hybrids ZP 666 and NS 6030. Field experiment was conducted on dry land farming at the Institute for Animal Husbandry Belgrade-Zemun in 2013. The tested hybrid NS 6030 had significantly higher ear height (EH), number of leaves (NL), buffering capacity of green mass (BC) and water soluble carbohydrates (WSC) and significantly lower dry matter content (DM) and crude protein content (CP) than hybrid ZP 666. Nitrogen levels have significantly effect on plant height (PH), NL, forage yield (FY), rain use efficiency (RUE), N use efficiency (NUE), CP, BC and WSC. PH and RUE did not differ between treatments fertilized with 60, 120 and 180 kg N ha^{-1}. Maximum NL and FY observed at 120 kg N ha^{-1}, CP at 180 kg N ha^{-1}, BC at control and NUE and WSC at 60 kg N ha^{-1}. NUE was significantly decreased with increased N rate from 60 to 180 kg ha^{-1}. NUE significantly depends on the availability of water, as indicated by the correlation coefficient between these parameters. Studied hybrids are suitable for ensiling. N rate of 120 kg N ha^{-1} can be recommended for growing studied hybrids in order to achieve high yields and quality of forage.

Key words: maize hybrid, N rate, forage yield, forage quality.

Introduction

Maize is used for human food, animal feed and as industrial raw material. In Serbia, about 80% of the total production of maize is used for feeding livestock. Maize grain can be used as feed for all farm animal species, while the silage or green fodder for ruminants (dairy and beef cattle, sheep and goats). Many positive parameters make maize a very good crop to ensile, such as high production of green mass per unit area, energy content of dry matter and quality of biomass *(Mandić et al., 2013)*. *Mandić et al. (2017)* report that in Province of Vojvodina average forage maize yield over longer period (2000-2015) was 22.7 t ha^{-1} and varied from 11.8 t ha^{-1} (2000) to 31.8 t ha^{-1} (2010). Also, their study shows that 43% of variation in maize forage yield could be explained by rainfall variability, while 57% by maize genetics, technical factors and other climatic factors. Their results indicated that forage maize yield had strong positive relationship with amount of rainfall during the growing season and average monthly rainfall for May and August. On the other hand, *Randjelovic et al. (2011)* report that amount of rainfall in June, July and August are important for maize biomass production. Yield of maize depends on the genetic potential, but climatic factors and nutrients has important role in rapid achieve their genetic potential. Generally, intensive maize production is based on the use of mineral nitrogen fertilizers, because nitrogen is a essential nutrient for plant growth, development and reproduction and affects the plant processes *(Mandić et al., 2016)*. Deficiency of nitrogen leads to loss green color in plants, because lower production of chlorophylls and proteins. This leads to lower photoasssimilate production and thus in lower green matter conversion. In this sense, *Jakelaitis et al. (2005)* concluded that genotypes with high NUE have greater capacity to assimilate CO_2 and synthesize carbohydrates in the photosynthesis, resulting in a higher biomass accumulation and grain yield. Maize requires large amounts of nitrogen inputs in order to produce large quantities of biomass. However, application of high level of N fertilizer may have negative effect on the environment, because nitrogen can be lost through soil erosion and runoff and can lead to contamination of ground and surface water. Therefore, nitrogen management in agriculture aims at achieving high crops and minimal N losses. *Amin (2011)* points that nitrogen frequently limits yield and plays an important role in forage quality of crops. *Zhao et al. (2005)* points that the nitrogen deficiency significantly reduces the yield because it is the main element for the synthesis of amino acids, nucleic acids and some organic acid and is necessary for plant growth. Many researchers report that the nitrogen fertilizer strongly affects the maize forage yield and quality, i.e. forage yield increases with increasing nitrogen level. So, *Cheema et al. (2010)* observe that an increase in the fertilizer nitrogen level increases the plant height, stem thickness, leaf area, leaf area index, dry matter accumulation; net assimilates ratio and yield per hectares. *Safari et al. (2014)* report that the highest values of plant height, stem diameter, crude protein, protein yield and forage yield of maize cultivar SC 704 are recorded at 150 and 225 kg N ha^{-1}. *Reddy and Bhanumurty (2010)* report that the highest values of green fodder yield, dry matter yield and crude protein of maize are recorded at 240 kg N ha^{-1}. *Almodares et al. (2009)* conclude that the highest values for biomass and protein content and lowest soluble carbohydrates and fiber contents of maize are recorded at an urea rate of 200 kg ha^{-1}. According to *Karasu et al. (2009)*, the highest forage and dry matter yield of maize are recorded in application of 300 kg N ha^{-1}. *Eltelib et al. (2006)* stated that nitrogen improves vegetative growth, FY and CP of forage maize. *Sheaffer et al. (2006)* report that increasing N rates from 0 to 200 kg ha-1 increases grain yield, whole plant dry matter yield and forage crude protein content of maize.

The aim of this paper was to estimate the effects of various nitrogen levels (0, 60, 120 and 180 kg ha^{-1}) on forage yield and quality in two Serbian maize hybrids of FAO 600 maturity group (ZP 666 and NS 6030).

Materials and methods

Field experiment was carried out in dry land farming at the trial field of the Institute for Animal Husbandry, Belgrade-Zemun (44° 49′ 10″ N, 20° 18′ 45″ E; 88 m a.s.l.), as a completely randomized design in four replications. The plot size was 16.8 m^2. Two maize hybrids FAO 600 maturity group (ZP 666 and NS 6030) were grown during 2013, at four N fertilization levels (0, 60, 120 and 180 kg N ha^{-1}). Preceding crop was winter wheat. Maize was planted on April 8th. Plant density was 64900 plants per hectare. Mineral nutrition *KAN* (27% N) was applied on May 15th when maize had from three to five expanded leaves (V3-V5 growth stage). The standard cultivation practice was applied.

The soil type was a calcareous Chernozem with pH in H_2O 7.18, 9.53% organic matter, 4.1% humus, 0.24% total N, 13.04 mg NH_4^+-N kg^{-1} and 4.12 mg NO_3^--N kg^{-1}. The P_2O_5 and K_2O were 5.03 and 14.1 mg 100 g^{-1} soil, respectively.

Climate diagram according to Walter and Lieth showed that in 2013 (total season rainfall 223 mm) drought periods were in April, when maize was at the initial stage of growth i.e. germination stage and early seedling growth, from late June until the harvest, i.e. from intensive growth of the stem to the milk-wax stage (Figure 1). The summer was dry and very hot. The wettest month was May with an average of 104.4 mm of rainfall. July was the driest month with rainfall of 2.9 mm. August was the hottest month with an average temperature of 25.3^{o}C. On hot summer days the air was very dry. Generally, in 2013, the climatic conditions were unfavorable (heat stress and drought).

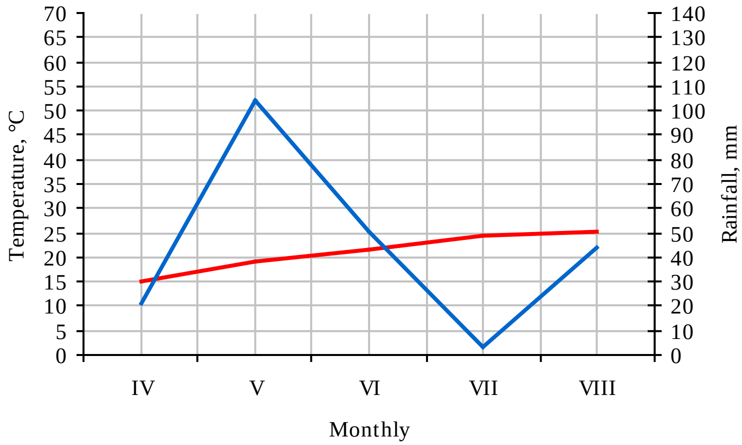


Figure 1. Climate diagram according to Walter and Lieth in the 2013 for Zemun, Serbia.

Plant height (PH), ear height (EH) and number of leaves per plant (NL) were recorded for 10 plants per each subplot. Maize hybrids were harvested during the second half of August at three-quarters milk line stage. Plants from each sub plot were cut on height 20 cm and chopped using maize forage combine harvester, and forage yield (FY) was measured. The FY was converted into t ha^{-1}. Rain use efficiency (RUE) was calculated according to formula FY/total seasonal rainfall (t ha^{-1} mm^{-1}) and N-use efficiency (NUE) according to formula FY/N applied (t forage mass kg^{-1} N applied). The dry matter (DM) was determined as the difference in the mass before and after the drying to constant mass in an oven at 105°C. The crude protein content (CP) was determined according to Kjeldahl *(AOAC 1990)*, buffering capacity (BC) according to method of *Playne and McDonald (1966)* and water soluble carbohydrate content (WSC) according to Luff-Schoorl method.

Data were processed using analysis of variance (ANOVA) using STATISTICA version 10. The significance level was set at $P \leq 0.05$ and $P \leq 0.01$ while differences between traits means were assessed using Duncan's Multiple Range Test at $P \leq 0.05$ level. The relationships between studied parameters were analyzed with simple Pearson correlation coefficients.

Results

Results of ANOVA indicated that hybrid had highly significant effect on EH and NL (Table 1). Hybrid NS 6030 had significantly higher EH (75.6 cm) and NL (10.7) than hybrid ZP 666 (63.8 cm and 10.1, respectively). Hybrid NS 6030 had lower PH (211.5 cm) and higher FY (32.26 t ha^{-1}) and NUE (0.26 t forage mass kg^{-1} N applied) than hybrid ZP 666 (215.2 cm, 30.62 t ha^{-1} and 0.25 t forage mass kg^{-1} N applied, respectively), but these differences were not significant.

Table 1. Hybrid and nitrogen fertilization level effects on maize traits, rain use efficiency and N use efficiency

Factor	PH	EH	NL	FY	RUE	NUE
	Hybrid (A)					
ZP 666	215.2 ± 6.7	63.8^{b} ± 5.9	10.1^{b} ± 0.4	30.62 ± 3.6	0.14±0.02	0.25 ± 0.12
NS 6030	211.5 ± 10.1	75.6^{a} ± 7.2	10.7^{a} ± 0.4	32.26 ± 3.7	0.14±0.02	0.26 ± 0.13
F test	ns	*	*	ns	ns	ns
	Nitrogen level, kg ha^{-1} (B)					
0	197.8^{b} ± 7.7	65.8 ± 7.1	10.1^{b} ± 0.4	25.86^{b} ± 5.2	0.11^{b} ± 0.10	0^{d}
60	214.9^{a} ± 8.4	68.5 ± 9.0	10.4^{b} ± 0.4	31.46^{b} ± 4.0	0.15^{a} ± 0.14	0.55^{a} ± 0.06
120	223.2^{a} ± 16.9	76.2 ± 15.2	10.8^{a} ± 1.1	36.56^{a} ± 1.1	0.16^{a} ± 0.15	0.30^{b} ± 0.04
180	217.6^{a} ± 9.7	68.2 ± 12.4	10.3^{b} ± 0.4	31.88^{b} ± 5.5	0.14^{a} ± 0.13	0.18^{c} ± 0.03
F test	**	ns	*	**	**	**
AxB	ns	ns	ns	ns	ns	ns
M	213.4	69.7	10.4	31.44	0.14	0.26

PH: plant height (cm); EH: ear height (cm); NL: number of leaves per plant; FY: forage yield (t ha^{-1}); RUE: rain use efficiency (t ha^{-1} mm^{-1}) and NUE: N use efficiency (t forage mass kg^{-1} N applied). Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range test ($p \leq 0.05$). *, **Significant at the 0.05 and 0.01 probability levels, respectively; ns: non-significant.

The nitrogen fertilization level had highly significant effect on NL, and very significant on PH, FY, RUE and NUE. However, results showed that PH and RUE did not differ between treatments fertilized with 60, 120 and 180 kg N ha^{-1}. The maximum PH (223.2 cm) and RUE (0.16 t ha^{-1} mm^{-1}) were recorded at 120 kg N ha^{-1}. Application of 120 kg N ha^{-1} gave significantly higher NL (10.8) and FY (36.56 t ha^{-1}) as compared to control (10.1 and 25.86 t ha^{-1}, respectively), 60 kg N ha^{-1} (10.4 and 31.46 t ha^{-1}, respectively) and 180 kg N ha^{-1} (10.3 and 31.88 t ha^{-1}, respectively). The N fertilization level (60, 120 and 180 kg ha^{-1}) had highly significant effect on NUE (0.55, 0.30 and 0.18 t forage mass kg^{-1} N applied, respectively) compared to control. NUE was significantly higher at 60 kg N ha^{-1} than 120 kg N ha^{-1} and 180 kg N ha^{-1}.

It was found that there is no significant interaction effect between hybrid and nitrogen level for parameters described in table 1.

Very strong positive correlation was found for FY with RUE (r = 0.88), table 3. Strong positive correlations were found for FY with NL (r = 0.63**) and NL with RUE (r = 0.62**). Moderate positive correlations were found for PH with EH (r = 0.47**), RUE with NUE (r = 0.59**) and FY with NUE (r = 0.41*). Weak, low positive correlations were found for PH with RUE (r = 0.40*) and NUE (r = 0.36*), PH with NL ($r = 0.27^{ns}$) and FY ($r = 0.27^{ns}$), and EH with NL ($r = 0.26^{ns}$). Very weak to negligible correlations were found for EH with FY ($r = 0.02^{ns}$), RUE ($r = 0.04^{ns}$) and NUE ($r = 0.08^{ns}$), and EH with NUE ($r = 0.08^{ns}$).

Table 2. Pearson correlation coefficient (*r*) between studied traits of two maize hybrids (ZP 666 and NS 6030 and four N fertilization levels (0, 60, 120 and 180 kg N ha^{-1})

	PH	EH	NL	FY	RUE
EH	0.47**				
NL	0.27^{ns}	0.26^{ns}			
FY	0.27^{ns}	0.02^{ns}	0.63**		
RUE	0.40*	0.04^{ns}	0.62**	0.88**	
NUE	0.36*	0.08^{ns}	0.27^{ns}	0.41*	0.59**

*, **Significant at the 0.05 and 0.01 probability levels, respectively; ns: non-significant.
PH: plant height; EH: ear height; NL: number of leaves per plant; FY: forage yield; RUE: rain use efficiency and NUE: N use efficiency.

Results of ANOVA in Table 3 showed that hybrid had highly significant effect on DM, CP, BC and WSC of the green forage mass. Hybrid ZP 666 had significantly higher DM (501.4 g kg^{-1}) and CP (g kg^{-1} DM) than hybrid NS 6030 (413.6 g kg^{-1} and 62.5 g kg^{-1} DM, respectively). Contrary, hybrid ZP had significant lower BC (38.5 meq 100 g^{-1} DM) and WSC (20.1 g kg^{-1} DM) than hybrid NS 6030 (43.0 meq 100 g^{-1} DM and 51.1 g kg^{-1} DM, respectively).

The nitrogen fertilization level had highly significant effect on CP, BC and WSC. Results showed that level of 180 kg N ha^{-1} lead to higher content of CP (98.6 g kg^{-1} DM) compared to other nitrogen treatments. CP did not differ between treatments fertilized with 0 (69.7 g kg^{-1} DM), 60 (71.8 g kg^{-1} DM) and 120 kg N ha^{-1} (78.5 g kg^{-1} DM). In treatment with 60 kg N ha^{-1}, BC was significantly lower and WSC significantly higher compared to other nitrogen treatments.

The interaction of hybrid and N fertilization level had highly significant effect on BC and WSC.

Table 3. Hybrid and nitrogen fertilization level effects on the quality green forage mass

Factor	DM	CP	BC	WSC
	Hybrid (A)			
ZP 666	501.4^{a}	96.8^{a}	38.5^{b}	20.1^{b}
NS 6030	413.6^{b}	62.5^{b}	43.0^{a}	51.1^{a}
F test	**	**	**	**
	Nitrogen level, kg ha^{-1} (B)			
0	428.7	69.7^{b}	42.7^{a}	42.0^{b}
60	473.4	71.8^{b}	38.5^{d}	51.8^{a}
120	449.5	78.5^{b}	41.6^{b}	26.5^{c}
180	478.5	98.6^{a}	40.2^{c}	22.3^{d}
F test	ns	**	**	**
AxB	ns	ns	**	**
M	457.5	79.7	40.8	35.6

DM: dry mater (g kg^{-1}); CP: crude protein content (g kg^{-1} DM); BC: buffering capacity (meq 100 g^{-1} DM) and WSC: water soluble carbohydrates (g kg^{-1} DM). Means followed by the same letter within a column are not significantly different according to Duncan's Multiple Range test ($p \leq 0.05$). *, **Significant at the 0.05 and 0.01 probability levels, respectively; ns: non-significant.

Discussion

Significant differences were recorded among maize hybrids for EH and NL. Hybrid NS 6030 had significantly higher EH than hybrid ZP 666. Ear height is very important trait for harvest of maize and the ears need to be at the same height within a population. If the ear height is greater, ear may bend the stem or break, but if it is lower, this further complicates harvesting and has a negative impact on yield because of more ear drop during harvest. Accordingly, the hybrid NS 6030 has fewer losses of ears during ensiling. *Zsubori et al. (2002)* report that environmental conditions and agronomic factor, such as plant density, fertilization, pests and diseases, influence the expression of EH. EH is a very important trait that is considered necessary in maize and is related to morphology, lodging, and yield *(Li et al., 2014)*. *Rao et al. (2014)* conclud that optimal PH and EH are critical parameters for improving plant density to maximize the utilization of fertilizer, moisture and photosynthetically active radiation. Generally, dry stress during stem elongation stage (in the second half of June) reduces the PH and EH, especially in hybrid NS 6030, which in the favorable climatic conditions has a PH of 300 cm and EH of 110 cm *(Jocković et al, 2010)*. The higher air temperature and lower amount of rainfall in this period reduce the stem cell expansion. Water deficit caused by drought slows down stem growth, and elongation of internodes is more inhibited. The result is that the internodes are drastically shorter. Also, *Mandić et al. (2015)* reported that lower amount of rainfall and the higher air temperature in June reduced the stem cell expansion. The NL depends on the genetic basis of hybrid. Hybrid NS 6030 has significantly higher NL than hybrid ZP 666. Also, *Mandić et al. (2015)* report that hybrid NS 6010 has significantly higher NL than hybrid ZP 684. In both researches, results show that hybrids of maize developed at Institute of field and vegetable crops, Novi Sad, have higher NL than hybrids developed at Maize Research Institute, Zemun Polje. On the other hand, PH, FY, RUE and NUE show no significant differences among studied hybrids, indicating their similar behavior in the environment conditions. Hybrid NS 6030 has higher FY for 1.64 t ha^{-1} than hybrid ZP 666, but the difference is not significant. Water resources in the Republic of Serbia are limited that's why for stable maize production rain use efficiency (RUE) is very important. The genotypes with improved RUE are particularly beneficial under low rainfall conditions. In our studies it is interesting that both hybrids have the same value for RUE (0.14 t ha^{-1} mm^{-1}). Also, hybrids ZP 666 and NS 6030 have similar values for NUE (0.25 and 0.26 t forage mass kg^{-1} N applied, respectively). Contrary, *Hokmalipour et al. (2010)* report significant variation in NUE among different maize genotypes, while *Cui et al. (2009)* conclude that the interaction between genotypes and nitrogen greatly influence NUE in maize.

Generally, the amount of rainfall and mean monthly air temperatures were not favorable for the production of maize. Drought periods were at the beginning of the growing period (sowing date), at the stage of intensive growth of the stem in second half of June, at the stage of flowering in July and at the stage of grain filling in August. Even short drought stress in the vegetative stage of maize can reduce PH, leaf area development and dry matter content of maize *(Çakir, 2004)*. Summer drought limits forage production by reducing stem growth and ear size. Ears were short with a small number of grains due to poor pollination. Also, *Mandić (2011)* shows that drought stress in August reduces the grain weight per ear, 1.000 grain weight and grain yield. Therefore, unfavorable climatic conditions contribute to low forage mass of maize. High FY was obtained in years with well distributed rainfall from June to August *(Mandić et al., 2015).*

PH, NL, FY, RUE and NUE were significantly affected by N fertilizer treatments. PH and RUE increased with increasing N rate from 0 to 120 kg ha^{-1}, while further increase of N rate to 180 kg ha^{-1} decreased the PH and RUE. However, there was no significant difference between the N rates of 60, 120 and 180 kg N ha^{-1}. Similar to *Amin (2011)*, our results indicate that increase in plant height is a result of increasing the length of internodes because nitrogen stimulates growth. The plants used N during active cell division to form building protein for cell elongation *(Iqbal et al., 2006)*. This effect is higher with applying N fertilizer than without N fertilizer. EH increased with increasing N rate from 0 to 120 kg ha^{-1}, and then decreased with the further increase of N rate to 180 kg ha^{-1}. However, there was no significant difference between the N rates. The highest NL and FY were recorded at 120 kg N ha^{-1} while the lowest value recorded at 0 kg N ha^{-1} which was statistically at par with 60 kg N ha^{-1} and 180 kg N ha^{-1}. Nitrogen fertilizer had stimulatory effect on stem elongation and leaf emergence of maize. Also, the increase in the NL could possibly be ascribed to the fact that nitrogen increases plant growth and height and this resulted in more nodes and internodes and more production of leaves. *Amin (2011)* report similar results. Nitrogen is a component of protoplasm, proteins, nucleic acids, chlorophyll and plays a vital role in vegetative phases of crop growth. Thus, *Safari et al. (2014)* conclude that nitrogen uptake increases with the increase in nitrogen fertilizer, and given in mind the role of nitrogen in the synthesis of chlorophyll and hence the process of photosynthesis and carbon dioxide assimilation leading to enhanced growth (plant height, stem diameter and number of leaves). Generally, forage yield is a function of growth parameters. The application of the nitrogen dose of 120 kg ha^{-1} stimulates vegetative growth by increasing the PH and NL, and thus the biomass (forage mass). *Witt et al. (2008)* point that NPK fertilizer application is the most important factor required for increasing fodder yield of maize. *Hassan et al. (2010)* have concluded that 140 kg N ha^{-1} has the most positive influence on stem diameter, leaf area index, green fodder yield and total dry matter of maize hybrid Akbar. According to them, this is the optimum nitrogen rate for maize fodder production. *Ali and Anjum (2017)* recommend that 180 kg N ha^{-1} is most economical strategy for obtaining best quality green and dry matter fodder maize yield. In compared to control, NUE was significantly higher at 60, 120 and 180 kg N ha^{-1}. The lower NUE (0.18 t forage mass kg^{-1} N applied) was recorded at 180 kg N ha^{-1} as a result of N loss in ecosystem. The lower NUE is characterized by a relatively low N output in forage mass, and relatively high N input. On the other hand, the higher values of NUE indicate the deficiency of the nutrient. In our case, application of 60 kg N ha^{-1} showed the highest NUE (0.55 t forage mass kg^{-1} N applied). Also, *Bernardi et al. (2011)*, *Amanullah (2014; 2016)* report that NUE decreases with increasing N rates. Results of *Cancellier et al. (2014)* show that for every kg of N applied in soil, the maize plant produces 264.1 kg of green mass.

DM, CP, BC and SS were significantly affected by hybrids. Hybrid ZP 666 had significantly higher DM and CP than hybrid NS 6030. Contrary, Hybrid ZP 666 had significantly lower BC and SS than hybrid NS 6030. Also, *Cox and Cherney (2001)*, *Sheaffer et al. (2006)*, *Bernardi et al. (2011)* and *Safari et al (2014)* conclude that N fertilizer increases whole plant CP content. In our results, nitrogen application of 180 kg N ha^{-1} gave the highest CP as compared with the other nitrogen rates. Generally, increasing content of CP with application of nitrogen, result is role which nitrogen has in the protein synthesis. The BC was significantly lower and WSC significantly higher in treatment with N rate of 60 kg ha^{-1} than other N treatments. This showed that maize hybrids have high sugar content and low buffering capacity at fertilization level of 60 kg N ha^{-1}. *Bijelić et al. (2015)* have found similar values of chemical composition of maize forages before ensiling on various N doses. Generally, forage mass of studied maize before ensiling meets the criteria for ensiling because of its low buffering capacity. *Cox and Cherney (2001)* and *Sheaffer et al. (2006)* reported that increasing N rate effects on silage maize quality components were inconsistent.

Conclusion

Selection of suitable genotypes is important factor for production of high FY. However, climatic factors and nutrients have important role for rapid achievement of genetic yield potential in maize hybrids. Hybrid NS 6030 had significantly higher EH, NL, BC and WSC and significantly lower DM and CP compared to hybrid ZP 666. Accordingly, the hybrid ZP 666 had a better quality of green mass; however both hybrids are suitable for ensiling. Results showed that PH, EH, NL, FY and RUE significantly increased with increase in fertilizer N rate to 120 kg ha^{-1}. Further increasing the N rate decreased values of these parameters.

Contrary, increasing N level from 60 to 180 kg N ha^{-1} significantly decreased the NUE. The NUE depends of the water availability. Nitrogen fertilization increased CP, but had less consistent effect on other forage quality parameters. Maize hybrids have high sugar content and low buffering capacity at fertilization level of 60 kg N ha^{-1}. Generally, the superiority N rate of 120 kg ha^{-1} may be attributed to the increase FY and quality. Also, results indicate that quality of maize green mass would be helpful in the identification and selection of genotypes for ensiling.

Uticaj različitih nivoa đubrenja azotom na prinos i kvalitet silokrme kukuruza

Violeta Mandić, Zorica Bijelić, Vesna Krnjaja, Dragana Ružić Muslić, Violeta Caro Petrović, Snežana Đorđević, Maja Petričević

Rezime

Optimalan unos azota je veoma važan faktor za postizanje visokih prinosa useva uz niske troškove proizvodnje na farmama. Cilj ovog istraživanja bio je procena efekta različitih nivoa azota (0, 60, 120 i 180 kg ha^{-1}) na prinos i kvalitet krme dva hibrida kukuruza ZP 666 i NS 6030. Poljski ogled je izveden u suvom ratarenju u Institutu za stočarstvo, Beograd-Zemun u 2013. godini. Hibrid NS 6030 imao je značajno veću visinu klipa (VK), broj listova (BL), puferni kapacitet (PK) i koncentraciju vodorastvorljivih ugljenih hidrata (VUH) i značajno niži sadržaj suve materije (SM) i sadržaj sirovog proteina (SP) od hibrida ZP 666. Nivo primenjenog azota značajno utiče na visinu biljke (VB), BL, prinos krme (PK), efikasnost korištenja padavina (kiše) (RUE), efikasnost korišćenja azota (NUE), SP, PK i VUH. VB i RUE nisu se razlikovali između tretmana đubrenih sa 60, 120 i 180 kg N ha^{-1}. Maksimalni BL i PK zabeleženi su na 120 kg N ha^{-1}, SP na 180 kg N ha^{-1}, PK u kontroli i NUE i VUH na 60 kg N ha^{-1}. NUE se značajno smanjuje sa povećanjem doze azota od 60 do 180 kg ha^{-1}. NUE značajno zavisi od dostupnosti vode, na šta ukazuje koeficijent korelacije između ova dva parametra. Ispitivani hibridi su pogodni za siliranje. Stopa azota od 120 kg ha^{-1} se može preporučiti za povećanje prinosa i kvaliteta krme kukuruza.

Ključne reči: hibrid kukuruza, stopa N, prinos krme, kvalitet krme

Acknowledgment

The research was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, project TR 31053.

References

ALI N., ANJUM M.M. (2017): Effect of different nitrogen rates on growth, yield and quality of maize. Middle East Journal of Agriculture Research, 6, 1, 107-112.

AMANULLAH (2014): Source and rate of nitrogen application influence agronomic N-use efficiency and harvest index in maize (*Zea mays* L) genotypes. Maydica 59, 80-89.

AMANULLAH (2016): Rate and timing of nitrogen application influence partial factor productivity and agronomic NUE of maize (*Zea mays* L) planted at low and high densities on calcareous soil in northwest Pakistan. Journal of Plant Nutrition, 39, 683-690.

AOAC (1990): Association of Official Analytical Chemists (AOAC), 1990. Official Methods of Analysis, Washington DC, USA, 1, 14, 684.

ALMODARES A., JAFARINIA M., HADI M. R. (2009): The effects of nitrogen fertilizer on chemical compositions in corn and sweet sorghum. American-Eurasian Journal of Agricultural and Environmental Sciences, 6, 441-446.

AMIN M.E.H. (2011): Effect of different nitrogen sources on growth, yield and quality of foddermaize (*Zea mays* L.). Journal of the Saudi Society of Agricultural Sciences, 10, 17-23.

BERNARDI A. C. C., SOUZA G. B., POLIDORO J. C., MONTE M. B. M., PAIVA P. R. P. (2011): Yield, quality components, and nitrogen levels of silage corn fertilized with urea and zeolite. Communication in Soil Science and Plant Analysis, 42, 1266-1275.

BIJELIĆ Z., MANDIĆ V., RUŽIĆ-MUSLIĆ D., TOMIĆ Z., KRNJAJA V., PETRIČEVIĆ V., GOGIĆ M., FILHO W. DE S. (2015): Effect of nitrogen fertilization and inoculant on nutritive value and fermentation characteristic of whole crop maize silage. Proceedings of the 4th International Congress New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, Serbia, 7-9 October 2015, 394-404.

ÇAKIR R. (2004): Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crops Research, 89, 1, 10, 1-16.

CANCELLIER L. L., PINHO, R. G. V., CANCELLIER L. E., PIRES L. P. M , AFFÉRRI, F. S., PELUZIO, J. M. (2014): Nitrogen use efficiency in forage yield of tropical maize populations. Ciências Agrárias, 35, 3, 1209-1220.

CHEEMA M.A., FARHAD W., SALEEM M.F., KHAN H.Z., MUNIR A., WAHID M.A., RASUL F., HAMMAD H.M. (2010): Nitrogen management strategies for sustainable maize production. Crop and Environment, 1, 1, 49-52.

ELTELIB H. A., HAMAD M. A., ELTOM E. A. (2006): The effect of nitrogen and phosphorus fertilization on growth, yield and quality of forage maize (*Zea mays* L.). Agronomy Journal, 5, 515-518.

COX W. J., CHERNEY D. J. R. (2001): Row spacing, plant density, and nitrogen effects on corn silage. Agronomy Journal, 93, 597-602.

CUI Z., ZHANG F., MI G., CHEN F., LI F., CHEN X., LI J., SHI L. (2009): Interaction between genotypic difference and nitrogen management strategy in determining nitrogen use efficiency of summer maize. Plant Soil, 317, 267-276.

HASSAN S.W., OAD F.C., TUNIO S.D., GANDAHI A.W., SIDDIQUI M.H., OAD S.M., JAGIRANI A.W. (2010): Impact of nitrogen levels and application methods on agronomic, physiological and nutrient uptake traits of maize fodder. Pakistan Journal of Botany, 42, 4095-4101.

HOKMALIPOUR S., JANAGARD M. S., DARBANDI M. H., ASHENAEE F. P., HASANZADEH M., SEIEDI M. S., SHABANI R. (2010): Comparison of agronomical nitrogen use efficiency in three cultivars of corn as affected by nitrogen fertilizer levels. World Applied Sciences Journal, 8, 1168-1174.

IQBAL A., AYUB M., ZAMAN H., AHMAD R. (2006): Impact of nutrient management and legume association on agro-qualitative traits of maize forage. Pakistan Journal of Botany, 38, 1079-1084.

JAKELAITIS A., SILVA A. A., FERREIRA L. R. (2005): Efeitos do nitrogênio sobre o milho cultivado em consórcio com Brachiaria brizantha. Acta Scientiarum Agronomy, Maringá, 27, 1, 39-46.

JOCKOVIĆ Đ., STOJAKOVIĆ M., IVANOVIĆ M., BEKAVAC G., POPOV R., ĐALOVIĆ I. (2010): NS hibridi kukuruza: danas i sutra. Ratarstvo i povrtarstvo/Field and Vegetable Crops Research, 47, 1, 325-333.

KARASU A., OZ M., BAYRAM G., TURGUT I. (2009): The effect of nitrogen levels on forage yield and some attributes in some hybrid corn (*Zea mays indentata* Sturt.) cultivars sown as second crop for silage corn. African Journal of Agricultural Research, 4, 166-170.

LI Z.Q., ZHANG H.M., WU X.P., SUN Y., LIU X.H. (2014): Quantitative trait locus analysis for ear height in maize based on a recombinant inbred line population. Genetics and Molecular Research, 13, 1, 450-456.

MANDIĆ V. (2011): Genotipski odgovor *stay green* hibrida kukuruza na povećanu gustinu useva. Doktorska disertacija, Poljoprivredni fakultet Univerziteta u Beogradu, Republika Srbija, 1-162.

MANDIĆ V., BIJELIĆ Z., KRNJAJA V., RUŽIĆ MUSLIĆ D., CARO PETROVIĆ V., OSTOJIĆ ANDRIĆ D., PETRIČEVIĆ M. (2017): Forage maize yield in function of rainfall in climatic conditions of Vojvodina (Republic of Serbia). The International Conference Agriculture for Life, Life for Agriculture, Bucharest, Romania, 8-10 June 2017, in press.

MANDIC V., DJORDJEVIC S., STANOJEVIC D., BIJELIC Z., KRNJAJA V., TOMIC Z., DRAGICEVIC V. (2016): Effect of Bacterial Seed Inoculation on Nitrogen Dynamics, Number of Bacteria in Soil under Maize, and Maize Yield. Journal of Environmental Protection and Ecology, 17, 3, 1003–1010.

MANDIĆ V., KRNJAJA V., BIJELIĆ Z., TOMIĆ Z., SIMIĆ A., STANOJKOVIĆ A., PETRIČEVIĆ M., CARO-PETROVIĆ V. (2015): The effect of crop density on yield of forage maize. Biotechnology in Animal Husbandry, 31, 4, 567-575.

MANDIĆ V., SIMIĆ A., TOMIĆ Z., KRNJAJA V., BIJELIĆ Z., MARINKOV G., STOJANOVIĆ LJ. (2013): Effect of drought and foliar fertilization on maize production. Proceedings of the 10th International Symposium Modern Trends in Livestock Production, Belgrade, Serbia, 2-4 October 2013, 416-429.

PLAYNE M. J., MCDONALD P. (1966): The buffering constituents of herbage and silage. Journal of the Science of Food and Agriculture, 17, 264-266.

RANDJELOVIC V., PRODANOVIC S., TOMIC Z., BIJELIC Z., SIMIC A. (2011): Genotype and year effect on grain yield and nutritive values of maize (*Zea mays* L.). Journal of Animal and Veterinary Advances, 10, 7, 835-840.

RAO P., SUBBAIAH G., VEERARAGHAVAIAH R. (2014): Agronomic responses of maize to plant population and nitrogen availability-a review. International Journal of Plant, Animal and Environmental Sciences, 4, 107-116.

REDDY D.M., BHANUMURTHY V.B. (2010): Fodder, grain yield, nitrogen uptake and crude protein of forage maize as influenced by different nitrogen management practices. International Journal of Bio-resource and Stress Management, 1, 69-71.

SAFARI A.R., HEMAYATI S.S., SALIGHEDAR F., BARIMAVANDI A.R. (2014): Yield and quality of forage corn (*Zea mays* L.) cultivar Single Cross 704 in response to nitrogen fertilization and plant density. International Journal of Biosciences, 4, 10, 146-153.

SHEAFFER C. C., HALGERSON J. L., JUNG H. G. (2006): Hybrid and N Fertilization Affect Corn Silage Yield and Quality. Journal of Agronomy and Crop Science, 192, 278-283.

ZHAO D., REDDY K.R., KAKANI V.G., REDDY V.R. (2005): Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. Europian Journal of Agronomy, 22, 391-403.

ZSUBORI Z., GYENES-HEGYI Z., ILLÉS O., PÓK I., RÁCZ F., SZŐKE C. (2002): Inheritance of plant and ear height in maize (*Zea mays* L.). Journal of Agricultural Sciences, University of Debrecen, 8, 34-38.

WITT C., PASUQUIN J.M.C.A., DOBERMANN A. (2008): Site-specific nutrients management for maize in favorable tropical environments of Saia. Proc. 5th Inter. Crop Sci. Cong., April 13-18, Jeju, Korea, 1-4.

ORALLY PRESENTED PAPERS

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

GENETIC DIVERSITY OF ALENTEJANO AND IBERIAN BREEDS ASSESED BY POLYMORPHISMS OF MAJOR GENES

María Muñoz[1,4], Riccardo Bozzi[2], Alessandro Crovetti[2], Rui Charneca[3], Jose M. Martins[3], Ana I. Fernández[4], Luca Fontanesi[5], Juan M. García-Casco[1,4], Cristina Óvilo[4]

[1] Centro de I+D en Cerdo Ibérico, Dpto. Mejora Genética Animal, INIA, 06300 Zafra (Spain).
[2] Department of Agrifood Production and Environmental Science - University of Firenze. Via delle Cascine, 5. 50144. Firenze (Italy).
[3] ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora (Portugal)
[4] INIA, Departamento de Mejora Genética Animal, 28040 Madrid (Spain)
[5] Department of Agricultural and Food Sciences, University of Bologna, Viale Fanin 46, 40127 Bologna (Italy)
Corresponding author: mariamm@inia.es
Original scientific paper

Abstract. Alentejano and Iberian breeds are biogeographically located in the southwest of the Iberian Peninsula. These breeds shared a set of common characteristics. Both are phenotypically very similar showing a low growth rate and a high intramuscular fat content and are reared under extensive systems in open-range fields. The aim of the current study, carried out within the framework of the TREASURE project, intends to characterize of the genetic diversity of Alentejano and Iberian breeds using the genotyping data of 32 polymorphisms located on 26 major genes. These polymorphisms were genotyped in a total of 950 animals belonging to Alentejano, Iberian and 18 additional European breeds. The low mean values of observed (Ho) and expected (Hs) heterozygosity and F_{IS} point out a low genetic diversity in the analyzed breeds. The genetic distances estimated using Ds and F_{ST} revealed values were close to 0 (0.005 and 0.030, respectively), suggesting that these breeds are genetically similar. The population structure analyzed with multivariate methods such as Discriminant Analysis of Principal Component and admixture analyses showed that both breeds were grouped within the same cluster. These results are supported by other authors based on mitochondrial sequences who reported short genetic distances between these breeds. Although complementary analyses using a larger number of markers should be performed, the results of the current analyses support the hypothesis of Alentejano and Iberian could be different strains of the same breed.

Keywords: Alentejano, Iberian, breed, polymorphisms, genetic diversity, multivariate analyses

Introduction

Alentejano and Iberian are Mediterranean pig breeds with a common origin located in the southwest of the Iberian Peninsula. While the population of Alentejano pig is located in Portugal, the population of Iberian pig is located in Spain. Although there have been interchange of individuals, mainly dams and sires, it has been limited due to a border effect. Both breeds have a lower growth rate compared to other commercial breeds like Duroc or Landrace, a higher fat deposition, and high-quality meats due to a higher intramuscular fat content. In addition, the optimal rearing conditions for Alentejano and Iberian animals are under extensive systems taking advantage of open range fields where the animals walk freely and are fed with acorns and grass. Both breeds are very appreciated for their high quality meat and are the basis for the production of local products *(Silió, 2000; Ramos et al., 2003).*

Although the breeding strategy of Alentejano has been substantially different to other autochthonous breeds, some crosses with Berkshire and Landrace breeds during the 1950s have taken place *(Fraçao, 1984)*. On the other hand, Iberian breed is not introgressed with lean European nor Chinese breeds *(Alves et al., 2003)*, but is actively crossbred with Duroc since this cross provides a higher daily gain and lean percentage in comparison to purebred animals *(Perez-Serrano, 2008; Sánchez-Esquilache, 2011).*

The TREASURE project is a multidisciplinary European project focused on the study of the diversity of local breeds and productions systems for high quality traditional products and sustainable products. Within the scope of TREASURE the objective of the current study was the characterization of the genetic diversity of Alentejano and Iberian pig breeds using the genotyping data of variants located in 26 major genes. These variants were genotyped both in Alentejano and Iberian and in 18 other autochthonous pig breeds.

Material and Methods

A total of 48 animals from Alentejano and Iberian breeds and 854 animals from 18 other European pig breeds were included in the study. Genomic DNA was extracted from blood samples with a standard phenol:chloroform protocol

(Sambrook et al., 1989). 32 variants located on 26 major genes (*MC1R*, *TYRP1*, *NR6A*, *PCK1*, *RYR1*, *IGF2*, *MC4R*, *LEPR*, *PHKG1*, *SCD*, *GBP5*, *TAS2R39*, *TAS2R4*, *TAS2R38*, *MUC4*, *ESR1*, *CYP2E1*, *LEP*, *CAST*, *MTTP*, *CYB5A*, *FTO*, *PPARGC1A*, *CAPN1*, *PPARD*, and *CTSL*) were genotyped using TaqMan® OpenArray® technology.

Genetic variability at the different loci in each population was measured with observed (Ho) and expected (He) heterozygosity *(Nei, 1987)*. Population structure was evaluated using the F_{IS} statistic *(Nei, 1987)*. Ds and F_{ST} distance measures were estimated according to *Takezaki and Nei (1996).* All the estimates were computed using the library hierfstat in R environment *(Goudet, 2005)*. In addition, in order to represent geometric relationships among the pig breeds, principal component analyses were carried out using the library FactoMineR in R environment *(Lê et al., 2008)*.

Population genetic structure was analyzed through Discriminant Analysis of Principal Component (DAPC) and the optimal number of clusters was identified through the Bayesian Information Criterion (BIC). Derivation of group membership probabilities has also been calculated using both α-score optimization and cross-validation (1000 replications). Both analyses were performed using the library Adegenet v.2.0.2 in R environment *(Jombart, 2008)*.

The algorithm implemented in STRUCTURE *(Pritchard et al., 2000)* was also used to determine the most likely number of partition in the dataset, irrespective of breed of origin. The assignment of the individuals to populations considered an ancestry model with no admixture and correlated allele frequencies. The burning period was set to 50,000 and the number of MCMC replications after burning was 200,000 for $2 \leq k \leq 25$ (k number of clusters).

Results and Discussion

A total of 26SNPs were successfully genotyped. The overall Ho and Hs values per locus ranged from 0.024 to 0.414 and from 0.025 to 0.415. The F_{ST} value from all loci was 0.27, pointing out that 27% of differences are due to breed and 73% caused by differences among individuals.

A total of 17 and 19 SNPs segregated in Alentejano an Iberian, respectively. Table 1 shows that both Alentejano and Iberian breeds have low mean values for Ho and Hs and also low values for F_{IS}, which suggests a low genetic diversity at the studied loci. In addition, the genetic distances estimated, Ds and $F_{ST,}$ were very low (0.005 and 0.030, respectively), pointing out that these breeds are genetically similar.

Table 1. Obseved (Ho) and expected (He) heterozygosity, F_{IS} statistic for each breed, and Nei's genetic distances (Ds and F_{ST}) between Alentejano and Iberian breeds

	Ho	Hs	F_{IS}	Ds	F_{ST}
Alentejano	0.121	0.129	0.052	0.005	0.030
Iberian	0.131	0.149	0.078		

Figure 1 shows the plots of the first versus the second component (Figure 1a) and the second versus the third component (Figure 1b) of the principal component analysis. In the Figure 1a) two clusters can be discerned being Alentejano and Iberian the main representatives of one of the clusters, while the rest of the breeds are on the other one. In addition, in the Figure 1b, Alentejano and Iberian are grouped again together in the same cluster.

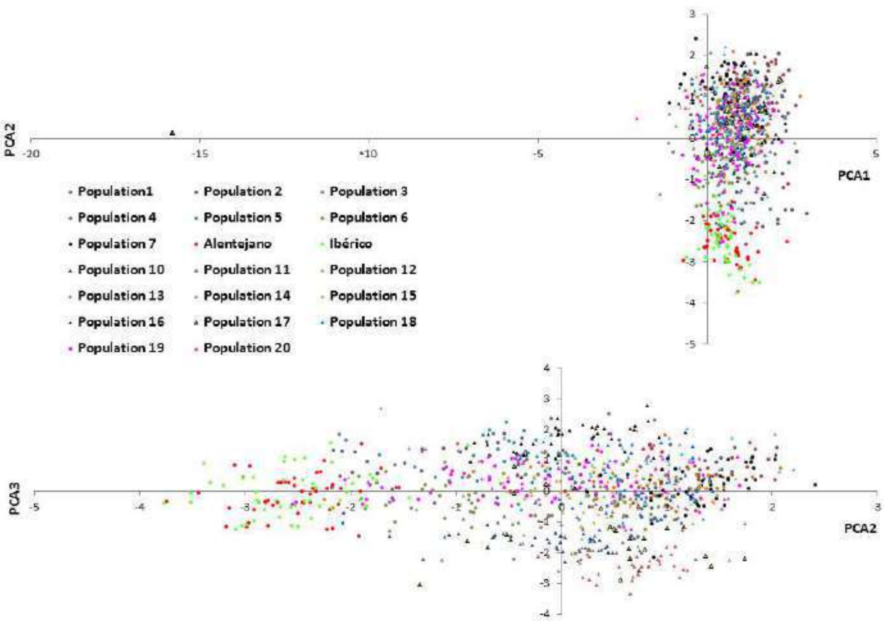


Figure 1. Diagram showing the relative position of the genotyped individuals defined by principal component factor scores.

DAPC analysis confirmed an original shared signal between Alentejano and Iberico which grouped in the same cluster (Figure 2a) and this result was supported by STRUCTURE where the across-run average of estimated ln probability of data (ln Pr(X|K)) reached a plateau at K=14 maintaining the two mentioned breeds in the same cluster (Figure 2b).

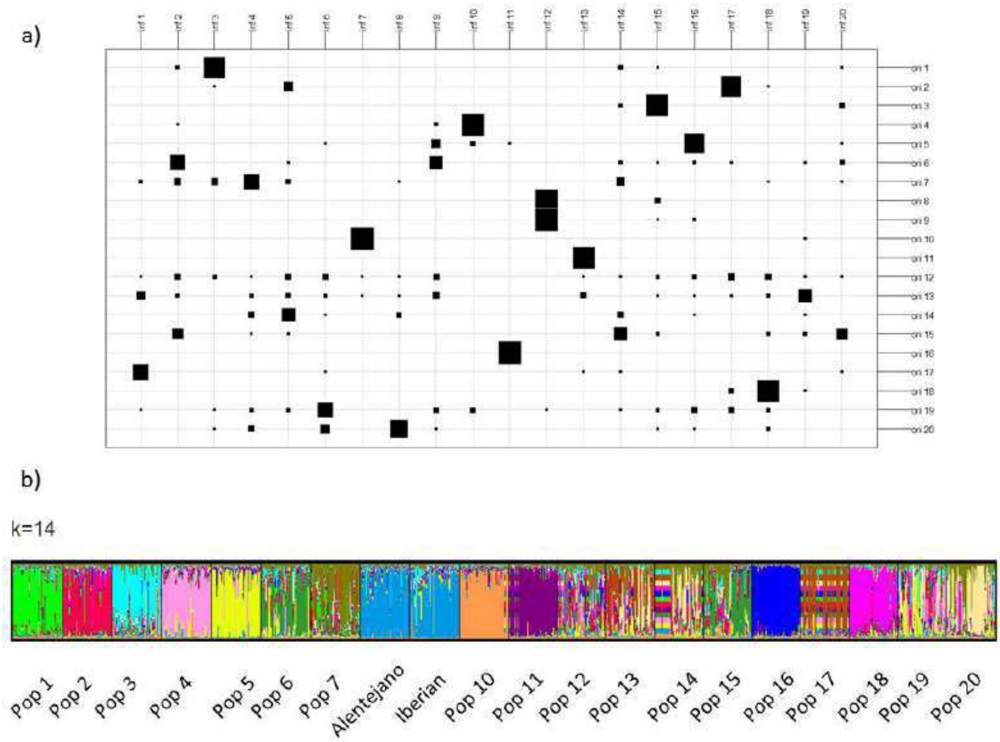


Figure 2. a) Diagram representing the number of individuals of each real population assigned to each inferred population. b) Results of admixture analyses with an optimal value of k=14

The most contributing alleles to diversity were *MC1R**3 and *MC1R**6*7 where the two investigated breeds showed intermediate and equal frequencies (MAF 0.25 and 0.10 for the two loci respectively).

Short genetic distances between Iberian and Alentejano breed were also reported by *Van Asch et al. (2012)* when they analysed the mtDNA control region (F_{ST} = 0.105). They also reported low genetic distances between Alentejano and

Iberian breeds and Iberian wild boars, suggesting an early intense gene flow between local wild populations and the domesticated herds in this region.

Although, additional analyses including a larger number of markers should be carried out to confirm these results in the whole genome, our results show a high genetic similarity between Iberian and Alentejano breeds in the analysed genes and they would be considered as different strains of the same breed.

Conclusion

Alentejano and Iberian breeds are genetically very similar for 26 SNPs located in major genes.

Genetička raznolikost alentehana i iberijske rasa ocenjena pomoću polimorfizma glavnih gena

María Muñoz, Riccardo Bozzi, Alessandro Crovetti, Rui Charneca, Jose M. Martins, Ana I. Fernández, Luca Fontanesi, Juan M. García-Casco, Cristina Óvilo

Rezime

Alentehano i iberijska rasa su biogeografski locirane na jugozapadu Iberijskog poluostrva. Ove rase dele skup zajedničkih karakteristika. Obe su fenotipski vrlo slične i pokazuju nisku stopu rasta i visok intramuskularni sadržaj masti i odgajaju se u ekstenzivnim sistemima, sa pripustima. Cilj ovog istraživanja, koji se sprovodi u okviru projekta TREASURE, je da se karakteriše genetička raznovrsnost alentehano i iberijske rase koristeći genotipske podatke od 32 polimorfizma koji se nalaze na 26 glavnih gena. Ti polimorfizmi su genotipirani u ukupno 950 životinja koje pripadaju alentehano, iberijskoj i 18 dodatnih evropskih rasa. Niske srednje vrednosti posmatranih (Ho) i očekivanih (Hs) heterozigotnosti i F_{IS} tačke ukazuju na nisku genetsku raznovrsnost u analiziranim rasama. Genetske udaljenosti koje su procenjene korišćenjem Ds i F_{ST} dale su vrednosti blizu 0 (0,005 i 0,030, respektivno), što ukazuje na to da su ove vrste genetički slične. Struktura populacije analizirana pomoću multivarijantnih metoda, kao što je analiza diskriminacije glavnih komponenata i analiza primesa, pokazuju da su obe rase grupisane unutar istog klastera. Ove rezultate podržavaju rezultati drugih autora, koji se zasnivaju na mitohondrijskim sekvencama koje su prijavile kratke

genetičke udaljenosti između ovih rasa. Iako treba izvršiti komplementarne analize pomoću većeg broja markera, rezultati sadašnjih analiza podržavaju hipotezu da alentehano i iberijska rasa mogu u stvari biti različiti sojevi u okviru iste rase.

Ključne reči: Alentehano, iberijska, rase, polimorfizmi, genetička raznovrsnost, multivarijantne analize

Acknowledgment

TREASURE project is funded under European Union's Horizon 2020 research and innovation programme, grant no. 634476. The content of this presentation reflects only the authors' view and Research Executive Agency is not responsible for any use that may be made of the information it contains.

References

ALVES E., OVILO C., RODRIGUEZ M. C., SILIO L. (2003): Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. Animal Genetics, 34,(5): 319-324.
FRAÇAO T. L. (1984): O porco alentejano melhorado. Boletim Pecuário
GOUDET J. (2005): Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Resources, 5,(1): 184-186.
JOMBART T. (2008): adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24,(11): 1403-1405.
LÊ S., JOSSE J., HUSSON F. (2008): FactoMineR: an R package for multivariate analysis. Journal of statistical software, 25,(1): 1-18.
NEI M. (1987). Molecular evolutionary genetics. Columbia university press.
PEREZ-SERRANO M. (2008): A study of factors that influence growth performance and carcass and meat quality of Iberian pigs reared under intensive management systems. Universidad Politécnica de Madrid.
PRITCHARD J. K., STEPHENS M., DONNELLY P. (2000): Inference of population structure using multilocus genotype data. Genetics, 155,(2): 945-959.
RAMOS A., MESTRE R., GOUVEIA S., EVANS G., ZHANG Y., CARDOSO A., ROTHSCHILD M., PLASTOW G.,RANGEL-FIGUEIREDO T. (2003): Use of Type I DNA markers for initial genetic characterization of two Portuguese swine breeds.
SAMBROOK J., FRITSCH E. F., MANIATIS T. (1989): Molecular cloning: a laboratory manual. Cold spring harbor laboratory press.

SÁNCHEZ-ESQUILACHE F. (2011): Metaanálisis de los resultados productivos de las fases de crecimiento y cebo del cerdo Ibérico. Universidad de Córdoba, Spain.
SILIÓ L. (2000): Iberian pig breeding program. In: Developing breeding strategies for lower input animal production environments. ICAR, (Galal S, Boyazoglou J, & Hammond K eds.): pp. 511-519.
TAKEZAKI N., NEI M. (1996): Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics, 144,(1): 389-399.
VAN ASCH B., PEREIRA F., SANTOS L., CARNEIRO J., SANTOS N., AMORIM A. (2012): Mitochondrial lineages reveal intense gene flow between Iberian wild boars and South Iberian pig breeds. Animal Genetics, 43,(1): 35-41.

FARROWING INTERVAL IN SELECTION OF DAM PIG BREEDS IN THE CZECH REPUBLIC

Zuzana Krupová, Emil Krupa, Josef Přibyl, Eliška Žáková

Institute of Animal Science, Přátelství 815, 10400, Prague, Czech Republic
Corresponding author: krupova.zuzana@vuzv.cz
Original scientific paper

Abstract: The current total merit index (TMI) of dam breeds of pigs (Czech Large white and Czech Landrace) consist of average daily gain (ADG), number of piglets born alive (NBA) and lean meat content (LMC). Farrowing interval (FI) was included among breeding objectives and the selection criteria of TMI to enhance reproduction performance. Genetic and economic response in complex of production, reproduction and carcass traits to selection on TMI was evaluated in the study. Current TMI gained high selection respond on piglets born alive (+0.31) with increase of ADG (+13.50 g) and keeping the LMC close to the current performance level (+0.02 %), all expressed per sow and per generation. Defining the FI as the breeding objective (leaving it omitted from the selection criterion) the trait value will be unfavourable increased by 0.07 day per sow and per generation. Direct incorporation of FI as the selection criterion would gain preservation of the length of interval (0.00 days per sow and per generation) with maintenance of the genetic and economic response in all other production and reproduction traits comparable with current TMI. Selection on current TMI caused correlated and not favoured response in the farrowing interval. Therefore, farrowing interval should be defined as the breeding objective and included among the selection criterion of the local dam pig breed population (thought TMI or a specialised reproduction index).

Key words: reproduction, selection, genetic response, economic response

Introduction

Breeding is considered as one of the most effective agricultural activities in terms of the use of inputs. Due to the specifics structure of the pig enterprise the trait improvement can be usefully transferred and vice spread from nucleus to commotional herds. Pig selection schemes usually give the highest pressure to reproduction and growth traits (*Sørensen, 2015; Houška et al., 2010*). The effect of selection is predicted to gain information needed for the next index application (*Serenius et al., 2007; Wallenbeck et al., 2015*) and provided to evaluate effectiveness of ongoing selection and response in traits (e.g. *Kasprzyk, 2007*).

In the Czech Republic, current breeding goal of dam breeds of pigs is aimed on reproduction (15.5 of pigs born alive), excellent growth (1 300 g per day from birth to end of performance testing) and maintenance the carcass quality (55 to 56% of lean meat content) (*CPBA, 2016*). Actually, reproduction performance is under the defined limit (13.05 of piglets born alive) and therefore should be improved. Next to the number of piglets born alive the farrowing interval can be used as a breeding tool for enhanced reproduction. Farrowing interval has been defined as important reproduction and selection trait by 13% of authorised breeders (*Krupová et al., 2017*). It is important for turnover of pig enterprise and for slightly positive (not favourable) correlation with the litter size (0.027; *Krupa et al., 2017b*). Moreover, methodology for its genetic evaluation in the local dam pig population has been developed (*Wolf, 2012*) and practically applied in the routine. Therefore, the aim of the study was to estimate the response in farrowing interval included into the current total merit index of the Czech dam pig's population.

Material and Methods

The total merit index (TMI) of dam breeds of pigs (Czech Large white and Czech Landrace) consist of three traits – average daily gain from birth to end of the test in grams (ADG), number of piglets born alive at the second and higher farrowing of a sow (NBA) and lean meat content in % (LMC) all weighted in the index (coefficient *b*) by 40:55:5. Farrowing interval expressed as days between two subsequent farrowings (FI) was included among the above mentioned breeding objectives and the selection criteria of TMI to enhance reproduction performance. The coefficients for traits of TMI and the appropriate selection response were calculated using the general principles of selection index theory. Altogether, three variants of TMI were investigated:

1) current TMI where coefficients *b* for ADG:NBA:LMC were 40:55:5;
2) current TMI where coefficients *b* for ADG:NBA:LMC were 40:55:5 and FI is included into the breeding objectives;
3) TMI with FI where coefficients *b* were 36:49.5:4.5:10 (based on preferences of authorised breeders, personal communication).

Genetic correlation among the traits and the reliability of estimated breeding values for traits were taken from former studies (*Krupa et al., 2016*; *Krupa et al., 2017b)* and calculated from the data provided by the Pig Breeders Association of the Czech Republic. To calculate the selection response in traits the standardised selection intensity of 1.0 was assumed. The economic response was calculated by multiplying the selection response with the economic value of the appropriate trait. Economic value of the trait was calculated as the partial derivative of the profit function with respect to the trait and breed using a bio-economic model of the program ECOWEIGHT. Average economic value of the trait calculated for both of dam breeds (Czech Large white and Czech Landrace) were applied in our study. More details to the economic values of traits and its calculation are given in *Krupa et al. (2017a,b)*. Base input parameters used for calculation of selection response are summarised in Table 1.

Results and Discussion

Genetic end economic response in complex of production, reproduction and carcass traits to selection on TMI and accuracy of the analysed selection indices are given in Table 2. Current TMI (**TMI no. 1**) gained high selection respond on piglets born alive (+0.31) with increase of ADG (+13.50 g) and keeping the LMC close to the current performance level (+0.02 %), all expressed per sow and per generation. Based on the requirements of authorised breeders, response in the NBA is acceptable. From the selection response calculated for **TMI no. 2** it can be seen that defining of FI only as the breeding objective (leaving it omitted from the selection criterion) the trait value will be unfavourable increased. As a selection result the interval between subsequent farrowing will be prolonged by 0.07 day per sow and per generation which would results to loss of nearly 25 euro cents per one sow and generation. Direct incorporating of FI as the selection criterion (**TMI no. 3**) would gain preservation of the length of interval (0.00 days per sow and per generation). From farmers point of view it is important that next to the stabilisation of FI the genetic and economic response in all other production and reproduction traits will be comparable with the current TMI (see Table 2).

Positive genetic change of the fertility (expressed as higher proportion of sows with interval ≤7 days between weaning and successful insemination) of 0.71% per generation was published for comprehensive breeding index of conventional pig farmers in Sweden (*Wallenbeck et al., 2015*). Favourable selection response in FI defined as a selection criterion of the comprehensive ‘Reproindex’ of the Czech dam pig breeds population was also founded (*Krupa et al., 2017b*). The ‘Reproindex’ consisting of four reproduction traits (number of piglets born total and weaned, NBA and FI) would gain positive selection response in litter size along with slightly reduced number of days between subsequent farrowing (-0.01 days per generation).

Compared to our results, similar selection response in ADG (9.2 and 30.4 g per day of piglets and of slaughter pigs, respectively) and lower selection gain in NBA (0.05 piglets), all expressed per generation was calculated to selection on Swedish comprehensive breeding index (*Wallenbeck et al., 2015*). Slightly higher genetic trend in NBA (0.17 pigs expressed per year) was found when evaluating the long term breeding process of the Polish Landrace saw population (*Kasprzyk, 2007*). Higher genetic improvement was also predicted in ADG (27.9 g per day and per generation) to the specialised selection index of Finish pig breeding system based on production and meat quality traits (without taking into account reproduction traits) (*Serenius et al., 2007*).

A contribution of the traits among indices evaluated in our study is stable (Figure 1). The most important selection criteria were ADG and NBA, which contributed to the response with 43 to 44% and 55 to 56%, respectively. Proportion of LMC and FI (in **TMI no. 3**) on the overall selection response not exceeds 1%. Based on the contribution of the traits and reliabilities of its estimated breeding values the final reliabilities of the evaluated TMI were relatively low (13% to 15%). Higher reliability (ranking from 40 to 70%) was estimated for index where reproduction traits were only included and coefficient *b* was calculated to maximise the response in the breeding objectives (*Krupa et al., 2017b*). Providing this optimisation in **TMI no. 1** in our study the reliability of TMI has increased to 61%. However, low selection response in NBA (only +0.039 piglets born alive per sow and per generation) probably would be not acceptable by authorised breeders. Selection on growth and reproduction as the first factors of efficiency remained for them constant.

Table 1. Economic weights (*ew*), genetic standard deviations (s_g), accuracy of estimated breeding values (on diagonal) and genetic correlations (above diagonal) among the traits.

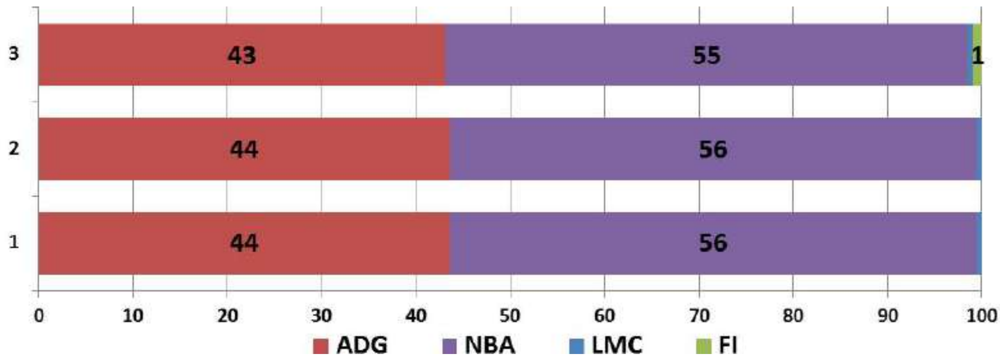
Trait	ew^1	s_g	g_{kor}			
			ADG	NBA	LMC	FI
ADG	2.26	27.5	0.65	-101	-89	70
NBA	23.57	0.719		0.42	23	27
LMC	40.26	0.82			0.64	10
FI	-3.52	1.30				0.22

[1]In € per unit of the trait per sow and per farrowing interval. For description of the traits included see Material and Method.

Table 2. Response on TMI selection and accuracy of the selection indices

TMI[1]	Response in trait[2]	ADG	NBA	LMC	FI	Accuracy of TMI (%)
1	genetic	13.504	0.315	0.025	-	14.8
	economic	30.53	7.43	1.00		
2	genetic	13.728	0.315	0.025	0.069	14.6
	economic	31.04	7.43	1.00	-0.24	
3	genetic	13.581	0.313	0.024	0.001	13.1
	economic	30.70	7.39	0.98	0.00	

[1]For description of TMI indices and the traits included see Material and Method. [2]Genetic response expressed per unit of the trait per sow and per generation. Economic value of response in € per sow and per generation (exchange rate was 27 CZK per €).



[1]Contribution of the trait is specified only when the appropriate trait participated on the total selection response by 1% at minimum.

Figure 1. Contribution of traits (%) in evaluated TMI indices[1]

Conclusion

Selection on current total merit index caused correlated and not favoured response in the farrowing interval. Farrowing interval should be defined as the breeding objective and included among the selection criterion of the local dam pig breed population (thought total merit index or a specialised reproduction index). A comprehensive total merit index consisting of growth, carcass and two reproduction traits would enhance reproduction performance thought the litter size and optimisation of the farrowing interval length. Growth and reproduction traits remained as the most important selection criterion in this index.

Acknowledgment

Thanks are due to the authorised breeders and the Pig Breeders Association of the Czech Republic for valuable comments and providing data, respectively. Study was supported by the Ministry of Agriculture of the Czech Republic, project QJ1310109.

Interval prašenja u selekciji materinskih rasa svinja u Češkoj

Zuzana Krupová, Emil Krupa, Josef Přibyl, Eliška Žáková

Rezime

Sadašnji ukupni priplodni indeks (TMI) materinskih rasa svinja (češkavelika bela i češki landras) sastoje se od prosečnog dnevnog prirasta (ADG), broja živorđene prasadi (NBA) i mesnatosti (LMC). Interval prašenja (FI) je bio uključen u odgajivačke ciljeve i kriterijume selekcije u okviru TMI radi poboljšanja performansi reprodukcije. Genetski i ekonomski odziv u kompleksu proizvodnih, reproduktivnih i osobina trupa za selekciju na TMI ocenjen je u ovom istraživanju. Sadašnji, tekući TMI je imao visok selekcioni odgovora na broj živorođene prsadi (+0,31) uz povećanje ADG (+13,50 g) i održavanje LMC blizu trenutnog nivoa performanse (+ 0,02%), sve izraženo po krmači i po generaciji. Definisanje FI kao odgajivačkog cilja (izostavljajući ga iz kriterijuma selekcije), vrednost osobine će biti nepovoljno povećana za 0,07 dana po krmači i po generaciji. Direktno uključivanje FI kao kriterijuma selekcije očuvaće se dužina intervala (0,00 dana po krmači i po generaciji) uz održavanje genetičkog i ekonomskog odgovora u svim drugim proizvodnim i reproduktivnim osobinama uporedivim sa trenutnim TMI. Selekcija u okviru trenutnog TMI izazvala je povezanu, ali ne i favorizovanu reakciju intervala prašenja. Prema tome, interval prašenja treba definisati kao odgajivački cilj i uključiti ga u kriterijume selekcije lokalne populacije materinskih rasa svinja (TMI ili specijalizovani indeks reprodukcije).

Ključne reči: reprodukcija, selekcija, genetski odgovor, ekonomski odgovor

References

CPBA. 2016. Plemenné standardy a chovné cíle (Czech language). Czech Pig Breeders Association. http://www.schpcm.cz/slechteni/metodiky/metodiky.asp.

HOUŠKA L., WOLFOVÁ M., NAGY I., CSORNYEI Z., KOMLOSI I. (2010): Economic values for traits of pigs in Hungary. Czech Journal of Animal Science**,** 55: 139-148.

KASPRZYK A. (2007): Estimates of genetic parameters and genetic gain for reproductive traits in the herd of Polish Landrace sows for the period of 25 years of the breeding work. Archive für Tierzucht, 50: 116-124

KRUPA E., KRUPOVÁ Z., WOLFOVÁ M., ŽÁKOVÁ E. (2017a): Estimation of economic values for traits of pig breeds in different breeding systems: II. Model application to a three-way crossing system. Livestock Science, (submitted).

KRUPA E., KRUPOVÁ Z., ŽÁKOVÁ E., PŘIBYL J. (2017b): Breeding objectives of dam pig breeds of the Czech national breeding program based on reproduction traits. Agriculturae Conspectus Scientificus, (accepted).
KRUPA E., ŽÁKOVÁ E., KRUPOVÁ Z., MICHALIČKOVÁ M. (2016): Estimation of genetic parameters for teat number and reproduction and production traits from different data sources for Czech dam breeds. Livestock Science, 19: 197-102.
KRUPOVÁ Z., ŽÁKOVÁ E., KRUPA E., MICHALIČKOVÁ M. (2017): New breeding objectives for the Czech pig population. Indian Journal of Animal Science, 6: 778-781.
Serenius T., Muhonen P. (2007): Economic values of pork production related traits in Finland. Agricultural and Food Science, 16: 79-88.
SØRENSEN A C. (2015): Subjective definition of traits and economic values for selection of organic sows in Denmark. 66th Annual meeting of the EAAP. Book of abstracts No. 21, August 29 – September 2, Warsaw, 465.
WALLENBECK A., RYDHMER L., RÖCKLINSBERG H., LJUNG M., STRANDBERG E., AHLMAN T. (2015): Preferences for pig breeding goals among organic and conventional farmers in Sweden. Organic Agriculture, 1-12.
WOLF J. (2012). Technical note: A general transformation formula for interval traits connected with reproduction in pigs. Journal of Animal Science, 90: 3695-3697.

BACKFAT FATTY ACID PROFILE AFTER GROWING PERIOD IN IBERIAN PIGS FED WITH OLIVE CAKE IN A DRY OR WET (SILAGE) FORM

Adrián López-García[1], Juan M. García-Casco[1], María Muñoz[1], José M. Martínez-Torres[2], Miguel A. Fernández-Barroso[1], Elena González-Sánchez[2]

[1] Centro de I+D en Cerdo Ibérico, Dpto. Mejora Genética Animal, INIA, 06300 Zafra (Badajoz).
[2] Escuela de Ingenierías Agrarias. Instituto Universitario de Investigación de Recursos Agrícolas (INURA), Universidad de Extremadura
Corresponding autor: adrian.lopez@inia.es
Original scientific paper

Abstract. The traditional production system of Iberian pig is characterized for having a finishing period called *montanera*, which is based on acorn intake. This kind of handling has an influence on both product quality and animal welfare issues. Acorn based diet during *montanera* has a positive effect on meat quality since increases the oleic content. However, *montanera* has an uneven effect on animal welfare, on one hand it is a semi-extensive system which is positive but, on the other hand, a restricted diet is required during pre-*montanera* period to prevent a non-desirable fatness percentage in final pig products. This restriction diet implies feeding stress. The use of olive by-products during the growing phase diet of Iberian pigs could be the solution to avoid this stress. Here, we studied the effect of three different dietary regimens given to Iberian pigs in growing period (42 kg to 95 kg) on backfat fatty acid composition. A control standard diet group (CD) was compared with two diets based on olive by-products, one incorporating dry olive pulp in the feed (DD), and the other one incorporating olive cake in wet form (WD). This last one consisted of olive cake in a silage presentation offered *ad libitum* and supplemented with a specific feed given once a day in a restricted regimen as the CD and DD diets. A significantly higher oleic acid values was observed for DD and WD diets compared with CD diet. Therefore, the diets based on olive cake seem to be an alternative to more expensive diets based on high-oleic raw material. However, before implementation in feeds used for the growing period, further studies analyzing their effects on meat quality and production costs should be carried out.

Key words: Iberian pig, olive by-products, growing period, backfat fatty acids

Introduction

Montanera is the finishing fattening period (up to 160 kg approx.) of the traditional Iberian pig production system. During this period, that lasts three to five months (from November to March), animal's diet is based on an *ad libitum* intake of acorns and grass *(López-Bote, 1998*). As the previous growing period (up to 100 kg approx.) lasts a long time, sometimes more than one year, feeding and management are aimed to prepare the pigs to that crucial fattening stage. A strongly restricted diet is required to decrease growing rate and prevent animals for getting a higher fatness percentage than desirable. However, this restriction causes a non-desirable feeding stress, as it means a negative effect on animal welfare. Low energy diets that allow a daily intake similar to an *ad libitum* one during the growing period could be an alternative to minimize this problem.

In Iberian pig production, olive-based by-products have been proposed as raw material of feed components for growing or finishing pigs *(Benito et al., 1998; Hernández-Matamoros et al., 2011; Joven et al., 2014; González-Sánchez et al., 2016)*. In addition, *Hernández-Matamoros et al. (2011)* reported changes in fatty acid profile on backfat at 104 kg live weight except for linoleic, producing an increase in the percentage of oleic and a decrease of the saturated fatty acids. However, there are no studies using *montanera* pigs bred into the traditional restricted period.

The objective of the current study was to analyze fatty acid profile (FA) at the end of the growing period in Iberian pigs fed with two different olive cake-based diets, in dry and wet form, respectively.

Material and Methods

Description of the by-products

Treatments and types of by-products along olive oil extraction are diverse. In the case of the material used in this work, olive oil and crude olive cake (COC) were obtained in the first place. COC consisted of olive pulp, skin and stone, containing a 72% of water and constituting a semiliquid paste. After that, a second extraction of the olive oil was made, and the main part of the olive stone was retired. The resulting pastry was then dehydrated, and the olive pulp (OP) obtained, which was composed by skin, pieces of olive stones and a small part of olive oil. Table 1 shows the fatty acid composition of the by-products and the remaining analytical composition of these by-products is described in a previous study (*García-Casco et al., 2017*).

Table 1. Fatty acid composition of the olive pulp (OP) and crude olive cake (COC) by-product, silage and the control (CD), dry olive pulp (DD) and wet crude olive cake (WD) experimental feeds (g/100g total lipids).

	By-products			Diets		
	OP	COC	Silage	CD	DD	WD
C14:0	0,05	0,16	0,46	0,76	0,41	0,40
C16:0	13,81	13,60	13,96	19,56	12,68	17,55
C16:1	0,85	1,04	1,07	1,06	0,72	0,26
C17:0	0,09	0,07	0,12	0,19	0,14	0,14
C17:1	0,11	0,11	0,23	0,17	0,16	0,10
C18:0	3,91	3,27	3,72	6,92	3,96	3,82
C18:1	69,17	65,98	66,09	36,15	59,14	25,51
C18:2	9,78	13,41	11,59	31,00	19,33	46,21
C18:3	0,87	1,08	1,00	2,80	1,95	4,96
C20:0	0,62	0,56	0,67	0,34	0,59	0,41
C20:1	0,42	0,33	0,68	0,85	0,58	0,64
C20:2	0,32	0,40	0,41	0,20	0,33	0,00

Animals, diets and samples

A total of 45 Iberian pigs were controlled from 6.5 months to commercial slaughter age, starting with an average weight of 42± 8.6 kg. Animals were evenly and randomly allocated in three different pens of 110 m^2, with both outdoor and covered areas. Three feeding systems were applied during the growing period: the analytical and ingredient composition of these diets is described in a previous study *(García-Casco et al., 2017)*. CD was based on a feed formulated in order to cover the protein and energy requirements for the growing period. DD was based on a feed with a 45% of OP by-product in a pelleted form. Lastly, WD was composed by two elements: COC by-product in a silage form to ease its conservation and use, and a specific feed (WD) as a complement. To prepare the silage, a mix with a 75% of COC and 25% of barley straw was made. This mix was packaged at a high pressure containing 42.5% of dry matter. Table 1 shows fatty acid profile analysis of the silage and the three feeds.

All these feeds were supplied in a pelleted form, once a day under a restriction feeding management. COC by-product of the WD regimen was supplied *ad libitum.* Pigs remained on these feeding conditions for 191 days, when they reached an average body weight (BW) of 95±13.7 kg.

At the end of the growing period, backfat biopsies were taken from the rear body part of each pig, close to the tail. Animal manipulations were performed according to the Spanish Policy for Animal Protection (RD1201/05), which meets the European Union Directive 86/609 for the protection of animals used in experimentation.

Fatty acid profile

Backfat from the biopsies was homogenized with chloroform until total dissolution of fat. Chloroform is collected and evaporated under nitrogen stream. Fatty acid profile was determined by gas chromatography after an acid transesterification in the presence of sulfuric acid *(Cava et al., 1997).* Fourteen fatty acids were analysed and the results are expressed as percentages of the total fatty acids.

Statistical analyses

Data were analyzed with the following linear model:

$$y = Xb+e$$

Where *b* represents the diet supplied during the growing period as a three-leveled factor for the fatty acids and *e* represents the residual effects. Analyses were carried out on R environment using the functions *lm, anova* and *Tukey HSD.* Fisher test was applied to test the effects of factors and Tukey test was used to make pair-wise comparisons. A *p-value* ≤ 0.05 was considered as a significant difference between the different diets.

Results

Table 2 shows the mean values of the 14 fatty acids analyzed and the sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Significant differences were observed between the treatment groups for most of them. Control diet (CD) showed higher values for the main saturated FA (C14:0, C16:0, C18:0). Dry and wet olive diets (DD and WD), with clearly higher levels of unsaturation than CD (C16:1, C18:1, C18:2, C18:3), are not very different among them. Only some less abundant FA mean values were significantly different

(C14:0, C17:0, C20:1, C20:2, C20:3) for DD and WD. An exception was found for the stearic acid (C18:0) for which CD showed a lower value than WD, but the sums of FA were similar.

Table 2. Comparison of backfat fatty acid composition between Control (CD), dry olive pulp (DD) and wet crude olive cake (WD) experimental diets. Standard error of the mean (SEM) and *p-value* corresponding to the Fisher test of the *ANOVA*.

Trait	CD	DD	WD	SEM	*p*
C14:0	1.40^{a}	1.14^{c}	1.27^{b}	0.15	1.90×10^{-06}
C16:0	24.30^{a}	21.54^{b}	20.87^{b}	1.90	1.35×10^{-09}
C16:1	2.67^{a}	2.36^{b}	2.29^{b}	0.25	1.76×10^{-05}
C17:0	0.41^{b}	0.50^{a}	0.42^{b}	0.08	4.21×10^{-04}
C17:1	0.42	0.41	0.39	0.05	0.476
C18:0	11.40^{a}	8.62^{c}	10.32^{b}	1.47	2.68×10^{-08}
C18:1	46.69^{b}	49.83^{a}	49.22^{a}	2.12	2.24×10^{-05}
C18:2	9.44^{b}	12.20^{a}	11.38^{a}	1.48	3.27×10^{-09}
C18:3	0.70^{b}	0.87^{a}	0.91^{a}	0.13	1.71×10^{-06}
C20:0	0.23	0.22	0.22	0.03	0.400
C20:1	1.36^{b}	1.31^{b}	1.51^{a}	0.14	1.37×10^{-05}
C20:2	0.63^{b}	0.65^{b}	0.75^{a}	0.08	2.28×10^{-05}
C20:4	0.18	0.19	0.19	0.03	0.577
C20:3	0.18^{b}	0.16^{c}	0.25^{a}	0.04	2.64×10^{-12}
SFA	37.75^{a}	32.03^{b}	33.10^{b}	3.12	8.54×10^{-10}
MUFA	51.13^{b}	53.90^{a}	53.42^{a}	2.06	1.93×10^{-4}
PUFA	11.12^{b}	14.07^{a}	13.49^{a}	1.64	5.53×10^{-09}

SFA, MUFA, PUFA: Sum of saturated, monounsaturated and polyunsaturated fatty acid, respectively

Discussion

Production of dry-cured Iberian pig products is oriented to high quality markets. The traditional *montanera* system, based on an acorn and grass feeding during the fattening period, conferred to fresh meat the ideal properties to resist a long curation period of hams and forelegs *(Ventanas et al., 2005)*. Fatty acid composition of subcutaneous fat is a key to this process and one of the main responsible of the unique organoleptic properties associated to Iberian pig products. High levels of oleic acid (> 53%) at slaughter weight are required from industries, as well as low values for palmitic (< 22%), stearic (< 10.5%) and linoleic (< 10.5%) acids.

In this scenario, fatty acid profile before *montanera* period must be controlled by farmers. Growing diets resulting in high SFA levels cannot be corrected with the acorn feeding, and the final quality of products could be affected. Formulation of pre-*montanera* feeds used for Iberian pigs take into account this situation and many of them have a high oleic composition obtained from raw material as sunflower seeds.

Our results of fatty acid profiles in subcutaneous fat point out to the use of diets based on olive by-products during growing period being an alternative to more expensive feeds to reach appropriate levels of oleic acid before *montanera*. Although linoleic acid levels were also higher in DD and WD diets than in CD one, the massive consume of acorn will likely result in a decrease of the linoleic fatty acid at the end of *montanera* period.

Fatty acid profile at the end of *montanera* and other meat quality traits are currently being analyzed to contrast potential undesirable influences of olive-based by product diets. Previous results on growth and slaughter traits *(García-Casco et al., 2017)* showed that they are not affected by DD and WD diets.

Conclusion

The incorporation of olive cake to the diet of Iberian pigs during the growing period has positive effect on fatty acid profile at the end of that period, increasing total unsaturation level. Although its use seems to be suitable, further studies analyzing final fatty acid profile and meat quality traits and production costs should be performed.

Profil masnih kiselina leđne slanine kod iberijskih svinja hranjenih maslinovom pogačom u suvom ili vlažnom (silažnom) obliku

Adrián López-García, Juan M. García-Casco, María Muñoz, José M. Martínez-Torres, Miguel A. Fernández-Barroso, Elena González-Sánchez

Rezime

Tradicionalni sistem proizvodnje iberijske svinje karakteriše završni period nazvan *montanera*, koji se zasniva na konzumiranju žira, što ima uticaj na kvalitet proizvoda i pitanje dobrobiti životinja. Ishrana zasnovana na žiru tokom *montanere* ima pozitivan efekat na kvalitet mesa, jer povećava oleinski sadržaj. Međutim, *montanera* ima nejednak efekat na dobrobit životinja, s jedne strane radi se o polu-ekstenzivnom sistemu koji je pozitivan, ali sa druge strane je potreban ograničena ishrana u periodu pre *montanere* kako bi se sprečila pojava neželjene masnoće finalnih svinjskih proizvoda. Ova restriktivna ishrana podrazumeva stres. Korišćenje maslinovih nusproizvoda tokom ishrane iberijskih svinja u fazi rasta može biti rešenje da se izbegne ovaj stres. Ovde smo proučavali efekat tri različita režima ishrane iberijskih svinja u periodu porasta (42 kg do 95 kg) na kompoziciju masnih kiselina leđne slanine. Kontrolna standardna dijetalna grupa (CD) je upoređena sa dve ishrane zasnovane na maslinovim nusproizvodima, od kojih je jedna sa suvom maslinovom pulpom u obroku (DD), a druga sa maslinovom pogačom u vlažnom obliku (VD). Ova poslednja se sastojala od maslinovoe pogače u silažnom obliku, *ad libitum* i dopunjena specifičnom hranom koja se daje jednom dnevno u ograničenom režimu, kao i CD i DD obrocima. Znatno veće vrednosti oleinske kiseline primećene su za DD i VD obroke, u poređenju sa CD obrokom. Dakle, ishrana zasnovana na maslinovoj pogači izgleda kao alternativa skupljim obrocima zasnovanim na visoko-oleinskoj sirovini. Međutim, prije implementacije u ishrani tokom perioda porasta, trebalo bi izvesti dodatne studije koje analiziraju njihove efekte na kvalitet mesa i troškove proizvodnje.

Ključne reči: periodi gajenja, maslinovi nusproizvodi, masne kiseline leđne slanine, iberijske svinje

Acknowledgements

This study has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634476 (project acronym TREASURE). The content of this paper reflects only the author’s view and the European Union Agency is not responsible for any use that may be made of the information it contains.

The authors are also grateful to the employees of the farm Puerto Lobo (Cabeza la Vaca, Badajoz, Spain) and especially to the owner Francisco de Borja Ramírez.

References

BENITO J., VÁZQUEZ-CISNEROS C., MENAYA C., FERRERA J. L., ROMERO A. AND GARCÍA CASCO J. M. (1998): Utilización del alperujo de aceituna como suplemento de la ración en el cerdo ibérico de primor. Solo Cerdo Ibérico, 1: 45-51.

CAVA, R, RUIZ CARRASCAL, J, LOPEZ BOTE, C, MARTIN, L, GARCIA, C, VENTANAS, J AND ANTEQUERA, T. (1997): Influence of finishing diet on fatty acid profiles of intramuscular lipids, triglycerides and phospholipids in muscles of the Iberian pig. Meat Science 45, no. 2.

GARCÍA CASCO J.M., MUÑOZ M., MARTÍNEZ-TORRES J.M., LÓPEZ-GARCÍA A., FERNÁNDEZ-BARROSO M.A. AND GONZÁLEZ-SÁNCHEZ E. (2017): Alternative feeding in Iberian pigs during growth period: incorporation of olive cake in a dry or wet (silage) form. 25th International Symposium Animal Science Days. Brandlucken (Austria), september 2017.

GONZÁLEZ-SÁNCHEZ E., FERNÁNDEZ-BARROSO M. A., CARABALLO C. AND GARCÍA CASCO J. M. (2016): Alimentación del cerdo ibérico con alperujo durante la recría. I Congreso Ibérico de Olivicultura, Badajoz.

HERNÁNDEZ-MATAMOROS A., PANIAGUA BREÑA M., IZQUIERDO CEBRIÁN M., TEJEDA SERENO J. AND GONZÁLEZ SÁNCHEZ E. (2011): Use of olive cake and tomato peel in the Iberian pig feed. XIV Jornadas sobre Producción Animal, Zaragoza.

JOVEN M., PINTOS E., LATORRE M., SUÁREZ-BELLOCH J., GUADA J. AND FONDEVILA M. (2014): Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. Animal Feed Science and Technology, 197: 185-193.

LÓPEZ-BOTE C. (1998): Sustained utilization of the Iberian pig breed. Meat Science, 49: S17-S27.

VENTANAS S., VENTANAS J., RUÍZ J. AND ESTÉVEZ M. (2005): Iberian pigs for the development of high-quality cured products. Recent Res. Devel. Agricultural & Food Chem., 6.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE EFFECT OF THE ACORN IN FEEDING ON THE PRODUCTION AND SLAUGHTER TRAITS OF CRNA SLAVONSKA PIG

Vladimir Margeta, Kristina Gvozdanović, Ivona Djurkin Kušec, Polonca Margeta, Goran Kušec, Žarko Radišić

[1]University of Josip Juraj Strossmayer, Agricultural faculty Osijek, Vladimira Prleoga 1, 31000 Osijek, Croatia
Corresponding author: Vladimir Margeta, vmargeta@pfos.hr
Original scientific paper

Abstract: The aim of the study was to estimate the how substitution of acorn in the meals for Black Slavonian pigs affects the production characteristics of finishing pigs and carcass traits. The research was carried out on 60 Black Slavonian pigs, male and female, kept on pasture under traditional keeping conditions. During the last period of fattening, 90 days before slaughter, pigs into two equal groups, A and B. Group A were fed the same meal as during the whole period of the fattening, and group B were fed only with oak horn. The results have shown that pigs fed with acorn in the finishing period had a statistically significant higher (P <0.001) average daily gain and final body weight compared to pigs fed by conventional meals. Statistical differences was found in the parameters of meat color, release of meat juice and the content of intramuscular fat in favor of the pigs fed with acorn. The obtained results indicate that acorn in meals for Black Slavonian pigs had positive effect on production and carcass traits.

Key words: Black Slavonian pig, acorn, carcass traits, production traits

Introduction

Black Slavonian pig is autochthonous pig breed in Croatia. It belongs combined type of pig breed. Main characteristics of this breed is strong constitution, good resistance and adaptability to extensive keeping conditions. Fertility ranges from 6 to 10 piglets in the litter, and the proportion of muscle tissue in the body ranges between 30 and 40%. Keeping condition and feeding are main factors that affects the fertility rates, there could be more than 10 piglets per litter, and proportion of muscle tissue in the body can reach over 45%. Black Slavonian pig is suitable for extensive and semi intensive production systems with low investments in facilities, equipment and food *(Margeta et al., 2013., Budimir et al.,* *2013., Margeta et al., 2016).* Due to its black color and pigmented skin, as well as its anatomical foot shape, it is suitable for keeping on open and softer-humid terrain (underwater and wetland soils, forests) i.e., on surfaces that are not suitable for other forms of animal husbandry and crop production *(Budimir et al., 2014).* On this type of soils, it can find other food, which reduces the proportion of conventional meals and hence the cost of feeding. Black Slavonian pig have ability to use voluminous fodder such as green alfalfa *(Gvozdanović et al., 2016, Živković et al., 2017),* livestock pea, black pea or pumpkin during the vegetation period, and during the winter period there are haystails of alfalfa and oak *(Budimir et al., 2014).* One of the most important characteristics of this breed is the extraordinary quality of muscle and fat tissue that is suitable for the processing and production of traditional dried meat products (kulen, ham, sausage, bacon, čvarak). The meat of Black Slavonian pigs by their qualitative and technological characteristics is far ahead of the meat of other breeds and types of pigs that was raise in Croatia under the conventional keeping conditions. It relates to properties such as color, water release, pH value (meat acidity), and intramuscular fat content that contributes to the particular taste and tenderness *(Gvozdanović et al., 2016, Margeta et al., 2016).* Acorn is one of the most important feed in extensive keeping conditions. Its importance is reflected in its chemical composition and antioxidant properties. The most common species of oak in Croatia is *Quercus robur* which production is about 270 kg / ha *(Gradečki-Poštenjak et al., 2011).* Feeding with acorn is carried out from December to February. Combination of pasture and acorn in pig feeding have influence on fatty acid content and lead to the increase of antioxidant capacity. This refers to the content of n-3 fatty acids, especially linoleic acid (C18: 3, n-3). Acorn have high concentration of tannin and it can contain around 65g / kg of dry matter. Tannins have s high antioxidant capacity and they are non-nutritional plant polymers that bind to proteins and reduce their digestibility *(Tejerina et al., 2011).* Feeding the pigs with acorns during the last phase of fattening period have influence on fatty acid profile; there was a high level of oleic acid (C18: 1, n-9) (54%) and low levels of palmitic (C16: 0) and stearic fatty acids (C18: 0) Sur., 2012). The content of acorn fatty acids affects the quality of meat and meat products of pigs fed during the last phase of fattening period *(Canellas et al., 2007).* Apart from the content of fatty acids in the carcass, acorn feeding have also effect on the health status of the pigs. The research conducted by *Salajpal et al. (2004)* suggests that feeding with acorn during the in the final phase of fattening affects the reduction of gastric-intestinal parasites and in this way reduce the need for anthelmintic.

Material and methods

A study was performed on 60 fattened Crna slavonska pigs of both sexes divided into two groups during the 90 days of ending fattening period. All pigs were kept outdoors, on pasture. During of the research the pigs of the first group received a standard meal consisting of grains used in Crna slavonska pigs (12 MJ / ME). Pigs of the second group during the research were given a meal made of oak acorn in an amount of 5 kg / day. The aim of the research was to simulate the traditional way of keeping and feeding the pigs of the Crna slavonska breed of oak acorn, which in the past was dominant in the area of eastern Croatia. During the investigation, pigs were weighed at intervals of 30 days. After 90 days, the pigs were taken to a slaughterhouse where, after 24 hours of rest, they were stuck with CO_2 and slaughtered according to the standard procedure. At the slaughter line and in laboratory, following carcass traits and meat quality traits were measured: carcass weight, carcass length, muscle and fat thickness, ham length, initial pH values (pH45) measured 45 minutes after slaughter and ultimate pH values measured after 24h of cooling at *m. longissimus dorsi* (MLD) and *m. semimembranosus* (MS) of primarily processed pig carcasses. Drip loss was measured by bag method according to *Honikel (1987)* afer 24h of cooling at 4 °C, while EZ drip was measured as described by *Christensen (2003).* Light reflectance scores for CIE L*, a* and b* were obtained using a Minolta CR-410 colorimeter (Minolta Camera Co. Ltd., Osaka Japan) with a D65 light source and eight-degree standard observer. Instrumental tenderness was measured on at least four subsamples of 2.54 cm thick LD chops. Prior to measurements chops were defrosted for 24h, sealed in plastic bags and cooked in water bath until an internal temperature of 73 °C. The samples were cooled at 4 °C overnight. Shear force was measured using a TA. XT plus Texture Analyser fitted with a 1 mm thick Warner-Bratzler shear attachment. The mean value of maximal strength necessary for cutting the samples was calculated with a Texture Exponent 4.0 Software (Stable Micro Systems Ltd., UK) and presented as Warner-Bratzler Shear Force (WBSF, N). Cooking loss was established from LD chops used for shear force determination. It was calculated from weights taken before and after cooking and expressed as a percentage. The intramuscular fat (IMF) in MLD were extracted with chloroform/methanol (2:1, vol:vol), according to the method described by *Pérez-Palacios et al. (2008).* Data were analysed using Statistica for Windows 8.0 software.

Results and discussion

Results of productive, carcass and meat quality traits are shown in next tables.

Table 1. Productive traits during fattening period

Trait	1st Group		2nd Group		Statistical significance
	Mean	Sd	Mean	Sd	
Initial weight, kg	101,73	4,81	102,01	4,14	n.s.
Final weight, kg	129,16	8,11	140,25	11,24	**
Average daily gain, kg	0,287	0,11	0,391	0,09	**

*n.s. – non significance, **P<0.01*

Table 2. Carcass traits

Trait	1st Group		2nd Group		Statistical significance
	Mean	Sd	Mean	Sd	
Cold carcass weight, kg	98,20	6,14	109,16	8,41	**
Muscle thickness, mm	53,45	5,24	55,12	5,21	n.s.
Fat thickness, mm	31,11	2,19	32,57	3,61	n.s.
Meatness, %	44,61	4,65	43,98	4,01	n.s.
Carcass length, cm	92,13	1,58	93,03	1,88	n.s.
Ham length, cm	41,38	2,02	42,99	1,85	n.s.
Ham scope, cm	75,40	3,36	75,68	2,97	n.s.
IMF, %	9,23	3,14	11,18	2,96	*

*n.s. – non significance, *P<0.05, **P<0.01*

Table 3. Meat quality traits

Trait	1st Group		2nd Group		Statistical significance
	Mean	Sd	Mean	Sd	
pH45, MLD	6,24	0,17	6,32	0,14	n.s.
pH45, MS	6,31	0,11	6,41	0,12	n.s.
pH24, MLD	5,64	0,09	5,69	0,08	n.s.
pH24, MS	5,66	0,13	5,68	0,13	n.s.
Drip loss, %	2,14	0,02	0,86	0,04	**
EZ drip, %	2,31	0,03	0,91	0,10	**
CIE-L	45,18	3,11	41,26	4,05	*
CIE-a	13,28	0,89	14,05	0,99	n.s.
CIE-b	6,58	0,13	6,10	0,21	n.s.
Cooking loss, %	24,14	2,14	23,92	2,05	n.s.
Shear force, N	57,26	3,25	59,31	2,62	n.s.

*n.s. – non significance, *P<0.05, **P<0.01*

As can be seen from Table 1, the oak acorn supplement in the final period of fattening time was statistically significant (P <0.01) improve the average daily gain, which resulting in significantly higher (P <0.01) final body weight. Similar results are also shown by *Garcia-Valverde et al. (2007)* and *Rodriguez-Estevez et al. (2011)* with Iberian pigs fed with oak acorn. No statistically significant difference was observed between carcass traits of slaughtered pigs, except for the weight of chilled half. These results are in line with the results obtained in the investigations of *Senčic and Samac (2016),* which found that different meal content in black pig slaughter pigs under extensive conditions does not affect the slaughtering properties of slaughtered carcasses.

However, it is interesting that, despite significantly higher body weight, there was no difference in the amount of muscle tissue in the carcass, indicating the beneficial effect of nutrition on oak acorn on the amount of meat in the half. The content of intramuscular fat (IMF) in the MLD was significantly ($P < 0.05$) higher in pigs fed with acorn compared to pigs that did not get a acorn in the meal. These results are consistent with the results of *Robin et al. (2013). Seiquer et al. (2013)* also state that feeding on the oak acorn has a positive effect on increasing intramuscular fat content in the muscle of the pig. In terms of the quality of muscle tissue quality, significant differences ($P < 0.01$) were found for the release of water holding capacity as well as significant difference ($P < 0.05$) in the muscle color, where the pigs fed by acorn had a higher water holding capacity and darker meat color.

This can be explained by the fact that it was observed during the experiments that the pigs that were fed with oak acorn were more likely to move than the others, that could explain the differences. *Clemente et al. (2012)* also state that Iberian pigs kept in the extensive keeping conditions and fed with acorn had a significantly darker muscle color and a lower rate of release of meat juice.

Conclusions

Based on the above, it can be concluded that the nutrition of oak acorn in the finishing Crna slavonska pigs has a positive effect on the average daily gain and final weight of the pigs, and on the most important traits of the quality of the carcass and muscle and fat tissues. The listed properties of muscle and fat tissue define the meat and fat of black Slavonian pigs as very good material for processing into high-quality traditional products. If, in addition to the above, the oak acorn are very cheap and easily accessible to pigs, then the feeding of the acorn has a positive effect on the economy and profitability of production.

Efekat žira u ishrani na proizvodna i klanična svojstva crne slavonske svinje

Vladimir Margeta, Kristina Gvozdanović, Ivona Djurkin Kušec, Polonca Margeta, Goran Kušec, Žarko Radišić

Rezime

Cilj studije je bio da se proceni kako zamena žira u ishrani crno-slavonske svinje utiče na proizvodna svojstva tovljenika, kao i osobine trupa. Istraživanje je sprovedeno na 60 crno-slavonskih svinja, muških i ženskih, koje se drže na paši pod tradicionalnim uslovima držanja. Tokom poslednjeg perioda tova, 90 dana pre klanja, svinje su podeljene u dve ravnopravne grupe, A i B. Svinje u grupi A su hranjene istim obrokom kao i tokom celog perioda tova, a grupa B je dobijala obrok sa hrastovim žirom. Rezultati su pokazali da su svinje koje su hranjene žirom u završnom periodu imale statistički značajno veći (P<0,001) prosečni dnevni prirast i završnu telesnu masu u poređenju sa svinjama hranjenim konvencionalnim obrocima. Statističke razlike su utvrđene u parametrima boje mesa, oslobađanje mesnih sokova i sadržaja intramuskularne masti u korist svinja koje su hranjene žirom. Dobijeni rezultati ukazuju na to da žir u obroku za crno-slavonske svinje ima pozitivan uticaj na proizvodne i osobine trupa.

Ključne reči: crna slavonska svinja, žir, osobine trupa, proizvodne osobine

References

BUDIMIR K., MARGETA V., MARGETA P. (2013): Breeding of the black slavonian pig in extensive conditions. Acta agriculturae Slovenic154a. 4; 93-96.

BUDIMIR K., MARGETA V., KRALIK G., MAGETA P. (2014): Silvo-pastoralni način držanja crne slavonske svinje. Krmiva : časopis o hranidbi životinja, proizvodnji i tehnologiji krme. 55, 3; 151- 154.

CANELLAS I., ROIG S., POBLACIONES M.J., GEA-IZQUIERDO, G., OLEA L. (2007): An approach to acorn production in Iberian dehesas. Agroforesty System 70:3-9.

GARCÍA-VALVERDE R., NIETO R., LACHICA M., AGUILERA J.F. (2007). Effects of herbage ingestion on the digestion site and nitrogen balance in heavy Iberian pigs fed on an acornbased diet. Livestock Science, Vol.112, pp. 63–77.

GRADEČKI-POŠTENJAK M., NOVAK AGBABA S., LICHT R., POSARIĆ D. (2011): Dinamika plodnošenja i kvaliteta uroda sjemena hrasta lužnjaka (Quercus robur) u narušenim ekološkim uvjetima. Šumarski list. 169-181.

GVOZDANOVIĆ K., GALOVIĆ D., MARGETA P., RADIŠIĆ Ž., MARGETA V. (2016): Zelena lucerna u hranidbi crne slavonske svinje. Zbornik sažetaka Krmiva, 20-21.

GVOZDANOVIĆ K., MARGETA V., DJURKIN KUŠEC I., MARGETA P., KUŠEC G. (2016): Meso crne slavonske svinje-fajferice. Knjiga sažetaka 9. međunarodnog znanstveno- stručnog skupa Hranom do zdravlja, 78-79.

MARGETA V., BUDIMIR K., MARGETA P. (2013): Održiva svinjogojska proizvodnja na obiteljskim gospodarstvima. Zbornik radova IX. savjetovanja uzgajivača svinja u Republici Hrvatskoj, 19-22.

MARGETA V., BUDIMIR K., KRALIK G., MARGETA P. (2013): Uzgoj crne slavonske svinje u funkciji zaštite okoliša. Agriculture in nature and environment protection. Osijek: Glas Slavonije d.d., 3. 42-48.

MARGETA V., GVOZDANOVIĆ K., MARGETA P., DJURKIN KUŠEC I., RADIŠIĆ Ž., GALOVIĆ D., KUŠEC G. (2016): Low input production system suitable for Black Slavonian pig breeding. Acta argiculturae Slovenica. 5; 122-126.

MARGETA V., GVOZDANOVIĆ K., GALOVIĆ D., GRČEVIĆ M., MARGETA P., RADIŠIĆ Ž. (2016): Proizvodna i klaonička svojstva crne slavonske svinje u tovu do visokih završnih tjelesnih težina. Zbornik sažetaka Krmiva, 67-68.

ROBINA A., VIGUERA J., PEREZ-PALACIOS T., MAYORAL A.I., VIVO J.M., GUILLEN M.T., RUIZ J. (2013): Carcass and meat quality traits of Iberian pigs as affected by sex and crossbreeding with different Duroc genetic lines. Spanish Journal of Agricultural Research 2013 11(4): 1057-1067.

RODRÍGUEZ-ESTÉVEZ V., SÁNCHEZ RODRÍGUEZ M., GARCÍA A.R., GÓMEZ-CASTRO A.G. (2011). Average daily weight gain of Iberian fattening pigs when grazing natural resources. Livestock Science, Vol.137, pp. 292–295.

SALAJPAL K., KAROLY D., BECK R., KIŠ G., VICKOVIĆ I., ĐIKIĆ M., KOVAČEVIĆ D. (2004): Effect of acorn (Quercus robur) intake on faecal egg count in outdoor reared Black Slavonian pig. Acta agricultuare slovenica 1: 173-178.

SEIQUER A., HAROJ F., AGUILERA R., NIETO A. (2013): Changes in fatty acid composition of intramuscular fat in growing Iberian and Landrace × Large White pigs under identical nutritional management. EAAP, volume 134.

SENČIĆ Đ., SAMAC D. (2016): Conformation and composition of Black slavonian pig carcasses and quality of Black slavonian pig meat of lower and higher meatness level. Meso: prvi hrvatski časopis o mesu, Vol.XVIII No.5.

TEJERINA D., GARCÍA-TORRES S., CABEZA DE VACA M., VÁZQUEZ F.M., CAVA R. (2011): Acorns (Quercus rotundifolia Lam.) and grass as natural

sources of antioxidants and fatty acids in the ‘‘ montanera” feeding of Iberian pig: Intra- and inter-annual variations. Food Chemistry 124: 997-1004.
TEJERINA D., GARCÍA-TORRES S., CABEZA DE VACA M., VÁZQUEZ F.M., CAVA R. (2012): Study of variability in antioxidant composition and fatty acids profile of Longissimus dorsi and Serratus ventralis muscles from Iberian pigs reared in two different Montanera seasons. Meat science 90(2): 414-419.
ŽIVKOVIĆ I., GVOZDANOVIĆ K., GALOVIĆ D., STEINER Z., MARGETA V. (2017): Lucerna kao bjelančevinasti dodatak u hranidbi crne slavonske svinje-fajferice. Zbornik radova 52. hrvatskog i 12. međunarodnog simpozij agronoma. Osijek, 589-593.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

FATTENING RESULTS AND BIOSECURITY LEVEL ON PIG FARMS WITH DIFFERENT CAPACITY

Anamaria Ekert Kabalin[1], Čedomir Radović[2], Ivan Vlahek[1], Marija Gogić[2], Nikola Brleković[3], Sandro Korent[4], Sven Menčik[1], Željko Pavičić[1], Mario Ostović[1], Velimir Sušić[1]

[1]University of Zagreb, Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia
[2]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[3]Veterinary Ambulance Merkur d.o.o., Klokočevik 2c, 35211 Trinjani, Croatia
[4]Bioinstitute d.o.o., Rudolfa Steinera 7, 40000 Čakovec, Croatia
Corresponding author: Anamaria Ekert Kabalin, Full Professor, PhD, anamaria.ekert-kabalin@vef.hr
Original scientific paper

Abstract: The profitable pig production, which includes animal welfare, is achieved by the breeding and production of healthy, highly productive animals. Since biosecurity presents one of the basic principles of pork production improvement, along with the improvement of animal production characteristics, management practices, nutrition, the precise and reasonable usage of preventive measures and antimicrobial usage, in this paper we presented and compared the production parameters and biosecurity levels on pig fattening farms according to their capacity. In one group were farms with 335 or less fattening pigs per turn (<1000 pigs annually), while other group comprised farms with more than 335 fattening pigs per turn (>1000 pigs per year). The survey was performed on 42 fattening farms in the northeast Croatia and central Serbia. The production data included: the duration of the fattening period, the average pig weight at the beginning/end of the fattening period, the total and average daily gain and conversion. The analysis of biosecurity level on farms was performed using the risk-based scoring system Biocheck.UGent. In addition to overall biosecurity level scoring, the main parameters of external and internal biosecurity of the farms are presented separately. Collected data were analyzed using Statistica v.13.2 reference program. There were no significant differences between fattening results on small / large farms, except for pigs' body weight at the beginning of the fattening period (lighter piglets were distributed to the small family farms, P<0.05). The biosecurity level was something lower on smallholder pig farms, but those differences were not significant, except for biosecurity measures conducted in fattening units (45.53% vs 82.33%, P<0.05). On the contrary, some indicators, such as the purchasing of animals were higher on small farms (100.00% vs 88.00%, P<0.05).

Although each production system pays more attention to particular biosecurity measures, the main principles should be present at minimal level on all farms.

Key words: fattening pigs, production parameters, biosecurity level

Introduction

Economically reasonable, intensive pig production implies the breeding and production of healthy, highly productive animals in accordance with their welfare. The farms for fattening pigs differ according to their capacities, the organization of production, the system of animal housing, as well as the level of biosafety (*FAO, 2010; Stanković et al., 2010; Dewulf, 2014*).

In accordance with the existing laws and guidelines for growing pigs (*Narodne novine, 2005, 2010; Službeni glasnik RS, 2010*), it is necessary to take into account the minimum requirements that ensure the protection of animal health and welfare. As the outbreak of diseases has a negative impact on the welfare and productivity of pigs and thus on the economic viability of production, the importance of the application of different measures and procedures in order to improve the biosecurity on farms is becoming increasingly evident. Moreover, the European regulation that covers the animal health and protection entails the responsibility of breeders to implement preventive measures, including the biosecurity measures on farms, in order to prevent and control infectious diseases within the EU (*Sternberg Lewerin et al., 2015*).

The biosecurity on pig farms involves the combined use of measures to reduce the risk of entry and spread of disease. Among the measures of special importance are those focused on the specific risks in pork production: the risk of farming different categories of animals in a relatively small area, the risk of purchasing pigs from different farms and the risk of breeding selected, high producing genotypes pigs with reduced resistance (*FAO, 2010; Koblentz, 2010; Sternberg Lewerin et al., 2015; Anonymous, 2017a, 2017b*). During the last years, in the EU and beyond, interest in the analysis of the implementation of biosecurity measures on pig farms constantly increases (*Bokland et al., 2003; Casal et al., 2007; Stanković et al., 2010; Nöremark et al., 2014; Bachans et al., 2015; Postma et al., 2016*). Although the implemented measures at the level of the herd reduce the possibility of pathogen entry into the herd and its spread among individuals, they may present a significant financial cost, particularly for smallholder farmers (*Sternberg Lewerin et al., 2015*). Therefore, it is important to analyze the level of biosecurity on existing farms for fattening pigs and to observe the major risk factors in the production systems of different capacities.

The biosecurity on pig fattening farms includes all measures taken to minimize the risk of new pathogens emergence into the herd (= external biosecurity), as well as measures that prevent the spreading of pathogens within the farm, i.e. between animals (=internal biosecurity) (*FAO, 2010; Koblentz, 2010; Laonen et al., 2013; Dewulf, 2014; Bachans et al., 2015; Sternberg Lewerin et al., 2015; Anonymous, 2017a, 2017b*).

The main aspects of external biosecurity refer to:

- the purchase of animals (it is preferred to limit the number of the farms of origin, to avoid buying of animals from suppliers with lower / unknown biosecurity level, and to abide the required quarantine period)
- the transport of animals, as well as the removal of manure and dead animals (it is necessary to take care of the cleanliness of the vehicles entering the farm and transporting pigs and/or dead animals; the disposal and transportation of manure should be in accordance with the prescribed procedures)
- food, water and equipment supply (reliable suppliers, the regular examination of drinking water, the regular cleaning of the drinking / feeding system)
- the movement of personnel and visitors on the farm (restrict to a minimum, compliance with the prescribed biosecurity measures for cleaning, washing and the disinfection of clothes, shoes and hands (to prevent / reduce disease transfer from humans to animals and vice versa, as well as the transferring of pathogens and animal excreta (manure, saliva, etc.) between pigs on farm)
- the appropriate vermin and bird control (to avoid the biological and/or mechanical transmission of pathogens);
- farm environment and region characteristics (swine farms density, the presence of wild boar nearby…)

The most important points of internal biosecurity on pig fattening farms are:

- disease management (compliance with the prescribed procedures of vaccination and the implementation of preventive measures)
- fattening unit (the entering of animals to facilities and individual boxes, procedures with animals, the hygiene conditions…)
- measures between compartments and the use of equipment
- the cleaning and disinfection of facilities, boxes, equipment etc.

As *Stanković et al. (2010)* stated, the choice and manner of the application of biosecurity measures is not unique on farms due to the differences referring to their location and epidemiological situation in region, then organization, the technology of production, the purchase of food and animals and employees as well.

The aim of this survey was to analyze the production parameters and biosecurity status on 42 pig fattening farms of different size and biosecurity level.

Material and methods

The survey was performed on 42 pig fattening farms of different capacity in north-east Croatia (29) and north Serbia (13). The farm capacity data (in one production turn and per year) and the production parameters (the body weight at the beginning / end of fattening period and its' duration, the total and average daily gain, conversion) were collected for the year 2016. The data were presented according to the farm capacity – in one group there were small family farms with less than 335 pigs (i.e. less than 1000 animals annually, n= 15), and in the other group were farms with the capacity of more than 335 fattening pigs per turn~~s~~ (>1000 fattening pigs per year, n = 27). The analyses of biosecurity level on farms were performed using the risk-based scoring system Biocheck.UGent™ (www.Biocheck.UGent.be). The statistical reference program Statistica for Windows v.13.2 *(StatSoft Inc., 2016)* was used for the data analyses. The obtained results are presented with mean and confidence interval, or with median, lower and upper quartile (parameters that were not normally distributed). The significance of differences between the groups was determined with the Student T-test or Mann-Whitney U-test (depending on the data distribution). The statistically significant differences are presented at level $P<0.05$.

Results and discussion

The results of fattening achieved on farms during 2016 are presented in Table 1.

Table 1. Fattening results achieved on the farms of different capacity

Production parameter	Farm capacity			
	<335 fattening pigs per turn (<1000 pigs annually) n = 15		>335 fattening pigs per turn (>1000 pigs annually) n = 27	
	mean	confidence interval	mean	confidence interval
No. of fattening pigs per turn	173.40*	123.23 - 223.57	862.41*	463.74 - 1261.08
No. of fattening pigs annually	583.63*	451.47 - 715.80	1717.50*	972.01 - 2462.99
Duration of fattening period (days)	117.97	102.58 - 133.35	126.14	117.85 - 134.42
Body weight at the beginning of fattening period (kg)	26.91*	25.79 - 28.02	28.72*	27.67 - 29.77
Body weight at the end of fattening period (kg)	118.46	110.90 - 126.03	128.79	121.54 - 136.04
Total gain (kg)	91.42	83.90 - 98.94	100.00	93.30 - 106.70
Average daily gain (g)	839.64	780.29 - 899.00	853.24	816.79 - 889.68
Conversion (kg)	3.09	2.91 - 3.27	3.13	3.03 - 3.23

*values in the same row differ significantly (P<0.05)

As is shown, there were no significant differences between the fattening results on small / large farms, except for the pigs' bodyweight at the beginning of the fattening period (lighter piglets were distributed to the small family farms, P<0.05). However, the pigs on large farms were fattened longer; they had something higher average daily gain and the final bodyweight, but also lower conversion. This was expected, having in mind that, with an increase in bodyweight over 100 kg, a daily gain (but mostly fat gain, which causes lower conversion rate) also increases.

In Table 2 and on Graph 1 the results of biosecurity scoring are presented.

Table 2. The overall biosecurity level and the indices of the external and internal biosecurity measures on different farms (based on their total capacity)

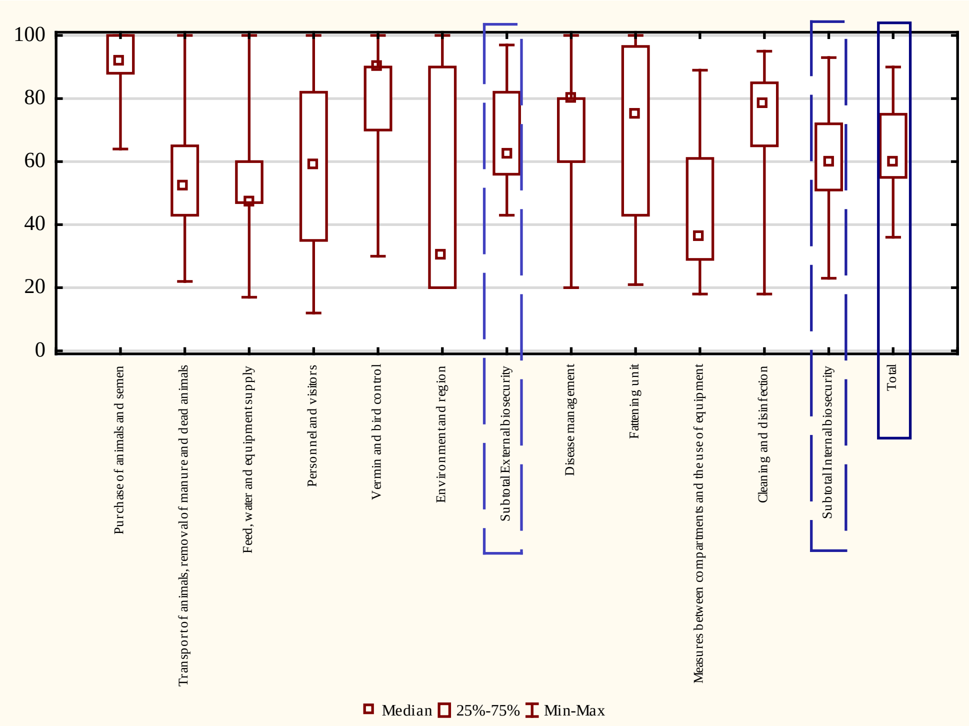
	Farm capacity			
	<335 fattening pigs per turn (<1000 pigs annually) n = 15		>335 fattening pigs per turn (>1000 pigs annually) n = 27	
	mean	confidence interval	mean	confidence interval
External biosecurity				
Purchase of animals and semen[a]	100.00*[a]	92.00-100.00	88.00*	72.00-100.00
Transport of animals, removal of manure and dead animals	53.40	43.00-63.80	60.67	52.54-68.80
Feed, water and equipment supply	52.20	46.05-58.35	52.56	44.90-60.21
Personnel and visitors	50.60	32.05-69.15	58.63	47.57-69.69
Vermin and bird control	81.33	72.24-90.42	80.74	74.16-87.32
Environment and region[a]	30.00[a]	20.00-100.00	30.00	20.00-90.00
Subtotal External biosecurity score	65.60	58.41-72.79	67.52	61.70-73.34
Internal biosecurity				
Disease management	80.00	71.63-88.37	71.82	63.00-80.70
Fattening unit	45.53*	33.09-57.99	82.33*	74.37-90.30
Measures between ompartments and the use of equipment	46.73	33.94-59.53	42.96	35.12-50.81
Cleaning and disinfection	73.00	63.44-82.56	74.59	66.78-82.40
Subtotal Internal biosecurity:	59.40	51.06-67.74	63.00	58.81-67.19
The total biosecurity score	**62.73**	**55.35-70.12**	**65.56**	**61.06-70.05**

[a] values were not normally distributed, so median and lower-upper quartile were presented
*values in the same row differ significantly (P<0.05)

According to the data summarized in Table 2, the most of biosecurity practices are poorly implemented on smallholder pig farms, but those differences were not significant, except for biosecurity measures conducted in fattening units as a whole (45,53% vs 82,33%, P<0.05). That is reasonable having in mind that the higher concentration of animals in facilities is one of the most important risk factors in pig production. Therefore, the large farms pay more attention to this specific biosecurity measure. On the contrary, some indicators were higher on small farms, as is the purchasing of animals (100,00% vs 88,00%, $P<0.05$), indicating that on small farms piglets are purchased mostly from one supplier, as well as some other parameters (vermin and bird control, disease management, measures between compartments and the use of equipment; not significant). The additional problem (especially on larger farms) is the purchase of pigs that are from different geographical origin and health background. Therefore, gathering pigs from different suppliers in a limited space poses a problem based on the fact that highly productive breeds are very sensitive to stress and more susceptible to infections (*Stanković et al., 2007*).

Very similar results were obtained by *Backhans et al. (2015)*. They've found the same relations of biosecurity levels between small and larger pig herds, except for the purchase of animals and semen (that was equal in both groups in their research), and vermin and bird control (these were lower in small herds) as part of external biosecurity. Concerning the internal biosecurity, the same authors obtained different results for disease management (that was higher in larger herds), cleaning and disinfection (higher on small farms).

Graph 1 shows the cumulative results for all observed fattening farms in northeast Croatia and central Serbia.



Graph 1. The average indices of external and internal biosecurity measures on all farms

Observed as a whole, the obtained results for external biosecurity were higher compared to overall internal biosecurity score. As farmers cannot influence on the subcategory of the environment and region, that was the most critical point in our research. The main risk factors that may be improved within external biosecurity are feed, water and equipment supply, the transport of animals, the removal of manure and dead animals and the control of personnel and visitors on farm. For internal biosecurity, there is room for improvement, especially regarding the measures taken between compartments and the use of equipment within the farm. *Bachans et al. (2015)* found that the most critical points of biosecurity for Swedish farrow-to-finish herds were the transport of animals and feed, the hygiene measures of personnel and visitors, the measures applied between compartments and the use of equipment within the farm.

Conclusions

The achieved productivity and biosecurity level may vary depending on the capacity of the farm, the organization of production cycle, as well as on the farming system. Based on our preliminary data, we may conclude that there were no significant differences in fattening results (except the bodyweight of pigs at the beginning of the fattening period), although on larger farms the higher final weights were achieved, but it took a bit longer. On larger capacity farms, with higher biosecurity level (non-significant), the main problems were mostly concentrated on environment and region control, as part of external biosecurity, as well as on measures between compartments and the use of equipment within internal measures. Within small farms, the lowest result was also observed for subcategory environment and region, while for internal biosecurity measures the poorest result was found for fattening unit as a whole. Although each pig production system requires some specific measures, the main principles of biosecurity standards should be present at minimal level an all farms to ensure the necessary level of herd health status.

Tovni rezultati i nivo biosigurnosti na farmama svinja različitih kapaciteta

Anamaria Ekert Kabalin, Čedomir Radović, Ivan Vlahek, Marija Gogić, Nikola Brleković, Sandro Korent, Sven Menčik, Željko Pavičić, Mario Ostović, Velimir Sušić

Rezime

Ekonomski efikasna (profitabilna) svinjarska proizvodnja, koja uključuje dobrobit životinja, postiže se uzgojem i proizvodnjom zdravih, visokoproizvodnih jedinki. Imajući u vidu da biosigurnost predstavlja jedan od osnovnih parametara poboljšanja proizvodnje, uz unapređenje proizvodnih svojstava životinja, menadžmenta proizvodnje, ishrane, kao i ciljane i opravdane upotrebe antimikrobnih preparata, u ovom smo radu uporedili proizvodne pokazatelje i nivo biosigurnosti na farmama za tov svinja različitog kapaciteta. Istraživanjem su obuhvaćene 42 farme za tov svinja s područja severoistočne Hrvatske, kao i Centralne Srbije. Prva grupa je obuhvatala farme kapaciteta ≤ 335 tovljenika po turnusu (ili <1000 životinja godišnje), dok su u drugoj grupi farme s više od 335 tovljenika po turnusu (>1000 svinja godišnje). Posmatrani proizvodni pokazatelji uključivali su: trajanje tova, prosečnu telesnu masu svinja pri stavljanju u tov i na kraju tova, ukupni i prosečni dnevni prirast, kao i konverziju. Procena nivoa bisigurnosti na farmama sprovedena je pomoću bodovnog sistema Biocheck.UGent. Osim procene ukupnog nivoa biosigurnosti, zasebno su prikazane vrednosti pojedinih pokazatelja spoljne i unutrašnje biosigurnosti na farmama. Prikupljeni podaci obrađeni su referentnim programom Statistica v.13.2. Nisu zabeležene značajne razlike proizvodnih pokazatelja ostvarenih na manjim i većim farmama, izuzev za telesnu masu prasadi pri stavljanju u tov, koja je bila značajno niža na manjim porodičnim gazdinstvima (p<0,05). Ukupni nivo biosigurnosti bio je nešto niži na manjim farmama, no navedene razlike nisu bile značajne, osim za biosigurnosne mere sprovedene u objektima za tov (45.53% vs. 82.33%, p<0,05). Suprotno tome, pojedini pokazatelji, poput biosigurnosnih mera pri nabavci životinja, bili su viši na manjim gazdinstvima (100.00% vs. 88.00%, p<0,05). Iako svaki proizvodni sistem pridaje više pažnje određenim biosigurnosnim merama, osnovni bi principi, barem na minimalnom nivou, morali biti zadovoljeni na svim farmama.

Ključne reči: tov svinja, proizvodni parametri, nivo biosigurnosti

References

ANONYMOUS (2017a): Animal Health. Farm Biosecurity [http://extension.psu.edu/animals/health/biosecurity] Pristupano: 12. veljače 2017.
ANONYMOUS (2017b): Biocheck.ugent® Pig [www.Biocheck.UGent.be] Pristupano: 10. siječnja 2017.
BACKHANS A., SJÖLUND M., LINDBERG A., EMANUELSON U. (2015): Biosecurity level and health management practices in 60 Swedish farrow-to-finish herds. Acta Veterinaria Scandinavica, 57, 14.
BOKLUND A., ALBAN L., MORTENSEN S., HOUE H. (2004): Biosecurity in 116 Danish fattening swineherds: descriptive results and factor analysis. Preventive Veterinary Medicine, 66, 49–62.
CASAL J., DE MANUEL A., MATEU E., MARTÍN M. (2007): Biosecurity measures on swine farms in Spain: Perceptions by farmers and their relationship to current on-farm measures. Preventive Veterinary Medicine, 82, 138-150.
DEWULF J. (2014): An online risk-based biosecurity scoring system for pig farms. Veterinary Ireland Journal, 4, 8, 426-429.
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS/WORLD ORGANISATION FOR ANIMAL HEALTH/WORLD

BANK (2010): Good practices for biosecurity in the pig sector – Issues and options in developing and transition countries. FAO Animal Production and Health Paper No. 169. Rome, FAO.

KOBLENTZ G. D. (2010): Biosecurity Reconsidered: Calibrating Biological Threats and Responses. International Security, 34, 4, 96–132.

LAANEN M., PERSOONS D., RIBBENS S., DE JONG E., CALLENS B., STRUBBE M., MAES D., DEWULF J. (2013): Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. The Veterinary Journal, 198, 508-512.

NARODNE NOVINE 136/05 (2005): Pravilnik o uvjetima kojima moraju udovoljavati farme i uvjetima za zaštitu životinja na farmama.

NARODNE NOVINE 119/10 (2010): Pravilnik o minimalnim uvjetima za zaštitu svinja.

NÖREMARK, M, STERNBERG LEWERIN, S. (2014): On-farm biosecurity as perceived by professionals visiting Swedish farms. Acta Veterinaria Scandiavica, 56, 28. doi:10.1186/1751-0147-56-28

POSTMA M., BACKHANS A., COLLINEAU L., LOESKEN S., SJÖLUND M., BELLOC C., EMANUELSON U., BEILAGE E. G., OKHOLM E., NIELSEN, K., STÄRK D. C., DEWULF J. (2016): Evaluation of the relationship between the biosecurity status, production parameters, herd characteristics and antimicrobial usage in farrow-to-finish pig production in four EU countries. Porcine Health Management, 2, 9**.** doi: 10.1186/s40813-016-0028-z

SLUŽBENI GLASNIK RS 41/2009 (2010): Zakon o dobrobiti životinja.

STANKOVIĆ B., HRISTOV S., JOKSIMOVIĆ-TODOROVIĆ M., DAVIDOVIĆ V., BOŽIĆ, A. (2007): Biosigurnost na farmi svinja. Međunarodna konferencija o doborbiti i biosigurnosti na farmama u Srbiji, 14.-15. novembar, Beograd-Zemun, 299-310.

STANKOVIĆ B., HRISTOV S., BOJKOVSKI T. J., MAKSIMOVIĆ N. (2010): Health status and bio-security plans on pig farms. Biotechnology in Animal Husbandry, 26, 1-2, 29-35.

STERNBERG LEWERIN S., ÖSTERBERG J., ALENIUS S., ELVANDER M., FELLSTRÖM C., TRÅVÉN M., WALLGREN P., PERSSON WALLER K., JACOBSON M. (2015): Risk assessment as a tool for improving external biosecurity at farm level. BMC Veterinary Research, 11, 171.

RABBITS BREEDING IN POLAND – POSSIBILITY OF IMPLEMENTATION OF MARKER ASSISTED SELECTION (MAS) IN BREEDING

Łukasz Migdał[1], Konrad Kozioł[1], Władysław Migdał[2], Sylwia Pałka[1], Michał Kmiecik[1], Anna Migdał[3], Józef Bieniek[1]

[1]Department of Genetics and Animal Breeding, Faculty of Animal Sciences, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland
[2] Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland
[3] Institute of Veterinary Sciences, Faculty of Animal Sciences, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland
Corresponding author: Łukasz Migdał, email: l.migdal@ur.krakow.pl
Original scientific paper

Abstract: Recently, use of molecular tools in husbandry is very common. Genomic selection has been implemented in cattle as well as it is advanced in pigs and chicken breeding. For rabbits there is little results regarding single nucleotide polymorphisms (SNPs) and their association with carcass/meat traits. So far most of those papers are linked with genes responsible for growth and with fatness traits. This paper will describe current knowledge and results obtained by authors as well as possibility of implementation of those results in breeding to improve quality of rabbit carcass in Poland. Currently analysis of possibility of use crossbreds rabbits as well as pure breeds, like Polish native breed – Popielno White rabbit with combination of genotyping of animals for favourable SNPs can lead to improvement of final product which is meat.

Key words: MAS, rabbits, meat quality

Introduction

Rabbit meat can provide abundant bioactive compounds for consumers, mainly by high protein level. Moreover, rabbit meat is considered as a good source of essential amino-acids (EAA) (*Dalle Zotte 2002*), moreover it can be described as meat with low fat content. Furthermore, rabbits meat has been considered as a functional food which is favourable for consumers and an effectively reduces public health costs (*Dalle Zotte and Szendro, 2011*).

Functional foods can be classified into two main categories according to their expected effects: first improving of human physiological functions, and second to reduce the risk of specific pathologies. *Diplock et al. (1999)* described functional food that it has to demonstrate its effects to the level normally expected as consumed with the regular diet and still be considered as food. Functional properties can be included in numerous different ways by adding functional ingredient to a traditional food matrix to obtain a fortified food, by modifying the technological process, through animal feeding, special growing conditions, or selecting new varieties not previously consumed (*Fogliano and Vitaglione, 2005*). Because of its properties e.g. high protein level and low fat content rabbits' meat can also be consider as functional (*Dal Bosco et al., 2001; Combes et al. 2003; Combes et al., 2010*). This meat is often described as meat for young, old and ill people thanks to it easy digestion and non-allergic properties (*Dalle Zotte and Szendro, 2011*).

Diversity of rabbit breeds results in different final product, which is caused mainly by genetic variability (Belgian Giant Grey - rabbit can be five times heavier than dwarf rabbits) (*Dalle Zotte et al., 2009)* Breeding strategy in rabbit husbandry focused in increased growth rates (*Rochambeau, 1997; Hernández et al. 1997).* This lead to decrease in the carcass quality, mainly because of increased fat deposition (*Maj et al., 2008). Zomeño et al. (2013)* reported that IMF level can be improved through selection of rabbits. Lipid content in rabbit carcass will vary depending on the part (loin, fore part or hind leg) as well as factors like feeding. *Pla et al. (2004)* reported different levels of fat content in carcass parts. But still there is few information available about IMF and meat and carcass response in rabbits (*Martínez-Álvaro et al., 2016*).

Improving of technology that enables discovery of genomic regions which control quantitative traits can be used to improve selection. Genotyping individuals and association of genotype with phenotype using statistical data can be very helpful in estimating breeding value. Gathering information of SNPs within candidate genes within different breeds and different condition can create database of SNPs that can be used in selection (*Dekkers, 2004*).

In rabbits there is little information regarding single nucleotide polymorphisms (SNPs) – so far genes associated with growth traits were analysed. found association of SNP within growth hormone (GH) (*Fontanesi et al., 2012a*) and insulin-like growth factor 2 (IGF2) (*Fontanesi et al., 2012b)* with market weight in commercial rabbits population. Within growth hormone receptor (GHR) (*Zhang et al., 2012*) analyses revealed that the 84-day-weight, eviscerated weight, semi-eviscerated weight, eviscerated slaughter rate, and semi-eviscerated slaughter. Within myostatin (MSTN) (*Sternstein et al., 2014*) association between SNP and carcass composition traits were found. *Zhang et al. (2013)* analysing fat and mass obesity gene (FTO) sequence identified SNPs associated with body weight at 35, 70, and 84 days of 28 age, average daily weight gain, and intramuscular fat content of *longissimus lumborum*.

This review will describe our experiments and possibility of use its results in rabbit selection

Materials and methods

Animals

The experiment was conducted under standardized conditions at the Experimental Station of the Department of Genetics and Animal Breeding, University of Agriculture in Kraków. All the available data like litter size, growth traits, carcass and meat traits were obtained from F2 New Zealand White (NZW) x Belgian Giant Grey (BGG) crossbred rabbits and purebreds: Popielno White rabbits, Termond White rabbits, New Zealand White rabbits. The animals were kept in a heated hall furnished with water supply (nipple drinkers), lighting (14L:10D) and exhaust ventilation. Water and feed were available *ad libitum*; the pelleted commercial diet contained 15% crude protein, 16.1% crude fibre and 3.5% crude fat.

Carcass traits

The rabbits were weaned at week 5 of life and slaughtered at week 12 (84th day BW). After 24h fasting, slaughter body weight (SW) was recorded, and animals were subsequently slaughtered. Experiment was conducted under a permit from the Local Ethics Commission. The rabbits were stunned, immediately bled, pelted and eviscerated. After slaughter, hot carcass weight (HCW) was recorded, and after 24h storage at 4ºC, chilled carcass weight (CCW) was recorded. The fore part (FP), intermediate part (IP) and hind part (HP) were weighted and dissected. The fore part meat (MF), fore part bone (BF), fore part dissectible fat (FF), intermediate part meat (MI), intermediate part bone (BI), intermediate part dissectible fat (FI), hind part meat (MH), hind part bone (BH) and hind part dissectible fat (FH) were weighted. Dressing out percentages (%) were calculated – both dressing out percentage hot (DPH) = [HCW/SW]*100 and dressing out percentage chilled (DPC) = [CCW/SW]*100. *M. longissimus lumborum* (loin) samples were collected for further physicochemical analysis and stored at -20ºC.

Colour and pH measurement

The lightness [L*], redness [a*] and yellowness [b*], with CIELAB system, of meat were determined using Konica Minolta CM – 600d spectrophotometer. The pH values in meat were determined using Consort C561 pH-metre. The colour and pH were recorded 45 minutes after slaughter and 24 hours after chilling on *m.longissimus lumborum* (loin) and *m.biceps femoris* (hind leg).

Meat quality traits

The meat samples were chemically analysed according to Polish Standards. Water content was determined, using the drying method in accordance with the PN-ISO-1442; protein content (%), using the Kjeldahl method in accordance with PN-A-04018:1975; fat content (%), using the Soxhlet method in accordance with PN-ISO-1444; and total ash content in accordance with PN-ISO-936.
Meat samples were roasted in an oven at 180ºC until they reached internal temperature of 78ºC. From these samples, shear force and texture parameters were measured using TA-XT2 Texture Analyser (Stable Micro System). Shear force was measured on cylindrical samples (14 mm diameter, 15 mm height) using a Warner-Bratzler cutting blade with a triangular notch in the blade. Blade speed during the test was 1.5 mm/s. Results are presented as force per area (kG/cm2).
Texture (hardness, springiness, cohesiveness, chewiness) was analysed using the attached cylinder of 50 mm in diameter. The cylindrical samples (14 mm diameter, 15 mm height) were subjected to a double pressing test, applying a force of 10 g to 70% of their height. Cylinder speed was 2 mm/s, and the interval between presses was 3 s.

Blood collection and DNA extraction

DNA was extracted using a GeneMATRIX kit (EURx) from 300 µl of blood collected during slaughter into tubes containing EDTA.

Primers design and sequencing

Primers for sequencing were designed in the Primer3 program using the genome assembly OryCun 2.0 available in the ENSEMBL database. DNA samples from rabbits showing the highest, lowest and intermediate fat content values were sequenced. PCR products were purified using Exonuclease I and shrimp alkaline phosphatase (SAP) (Fermentas). PCR sequencing was performed for forward and reverse strands with a BigDye terminator v3.1 sequencing kit (Applied Biosystems) on an ABI 3730xl automatic sequencer (Applied Biosystems).

Sequences analysis and genotyping

All sequences were visually inspected in FinchTV. BLAST tool were used to find homology with known sequences. Quality of sequences was inspected using CodonCode Aligner (CodonCode Corporation, www.codoncode.com). SNPs were identified by aligning reference sequence of OryCun 2.0 and aligned sequencing reads in MEGA6 *(Tamura et al. 2013).*

Statistical analysis

The chi-square test for Hardy–Weinberg equilibrium was applied to assess deviation of the number of observed vs. expected genotypes. Genotype distribution was tested for Hardy–Weinberg equilibrium (P > 0.05) using a Web application (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Associations between SNPs and quantitative traits were investigated, by performing the analysis of variance through the general linear model (GLM) procedure of SAS 9.4 (SAS Institute Inc, 2014), using the following model:

$$Yijk = \mu + Gi + Sj + (G*S)ij + \beta Mijk + eijk$$

where Yijk – studied traits; μ –mean value of the trait; Gi – fixed effect of i-th genotype (i=1,2,3); Sj – fixed effect of j-th gender (j=1,2); (G*S)ij – the interaction between genotype and gender; βMijk – linear regression of litter size (the litter size was from 4 to 13 individuals); eijk – error term.

Tukey’s test was used for multiple comparisons. For calculations of multiple testing, the Bonferroni correction was used. The Bonferroni threshold of significance was calculated as a ratio between the significance level (P = 0.05) and the number of hypothesis tests (number of analyzed traits × number of SNPs). This means that the Bonferroni threshold was P = 0.05/(51×2) = 0.00049. The correlations between analyzed traits were calculated using CORR procedure in SAS *(SAS, 2014).*

Results and discussion

Many papers described rabbit meat as well as its good quality, possibility of modification and usage of different breeds/lines in husbandry. Moreover development of molecular biology tools and available rabbit genome information allowed to describe SNP (single nucleotide polymorphism) within candidate genes for growth rate and fatness traits. This research focused on genes related with fatness traits like fatty acids binding protein 3 and 4 (FABP3 and FABP4) and leptin (LEP). There were identified within those genes - SNPs associated with intramuscular fat content (IMF) and dissectible fat weight. There were performed analyses of associations between identified SNPs and carcass and meat traits in New Zealand White and Belgian Giant Grey crossbreed rabbits. All three SNPs (*g.16081633T>C*; *g.16079636C>G, g.16081420C>T)* were associated with IMF and missense mutation *g.16081633T>C* and affected dissectible fat percentage in carcass and dissectible fat weight in intermediate part (loin) (*Migdał Ł. et al., paper* *in press*). For FABP4 gene there were identified *g.97156738 G>A; g.97156696 A>G; g.97156168 G>A and g.97156084 G>A.* Statistical analysis showed association of *g.97156025 G>A* for L* values obtained 45minuts after slaughter and after 24 hours chilling for *m. longissimus lumborum*. After Bofferoni correction statistically significant differences were found for meat weight of loin, IMF and shear force for g.*97156692 C>A* polymorphism (*Migdał et al., paper in press*).

All above presented results proved that in rabbits breeding selection using favourable SNPs within candidate genes for growth rate of carcass traits can possibly improve rabbit carcass dressings.

In Poland we have only one local, primitive rabbit breed – Popielno White rabbit – selected between 1950 – 1970, mainly for backyard raising. That breed of rabbits can be described by good immunity, good fertility and litter size about 7-8 rabbit at the weaning. Popielno White rabbits can be described by highest body weight (g) in 84 days of life (2736±282) compared to Californian (2509±298) and Termond White (2707±212) rabbits. This results were obtained while at the weaning at 35 days of life Popielno White rabbits had lowest body weight (804±134) compared to Californian (829±179) and Termond White (909±128) (*Kmiecik et al., 2016*).

Additionally milk yields were calculated for all above mentioned breeds and Belgian Giant Grey. Highest milk yield value was found in Termonde White rabbits (3.76±0.2). High values were also found for Popielno White rabbits (3.73±0.25) and New Zealand White (3.72±0.25) while the lowest values were found for Belgian Giant Grey (3,18±0,62) (article in press). On the other hand, Belgian Giant Grey were characterized by highest average litter size (9.09±0.21) followed by Termonde White (7.78±2.11), Popielno White (7.46±2.36), Californian (7.50±1.97) and New Zealand White (6.47±1.,93) (article in press). Those results can clarify why Belgian Giant Grey breed are not widely used in crossbreeding for slaughter rabbits. Despite that, their high litter size and low milk yield ended with average 4-5 rabbits weaned.

There are many parameters influencing meat quality. Many of them, like sex, breed feeding, transport, pre-slaughter stress, proper chilling of carcass, guarantee final product of good quality that fits consumers demands. Same important traits were the shear force and texture profile analysis influenced by e.g. way of meat preparation. Selected textural analyses performed on cooked and roasted rabbt meat (*Kozioł et al., 2016*) showed that cooked meat of Termonde White rabbits had the lower shear force value (1.65±0.43 kg/cm^2) compared to roasted meat (1.76±0.6 kg/cm^2). Those obtained values were lower in comparison to results of other authors: *Ariño et al. (2006)* – 3.57 kg/cm^2 and *Gil et al. (2006)* – 3.48 kg/cm^2. Average hardness value obtained in our study reached 9.65 kg and was similar to hardness obtained by other authors. Differences, in shear force values, between this experiment and other authors results, were probably due to different breeds used in experiment. In this study there were used the pure breed rabbits while *Ariño et al. (2006)* and *Gil et al. (2006)* used synthetic lines selected for growth rate. Selection for growth rate can decrease quality of meat (*Maj et al., 2008)* and therefore use of molecular markers can help in selection of animals with favourable genotype and use them in crossing for slaughter rabbits

Conclusion

As a final finding based on this experiment results the usage of Popielno White rabbits as a component in crossing with crossbreed females (Termonde White x Californian Black) or as a component for crossbreed females (New Zealand White x Popielno White) crossed with Termonde White is recommended for obtaing good quality crossbreeds and proper traits of rabbits carcasses. Moreover our analysis showed that use of SNPs associations results can lead to selection of animals with better traits.

Uzgoj zečeva u Poljskoj - mogućnost primene MAS selekcije u uzgoju

Łukasz Migdał, Konrad Kozioł, Władysław Migdał, Sylwia Pałka, Michał Kmiecik, Anna Migdał, Józef Bieniek

Rezime

Nedavno je upotreba molekularnih alata u uzgoju je postala veoma česta. Genomska selekcija se primenjuje kod goveda, a takođe se razvija i kod svinja i odgajivača pilića. Za zečeve ima malo rezultata u vezi sa pojedinačnim nukleotidnim polimorfizmima (SNPs) i njihovom povezanošću sa osobinama trupa/mesa. Do sada je većina tih dokumenata povezana sa genom odgovornim za rast i sa osobinama debljine. Ovaj rad će opisati aktuelno znanje, rezultate koje su dobili autori, kao i mogućnost primene ovih rezultata u uzgoju kako bi se poboljšao kvalitet trupova zeca u Poljskoj. Trenutno analiza mogućnosti korišćenja ukrštenih zečeva, kao i čistih rasa, kao što je poljska autohtona vrsta - Popijelno beli zec sa kombinacijom genotipizacije životinja za povoljne SNP može dovesti do poboljšanja finalnog proizvoda koji je meso.

Ključne reči: MAS, zečevi, kvalitet mesa

References

ARIÑO B., HERNÁNDEZ P., BLASCO A. (2006): Comparison of texture and biochemical characteristics of three rabbit lines selected for litter size or growth rate. Meat Science 73, 687-692. 2.

BARABASZ B., BIENIEK J. (2003): Króliki. Towarowa produkcja mięsna. PWRiL, Warszawa.

BIELAŃSKI P. (2004): Wpływ rasy i systemów utrzymania na cechy produkcyjne brojlerów króliczych. Roczniki Naukowe Zootechniki 18, 5-86.

COMBES S., LEPETIT J., DARCGE B., LEBAS F. (2003): Effect of cooking temperature and cooking time on Warner-Bratzler tenderness measurement and collagen content in rabbit meat. Meat Science 66, 91-96.

COMBES S., POSTOLLEC G., CAUQUIL L., GIDENNE T. (2010): Influence of cage or pen housing on carcass traits and meat quality of rabbit. Animal 4, 2, 295-302.

DAL BOSCO A., CASTELLANI C., BERNARDINI M. (2001): Nutritional quality of rabbit meat as affected by cooking procedure and dietary vitamin E. Journal of Food Science 66, 7, 1047-1051.

DALLE ZOTTE A. (2002): Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Science 75, 11-32.

DALLE ZOTTE A., PRINCZ Z., METZGER SZ., SZABÓ A., RADNAI I., BIRÓ-NÉMETH E., OROVA Z., SZENDRŐ Z.S. (2009): Response of fattening rabbits reared under different housing conditions. 2. Carcass and meat quality. Livestock Sciences, 122; 39-47.

DALLE ZOTTE A., SZENDRŐ Z. (2011): The role of rabbit meat as functional food. Meat Science 88, 319-331.

DEKKERS, J.C.M. (2004): Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. Journal of Animal Sciences, 82; 313–328.

DIPLOCK A.T. AGGETT P.J., ASHWELL M., BORNET F., FERN E.B., ROBER-FROID M.B. (1999): Scientific concepts of functional foods in Europe: Consensus document The British Journal of Nutrition, 8; S1-S27.

FONTANESI L., DALL'OLIO S., SPACCAPANICCIA E., SCOTTI E., FORNASINI D., FRABETTI A., RUSSO V. (2012a): A single nucleotide polymorphism in the rabbit growth hormone (GH1) gene is associated with market weight in a commercial rabbit population. Livestock Science,147; 84–88.

FONTANESI L., MAZZONI G., BOVO S., FRABETTI A., FORNASINI D., DALL'OLIO S., RUSSO V. (2012b): Association between a polymorphism in the IGF2 gene and finishing weight in a commercial rabbit population Animal Genetics, 43, 646–652.
FOGLIANO V., VITAGLIONE P. (2005): Functional foods: Planning and development. A review. Molecular Nutrition & Food Research, 49; 256-262.
GIL M., RAMÍREZ J.A., PLA M., ARIÑO B., HERNÁNDEZ P., PASCUAL M., BLASCO A., GUERRERO L., HAJÓS G., SZERDAHELYI E., OLIVER M.A. (2006): Effect of selection for growth rate on the ageing of myofibrilis, meat texture properties and the muscle proteolytic potential of m. longissimus in rabbits. Meat Science 72, 121-129.
HERNÁNDEZ P., PLA M., BLASCO A. (1997): Relationships of Meat Characteristics of Two Lines of Rabbits Selected for Litter Size and Growth Rate. Journal of Animal Science, 75; 2936–2941.
KMIECIK M., PAŁKA S., KOZIOŁ K., OTWINOWSKA-MINDUR A., MIGDAŁ Ł., BIENIEK J. (2016): Porównanie przyrostów dziennych i masy ciała samic i samców królików ras średnich. Roczniki Naukowe Zootechniki, 43, 2, 183-189 [In Polish]
KOZIOŁ K., PAŁKA S., MIGDAŁ Ł., DEREWICKA O., KMIECIK M., MAJ D., BIENIEK J. (2016): Analiza tekstury mięsa królików w zależności od sposobu obróbki termicznej. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, t. 12, nr 1, 25-32 [In Polish].
MAJ D., BIENIEK J., ŁAPA P. (2008): Jakość mięsa królików rasy białej nowozelandzkiej i kalifornijskiej oraz ich mieszańców. Medycyna Weterynaryjna, 64; 351-353 [In Polish].
PLA M., PASCUAL M., ARINO B. (2004): Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. World Rabbit Science 12; 149–158.
ROCHAMBEAU DE H. (1997): Genetics of a rabbit for meat production: What's new since the World Rabbit Congress held in Budapest in 1988? A review. World Rabbit Science 5, 77-82.
STERNSTEIN I., REISSMANN M., MAJ D., BIENIEK J. BROCKMANN G.A. (2014): A new single nucleotide polymorphism in the rabbit (*Oryctolagus cuniculus*) myostatin (MSTN) gene is associated with carcass composition traits. Animal Genetics, 45(4); 596-599.
ZHANG W.X., ZHANG G.W., PENG J., LAI S.J. (2012): The polymorphism of GHR gene associated with the growth and carcass traits in three rabbit breeds. World Rabbit Science Association Proceedings 10 th World Rabbit Congress – September 3 - 6, 2012– Sharm El- Sheikh –Egypt,75- 78.
ZHANG G.W., GAO L., CHEN S.Y., ZHAO X.B., TIAN Y.F., WANG X., DENG

X.S., LAI S.J. (2013): Single nucleotide polymorphisms in the FTO gene and their association 2 with growth and meat quality traits in rabbits. Gene, 25;527(2); 553-557.

ZOMEÑO C., BLASCO A., HERNÁNDEZ P. (2013): Divergent selection for intramuscular fat content in rabbits. II. Correlated responses on carcass and meat quality traits. Journal of Animal Science, 9; 4532-4539.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

BREEDING DISEASE AND WELFARE CALVES ON DAIRY FARMS TIED BREEDING SYSTEM

Jovan Bojkovski[1], Radiša Prodanović[1], Ivan Vujanac[1], Sreten Nedić[1], Renata Relić[2], Danijela Bojkovski[3] Dejan Bugarski,[4] Dušica Ostojić-Andrić,[5] Ivan Pavolović,[6] Dubravka Milanov[4]

[1] University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia
[2] University of Belgrade, Faculty of Agricultural, Belgrade- Zemun, Serbia
[3] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
[4] Scientific Veterinary Institute, Novi Sad, Serbia
[5] Institute for Animal Husbandry, Belgrade, Zemun
[6]Scientific Veterinary Institute Serbia, Belgrade, Serbia
Corresponding author: Jovan Bojkovski, bojkovski@vet.bg.ac.rs
Review paper

Abstract: Intensive breeding of cattle includes a series of technological procedures that should enable continuous production and optimal use of production capacities. Under such conditions, new-born calves must be adapted to different environmental factors, which primarily relate to diet and housing conditions. The state of health and well-being of calves in the first months of life are most often endangered by diseases of the digestive and respiratory organs. The most significant causes of gastrointestinal disease in calves are enteropathogenic strains of Escherichia (*E. coli*), Clostridium (*C. Perfrngens*), Gastrointestinal diseases of viral etiology (Rotaviruses, Coronaviruses), when the determinant factor is determined by the strain of virus defending the mechanics of defense of the local immune system in the intestine Reduces the natural defense of the digestive tract microbitis. In summer months, on our farms, there is a frequent occurrence of infectious bovine keratoconjunctivitis in calves. From four-month old calf swabs, isolates of *Moraxella bovis*, *Pasteurella multocida*, different species of bacteria from the genus *Streptococcus* and *Nocardia* sp. The most common primary causes of infectious diseases of the respiratory system are mycoplasma (*Mycoplasma bovis*), and these infections are usually complicated by secondary infections caused by different types of bacteria. In this paper, the most frequent health problems of calves on farms of highly milky cows of the related system of keeping are considered.

Key words: calves, health disoders, welfare

Introduction

High production in cattle breeding is based on the successful cultivation of calves, especially the youngest categories. Planting conditions should allow for the satisfaction of their basic life needs, such as optimal air quality, sufficient space for movement and rest, and proper nutrition and power (*Broom, 1991*). If life needs are not fully met, the level of calves' welfare is questionable. Consequences of disturbed welfare are usually expressed as changes in the state of health of calves, or the occurrence of various diseases and deaths. The loss of calves is primarily due to diseases of the digestive organs and respiratory organs (*Bojkovski et al., 2005a; 2007; 2008a; 2010; 2011b; Bugarski et al., 2005*). In our dairy farms, the biggest losses are related to the period of transferring calves from one phase to another (*Bojkovski et al., 2007a; b).* Benefits regarding accommodation conditions are most often endangered by inadequate ventilation of objects, flow rate and air temperature, as well as exposure to pathogenic microorganisms and parasites. In addition to the accommodation conditions, a full picture of the state of well-being is taken into account for nutrition and certain management procedures, such as feeding or rejections of calves. An analysis of potential risk for well-being on one farm indicates possible causes of already present health and other problems in calves, as well as problems that may arise in the future (*Relić and Bojkovski, 2010*). This paper presents the most common diseases of calves that occur in breeding farms of -dairy cows of thtied breeding system, whose occurrence is the result of failure in conditions of accommodation and technology of breeding.

Breeding disease related to breeding technology and calves nutrition

Calf is susceptible to the appearance of cardio-respiratory and metabolic disorders that can be diagnosed during the first weeks of life. The reason for their occurrence is related to the fact that the organism of the new-born calves is not fully formed, and the adaptation to the extrauterine life style in conditions of modern cattle production must be done very quickly. The vitality of calves may be affected by errors in the management of pregnancy, in particular the nutrition of cows in the last third of the steady state. In the nutrition of steamed cows, the need for optimal amounts of ß-carotene should be emphasized, since calves diarrhea often occurs in the zones where the concentration of ß-carotene in the blood serum of highly steep cows was very low (*Ivanov et al., 2001*). It is well known that for the survival of calves and their further progress it is crucial to take adequate colostrum for the time being. Through colostrum, newborn calves are supplied with nutrients (proteins, amino acids, fatty acids, lactose, vitamins, macro and microelements), as well as immunoglobulins, hormones, enzymes, and other life essentials (*Arthington et al., 2000; Bondi, 1987*). The concentration of the most important components of colostrum of the cow was examined in a number of previous studies (*Bojkovski et al., 2003; 2005; 2008a*). During the first week of life, in order to protect the health of calves and satisfy their nutritional needs, gaps that, unfortunately, are often found, such as giving inadequate or excessive colostrum, should be avoided. The supply of excess colostrum occurs in cases where the calf did not receive colostrum 3-4 times in the first 24 hours of life, but only after 10-12 hours. In such cases, severe gastritestinal disorders may occur. Also, it is wrong to supply calves older than 3-4 days with a colostrum of freshly-cowed cows, and the possibility of feeding calves with colostrum from cows suffering from mastitis and a colostrum containing toxins should be completely excluded. Calves may be due to the presence of mycotoxins in the colostrum (*Ožegović, 1995*). During the calorie feeding period, the spacing between the two power supplies should be correct. Otherwise, the calf at the next power supply due to starvation, too quickly takes too much milk. Due to the insufficiently developed esophagogastric reflex, the entire amount of milk (3, 3,5 L) is not conducted to the point of origin, so a part of the milk is returned to the thistle where the turf processes begin.Insufficient hygienic conditions in the supply, insufficiently clean and inadequate containers and cures, especially detergent or disinfectant residues on their surfaces, may damage the mucous membrane of the digestive tract and lead to gastric disturbances.Functional disorders and infectious diseases of the gastrointestinal tract are considered the most important problem of calving breeding. Calf diarrhea is an etiologically complex syndrome in which, besides infectious agents, the environment factors affecting housing conditions and diet also play an important role. According to previous studies *(Bojkovski et al., 2004; 2007; 2009; Šamanc, 2009*), the most significant causes of gastrointestinal diseases in calves are enterotoxic strains of *E. coli* and *C. perfrigens*. Enterotoxic strains of *E. coli* (ETEC) are the most common causes of neonatal calves in the first 3-7 days of life. Bacteria of this group have the ability to produce at least one of two potent enterotoxins: thermolabile (LT) and thermostable (TS). The effect of these toxins is clinically manifested by the appearance of aqueous diarrhea, rapid dehydration, the development of metabolic acidosis, and the death can occur within 24 hours. The fluid and electrolyte recovery must be carried out rapidly, orally or intravenously depending on the severity of the clinical picture. It is of utmost importance that calves do not deny milk during the treatment period. Calf that is treated with oral electrolytes should not be without milk for more than 24 hours. Oral electrolyte solutions containing bicarbonate and citrate are excellent alkalizing agents, but they reduce the digestibility of milk, which is why they should not be used in calves that have received milk. Solutions containing acetates can be used in combination with milk. Vaccines containing the K99 antigen and administered by cows 6 and 3 weeks before calving provide immunity to other *E. coli* types. Vaccines may contain a combination with rota and corona viruses. But calves must receive a colostrum for the first five hours of life, so that the vaccines are effective.Gastrointestinal diseases caused by clostridia are particularly characteristic for calves younger than three months, which are more susceptible than adults. Toxo-types B and C *C. perfringens* are the most common causes of calves' enterotoxemia. Clostridial infections of calves are characterized by a sudden onset of disease or sudden death, preceded by colic or nerve disorders. Gastrointestinal diseases of viral etiology (Rotaviruses, Coronaviruses) occur when the influence of virulence in a particular strain of the virus suppresses local intestinal immunity and reduces intestinal bacterial mycophlora. Viruses are not considered the primary causes of the disease, even when they are isolated from feces of calves to diarrhea. The mode of nutrition and the type of nutrients in the period prior to rejection significantly influence the development of digestive organs and the occurrence of pathological processes in them (*Mattiello et al., 2002; Quigley et al.,2006*). The synergistic activity of bacteria and viruses is also significant in the occurrence of respiratory diseases of calves, especially in overpopulated objects where the microclimate is inadequate (combination of low temperature and high humidity, proliferation, exposure to adult animals, high ammonia concentration) and in animals with impaired immunity, especially inadequate passive immunity, due to mistakes in feeding calves colostrum. Breathing infections present a permanent problem with seasonal delusions, especially on farms where poorly-operated zoohigenic measures are taken. Mild forms of respiratory tract infection were also observed in calves that live in open and semi-coniferous areas (*Bugarski et al., 2005).*The most common causes of respiratory infections of calves are viruses (*Bugarski et al., 2005*), bacteria and mycoplasmas (*Mycoplasma bovis*). Pathogenic action of the virus and mycoplasmas allow later pathogenic action of the bacteria.The following types of bacteria are most often isolated from the lungs of calves with respiratory infections: *Pasteurella multocida, Mannheimia haemolytica (Pasteurella haemolytica), Trueperella pyogenes, Haemophilus* sp. and *Klebsiella pneumoniae* (*Bojkovski et al., 2011).* Also, frequent occurrence on our farms, especially in the summer period, is infectious bovine keratoconjunctivitis of calves. In swabs taken from the eye of calves aged 4 months, isolates of *Moraxella bovis, P. multocida, Streptococcus* spp. and *Nocardia* spp. *(Bojkovski et al., 2008).*From parasitic diseases, in intensive cattle production, the problem is caused by protozoa and ectoparasites. Poor hygienic and microclimate conditions in buildings, as well as the overcrowding of buildings, favor the retention of parasites and their developmental forms in the building, as well as the appearance and spread of the disease.Protozoal infections are very common in calves, and are mostly coccidiosis and cryptosporidiosis. Helminth infections rarely occur in this species, and in intensive (holding) posture, they are almost eliminated *(Pavlović i sar, 2011)*. Bovine coccidiosis is an acute or chronic disease that is, in some countries, of its importance at the top of parasitic diseases. It occurs mainly in younger categories of animals, and in the elderly, in conditions that lead to a decline in immunity (stress, transport, some of the gastrointestinal diseases of other etiology, mycotoxins in food and the like) *(Pavlović et al., 2011).*Types of coccidias with the greatest pathogenic potential are *E. bovis* and *E. zurni* and pathogenic potential are also found in *E. alabamensis* and *E. auburensis*. The parasitization site is a small intestine. The first symptoms occur 2-3 weeks after the festivity, in the form of a long-lasting, persistent diarrhea of unpleasant odor, with the addition of blood and mucus. Diarrhea is accompanied by loss of appetite, general weakness and rapid weight loss, and if the illness persists, anemia occurs. Crestvial peristalsis is accelerated, followed prsistent tessems that can lead to a rectum prolapse. In calves, the course of the disease is usually acute and severe than in older animals *(Pavlović et al., 2011*).Cryptosporidiosis is a protozoal anthropozoonosis caused by the Cryptosporidium parvum coccyxide. The disease is characterized by high morbidity and mortality. The location of the parasite is in the flute epithelium of the jejunu and ileum reservoirs and the plate-like epithelium of the column. The operation of the parasite in a short time leads to the destruction of these cells, which results in a reduction or completely preventing the physiological function of the bowel, ie, reduced digestion, poor absorption and diarrhea. In infected calfs of 1-3 weeks, clinical signs vary from moderate enteritis to severe diarrhea (yellowish, aquatic or mucous feces, rarely with blood), with anorexia, weight loss, dehydration, depression, nausea and vomiting (*Pavlovic and Anđelić -Buzadzic, 2011)*

Of the ectoparasitic infections most commonly found is the scab, caused by Sarcoptes scabiei var. bovis, Psoroptes equi var. bovis and Chorioptes bovis var. bovis. Sarkoptes scabies usually involve the skin of the head, from where it spreads to the neck and chest. The skin becomes dry and heavily wrinkled, and later ragweed and scabies appear. Due to the pronounced itchiness, the animals are scratching and lubricating the skin, and secondary bacterial infections often occur in these places. Psoroptes sores usually involve the skin around the base of the tail, the dorsal side of the neck, the perineal region, the wrinkles, the scrotum, the medial and lateral sides of the extremities, and rarely the whole body. On the skin there may be thickening, dry shells and scabs, as well as inflammatory processes due to which the hair falls. Secrets of the pigs cause itching, and the animals are crushed, and bleeding and secondary infections can occur. Horioptes juveniles live on the surface of the skin and cause irritation and itching. These pigs are more difficult to transmit from one animal to another, so the number of patients slowly increases. The parasit site is the base of the tail, from where it spreads to the perineal region, the udder, the scrotum and the distal parts of the hind legs, and can extend to the area of the back and neck. It rarely involves the whole body. Skin changes are manifested by the appearance of erythema, papillae, scab, scaly, and hair loss. The disease has a mild flow, and usually occurs during the winter period (*Pavlović, 2005; Pavlović and Anđelić-Buzadžić, 2011)*

Conclusion

Diseases of calves in intensive farming are mostly multifactorial etiologies, and as the most significant health problems on our farms, diseases of the digestive and respiratory tract, as well as eye infections, are distinguished.

In order to develop infectious and parasitic diseases of calves, unfavorable hygienic and microclimatic conditions act as disposing factors. Inadequate conditions of accommodation and breaches in the technology of education, especially the supply of colostrum calves, are of particular importance.

Intensive rearing of calves requires continuous monitoring of their health condition, as well as adequate and timely application of veterinary-prophylactic, hygienic-sanitary and zootechnical measures. Improving health conditions positively affects production results and the well-being of calves

Bolesti uzgoja teladi i doborbit na farmama visko-mlečnih krava vezanog sistema držanja

Jovan Bojkovski, Radiša Prodanović, Ivan Vujanac, Sreten Nedić, Renata Relić,Danijela Bojkovski Dejan Bugarski, Dušica Ostojić- Andrić, Ivan Pavolović, Dubravka Milanov

Rezime

Intenzivan uzgoj goveda obuhvata niz tehnoloških postupaka koji treba da omoguće kontinuiranu proizvodnju i optimalno korišćenje proizvodnih kapaciteta. U takvim uslovima novorođena telad mora da se prilagodi različitim činocima iz okoline, uključujući način ishrane i uslove smeštaja. Zdravstveno stanje i dobrobit teladi u prvim mesecima života najčešća ugrožavaju oboljenja organa za varenje i disanje. Najznačajniji uzročnici gastrointestinalnih oboljenja kod teladi su enteropatogeni sojevi *E. coli* i *C. Perfrigens.* Gastrointestinalna oboljenja virusne etiologije (*Rotavirusi,)* nastaju kada je uticaj virulence određenog soja virusa

potisnuo lokalani crevni imunitet i redukovao crevnu bakterijsku floru. Česta pojava u letnjim mesecima na našim farmama, naročito u letnjem periodu je infektivni bovini keratokonjunktivitis kod teladi. U brisevima uzetim iz oka teladi uzrasta od 4 meseca izolovana je *Moraxella bovis*, *P. multocida, Streptcoccus spp.* i *Nocardia spp.* Kao najčešći uzročnici oboljenja organa za diasnje navode se bakterije i mikoplazme (*Mycoplasma bovis*). Izolati iz nosnih briseva teladi obolelih od infekcije disajnih puteva najčešći bili su: *Pasteurella multocida, Mannheimia haemolitica (Pasteurella haemolytica)*, *Arcanobacterium pyogenes, Haemophilus sp.*, kao i *Klebsiella pneumoniae*. U ovom radu razmatrani su najčešći zdravstveni poremećaji teladi na farmama vezanog sistema držanja.

Ključne reči: telad, zdravstveni poremećaji , dobrobit

Acknowledgment

This resarch is part of project TR 31071, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

References

ARTHINGTON J.D., CATTELL M.B., QUIGLEY J.D. *(*2000): Effects of dietary IgG source (colostrum, serum or milk derived suplememt) on the efficiency of Ig absortion in newborn Holstein calves. Journal of Dairy Science, 83, 1463-1467.

BROOM D.M. (1991): Needs and welfare of housed calves. In: New Trends in veal calf production. Proceedings of the International Symposium on Veal Calf Production, ed.by Metz, J.H.M. and Groenestein, C.M. EAAP Publications No.52, Pudoc, Wageningen, 23-31.

BONDI A.A.(1987*):* Animal Nutrition, Chichester, J. Eiliey and sons.

BOJKOVSKI J., BOROZAN S., JOŽEF I., ŠAMANC H. (2003): Sastojci kolostruma pre i posle telenja krava Holštajn-Frizijske rase, Veterinarski Glasnik, 376.

BOJKOVSKI J., BOROZAN S., JOŽEF I., ŠAMANC H. (2005): Colostrum ingredients before and after calving in Holstein-Frisian Cows. Veterinary Record, 4:156(23): p 744-5.

BOJKOVSKI J., RADOJIČIĆ B. (2004): Najčešći poremećaji zdravlja teladi u ranom neonatalnom periodu i do četri meseca starosti sa jedne farme visoko-mlečnih krava. Zbornik kratkih sadržaja 16. Savetovanje veterinara Srbije sa međunarodnim učešćem, Zlatibor, 8-10 septembar, 50-51.

BOJKOVSKI J., BOROZAN S., VUJANAC I., ŠAMANC H. (2005): Prilog poznavanju sastojaka kolostruma krava Holštajn-Frizijske rase i njihovog zdravstvenog stanja u puerperijumu, Veterinarski Žurnal Republike Srpske, vol. V, 1 -2, 46-50.

BOJKOVSKI J., RADOJIČIĆ B. (2007*):* Najčešći patogeni uzročnici oboljenja goveda mlečnih rasa. Zbornik kratkih sadržaja 2. Kongres veterinara Republike Srpske samеđunarodnim učešćem, Banja Luka, 85.

BOJKOVSKI J., RADOJIČIĆ B., PETRUJKIĆ T. (2007A): An outline of clinical caseses of disease in Holstein Frisian cattle on dairy farms in Serbia. 15th International Congress of Mediterranean federation for health and production of ruminants, Abstract, book, 15-19 may Kushadasy, Turkey, 38.

BOJKOVSKI J., BOROZAN S., HRISTOV S., STANKOVIĆ B., JOKSIMOVIĆ-TODOROVIĆ M., DAVIDOVIĆ V. (2007B*):* Uticaj kontaminenata na zdravstveno stanje farmskih životinja. Dobrobit životinja i biosigurnost na farmama, Međunarodna konferencija o dobrobiti i biosigurnosti na farmama u Srbiji, Zemun 14. i 15. novembar. Univerzitet u Beogradu, Poljoprivredni fakultet, 251-258.

BOJKOVSKI J., RADOJIČIĆ B., PETRUJKIĆ T., BOROZAN S.(2008): Prilog poznavanju etioloških činilaca koji utiču na zdravlje farmskih životinja. 20 Savetovanje veterinara Srbije, sa međunarodnim učešćem, Zlatibor, 24-27.09. Knjiga kratkih sadržaja i celih radova, 405-412.

BOJKOVSKI J., RADOJIČIĆ B. PETRUJKIĆ T., BOROZAN S. (2008a): A contribution to insight of the most important etiological factors with influence of farm animal health in Serbia Proceedings of the International Symposium on new research in biotehnology, Bucharest, Romania, Biotehnology, seria F, Special volume, 101- 114.

BOJKOVSKI J., VALČIĆ M., MIRILOVIĆ M., STANKOVIĆ B. (2009): Enterotoksemije teladi visoko-mlečnih krava uzrokovane sa *Clostridium perfringens* tip B. Zbornik naučnih radova Institut PKB, Agroekonomik, Beograd, vol. 15, 3-4, 205- 214.

BOJKOVSKI J., PETRUJKIĆ T., STANKOVIĆ B., ĐOKOVIĆ M., VALČIĆ M., PAVLOVIĆ I., SAVIĆ B. (2010): Prilog poznavanju zdravstvenih i reproduktivnih, biosigurnosnih i ekoloških problema u intenzivnoj govedarskoj i svinjarskoj proizvodnji. Zbornik radova sa XXIV savetovanja agronoma, veterinara i tehnologa, vol.16, 3-4, 105-115.

BOJKOVSKI J., SAVIĆ B., PAVLOVIĆ I. PETRUJKIĆ T., RELIĆ R. ROGOŽARSKI D. (2011*):* The most common pathogenic causes of disease in dairy breed cattle and pigs in farm. Lucrări stiinłifice medicină veterinară Timisoara, vol. XLIV(1), 149-156.

BUGARSKI D., LAZIC S., PETROVIĆ T., MILOVANOV D., BOJKOVSKI J., VUJANAC I. (2005): Infekcije disajnih puteva teladi. Veterinarski Žurnal Republike Srpske, vol. V, 3-4, 107-164.

IVANOV I., JANKOVIĆ D., ŠAMANC H., BOJKOVSKI J.(2001): Novi pristup terapiji i profilaksi dijareje teladi,Zbornik radova trećeg savetovanja iz klinčke patologije i terapije sa međunarodnim učešćem, Budva11-15 jun, 137-141.

MATTIELLO S., CANALI E., FERRANTE V., CANIATTI M., GOTTTARDO F., COZZI G., ANDRIGHETTO I., VERGA M. (2002):The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. J. Anim. Sci., 80, 367-375.

OŽEGOVIĆ L., PEPELJNJAK S.(1995): Mikotoksikoze, Školska knjiga Zagreb

PANCIERA R.J., BOILEAU M.J., STEP D. L. (2007): Tympany, acidosis, and mural emphysema of the stomach in calves: report of cases and experimental induction J. of Vet. Diag. Invest., 19, 392–395.

PAVLOVIĆ I. (2005): Šuga goveda. Veterinarskiinformator, 18/19, 67.

PAVLOVIĆ I. (2006): Paraziti goveda – zoonotski paraziti u mesu goveda. Govedarski glasnik, IV (9), 31-32.

PAVLOVIĆ I**.,** ANĐELIĆ-BUZADŽIĆ G. (2011): Parazitske bolesti sa osnovama parazitologije.Visoka poljoprivredna škola strukovnih studija u Šapcu, 1-154.

RELIĆ R., BOJKOVSKI J. (2010):Housing conditions in calves welfare risk assessment. Journal of Agricultural Sciences. Vol. 55, No. 3, 283 – 292.

QUIGLEY J.D., WOLFE T.A., ELSASSER T.H. (2006): Effects of aditional milk replacer feeding on calf health, growth, and selected blood metabolites in calves.J. Dairy Sci. 89, 207–216.

ŠAMANC H.(2009): Bolesti organa za varenje, Naučna, Beograd.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE MOST IMPORTANT FAILURES IN MAINTAINING THE HYGIENE OF MILKING, COOLING SYSTEMS AND TRANSPORTATION OF DAIRY MILK

Slavča Hristov[1], Jelena Miočinović[1], Branislav Stanković[1], Zorica Radulović[1] Dušica Ostojić Andrić[2], Zvonko Zlatanović[3]

[1]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
[2]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[3]High Agriculture Food College, Ćirila i Metodija 1, 18400 Prokuplje, Serbia
Corresponding author: Slavča Hristov, hristovs@agrif.bg.ac.rs
Review paper

Abstract. Occasional increases in the number of somatic cells and the total number of microorganisms in raw milk of dairy cows indicate a need for a comprehensive overview the most important failures in maintaining the hygiene of milking, cooling systems and transportation of dairy milk in our country. In order to analyze current situation, the project financed by Serbian Ministry of agriculture, forestry and water management took place in 2016, through detailed surveys in comprehensive questionnaire for stakeholders. Current overviews revealed that the most common failures are related to barns conditions, bedding hygiene, application of hygiene measures before and after milking, especially cleaning, wiping and disinfection of teats. Summer season crops production field activities have bad impact on milking personnel hands, udders and milking machines hygiene maintenance. In addition, failures are related to milk cooling and maintaining hygiene in room for milk cooling tank. In order to overcome these problems, as one of the project results, the manual was prepared, containing recommendations for milking, cooling and milk transport procedures improvement. Total number of microorganisms and somatic cell count in the production of the raw cow's milk have very important role. Poor milking hygiene, inadequate cooling and transportation of milk increase their value. These aspects are closely related, which is obvious not only in small and medium-sized family dairy farms, but in high-capacity intensive production as well. Success in the production of milk is practically dependent on the adequate implementation of hygiene measures during milking and milk procedures immediately after the milking. Therefore, the knowledge of the basic aspects of hygiene of cows milking and procedures during milk cooling and transport to the processing of milk has exceptional importance.

Key words: raw milk, hygiene, milking, cooling, transportation

Introduction

Maintaining a high standard of hygiene is one of currently the most important milk production objectives primarily for the protection of human health. Consumers are concerned worldwide about the safety of dairy products and the conditions under which these are produced (*Katić and Stojanović, 2003; Chye et al., 2004; Hill et al., 2012; Smigić et al., 2012*). Because the milk hygiene level directly influences the production economical results, dairy industry steadily enforce raising their quality requirements for raw milk (*Petrović et al. 2006*). It is therefore critically important to ensure high quality of raw milk which can be produced by healthy cows under good hygienic conditions and that all control measures have to been applied continuously to protect human health (*Tucović i Nakić, 2002; Ibtisam et al., 2007; Kalmus et al., 2015*).

Factors influencing raw milk quality and dairy products were described by *Chirlaque* (2011) and monitoring the hygiene of raw milk from farms to milk retailers by *Ruangwittayanusorn et al.* (2016). There are many factors influencing raw milk quality including spatial, microclimatic and hygienic conditions in the barns, especially quality of bedding material, nutrition of cows, udder condition, milking hygiene and procedures during milk cooling, collecting and transportation to the milk plants (*Ruegg, 2006; Anon., 2013*). All these factors contribute in a variety of ways to the hygienic qualities of cow raw milk. The impact of these factors is complex and most often vary in different dairy cattle farms (*Bašić et al., 2012; Godič Torkar and Golc Teger, 2012; Kalmus et al., 2015*).

Dairy milk can be contaminated from many sources at any point of the milk production process. It is the responsibility of the milk producer to identify these points, firstly the key ones, and implement control measures to protect milk from contamination which was determined by the EU regulation 853/2004 (*Anon., 2004*) and regulation in our country (*Anon., 2009*).

The key sources of contamination of raw milk are: faeces, soiled animals, especially their teats, udders and tails, poor milking practices, dirty milker hands, inadequately cleaned and disinfected equipment (including bulk milk tanks), and failure to clean and disinfect teats prior and after milking (*Hristov et al., 1995; Hristov et al., 1997*). In addition, the important causes that contribute to milk contamination are failure to detect abnormal milk (mastitis pathogens, blood and clots), foreign bodies, especially from corroded components in milking machines and bulk tanks, dust, bedding materials, dung, insects, rodents, animal hair, metals, organic material, veterinary product residues, cleaning chemicals and use of non-food grade equipment (*Hristov et al., 2002*).

In our country, there is a obvious need to consider the current situation of the hygienic quality of raw milk. Having this in mind, the project *"Hygiene of milking cows, usage of milk transport systems and milk cooling devices"* was founded by Directorate of Agrarian Payments at Ministry of Agriculture, Forestry and Water Management, Republic of Serbia in 2016.

The project objectives

In cooperation with agricultural extension services, farms and cooperatives, the detailed analysis of the current situation in terms of milk hygiene, as well as regarding the application of modern preventive and corrective measures of cows hygiene in barns in relation to body hygiene, udder hygiene before and after milking, parlour hygiene, handling of cooling systems and the transport of milk with emphases on implementation of effective preventive and corrective measures in milk production has been made during realisation of the projects.

For the purpose of the analysis of the current situation, detailed surveys and interviews that are applied in the form of a comprehensive questionnaire were prepared. Using the questionnaire, the detailed qualitative assessment of the current state of application of hygienic measures through activities that included testing of selected survey of milk producers in the field, as well as the assessment from electronically testing, was carried out. Consideration of the current situation on the field has been thoroughly carried out on 15 farms of small, medium and large capacity.

Special attention in the comprehensive consideration of the application of hygiene measures was focused on the evaluation of awareness and perception of milk producers about potential hygiene and biosecurity risks that may arise in the production of dairy milk, their impact on the health of the consumers, awareness of modern hygienic measures and the degree of their implementation, as well as consideration of hygiene during milking, milk cooling and transport through the questionnaire based on a tool box for assessing cow, udder, teat hygiene, described by *Reinemann and Cook (2007),* and methodology for assessment of hygiene of barns and body, udder, teats, leg, flank and abdomen hygiene described by *Hristov, (2002)* and *Relić and Hristov (2016).* In the assessment of hygienic conditions in the stalls was taken into account biosecurity plans application in dairy farm production (*Stanković et al., 2013*).

In addition, the analysis of the current situation included a detailed review of the available scientific and professional literature on the hygiene of milk, both in our country and in the region and developed countries, and the comparison with project obtained data was completed.

Notes and recommendations of the project

The results of the project were published in two studies (*Hristov et al., 2016a; Hristov et al., 2016b*) and recommendations regarding the application of hygienic measures in order to improve the quality of dairy milk in the manual *(Hristov et al., 2016c).* Major determinants of the hygienic quality of milk are the number of somatic cells and total count of microorganisms (*Heeschen, 1997*) and proper udder and teat preparation for milking are the primary condition that have to be fulfilled for the production of milk of high hygienic standard.

The results of the project revealed that in our country, raw cow milk that arrives in the plants sporadically has an increased counts of microorganisms and somatic cells, which is primarily influenced by mastitis, failures in hygiene during milking and milk collecting. Most often, this happens on small and medium-sized dairy farms during the summer season. The most important aspects of determining the total number of microorganisms and number of somatic cells in raw milk of cows were described by *Hristov (2002b)* and *Hristov (2002a)*, respectively.

Very important practices before every milking are forestripping, and udder and teats cleaning. Forestripping is recommended due to the fact that prior to milking, the highest concentration of somatic cells and microorganisms are in the milk of the teat cistern. Thus, the removal of this milk results in significant reduction in the counts of these elements in the milk. At the same time, forestripping facilitates prompt identification of the clinical form of mastitis in individual quarters and enables immediate treatment of diseased cows and their milking using separate equipment. For these reasons, and as a result of separating the milk of healthy cows from diseased ones, the somatic cell and microorganism counts in bulk tank milk will be reduced (*Pankey, 1989; Pankey and Drechsler, 1993; Anon., 2013).*

The basic problems faced by small and medium-sized households involved in milk production in our country are: poor hygiene in barns, low quality of bedding material and poor hygiene during the milking of cows, inadequate milking units, inadequate transport and milk cooling processes, which are certainly related to many technologic aspects of dairy cattle production, as well as the numerous economic and financial burdens that accompany them (*Hristov et al., 2016a; 2016b).*

The detail evaluation of the procedures before the cow's milking including the preparation of cows for milking, visual examination of the mammary gland for the presence of signs of inflammation or any other damage, visual examination of the teats and udder base on their contamination, proper washing and disinfection of teats, application of premilking and mastitis test, evaluation of milking procedures, as well as hygiene procedures before and during use of milking units pointed out many failures. For instance, it was noticed the absence of minimum contact time of the disinfectant and the teats for at least 30 seconds which should be wiped afterwards, as well as proper set up of milking units (*Hristov et al.,* 2016c).

It is well known fact that a good milking procedure is essential for the production of safe cow raw milk. First of all, teats, udder and adjacent parts must be clean before teats cups attachement. Cleaning of teats before milking is important procedure to remove both visible soiling (e.g. faeces, bedding, mud, residual post milking disinfectants) and bacteria which could contaminate the milk. In addition, teat dips or sprays must be used in accordance with manufacturer instructions. It is important to always keep in mind the research results which proved that the number of bacteria on teats is not necessarily linked to visual cleanliness, so all cows should be treated, not only those with visibly dirty teats. Thorough washed and dried udder should be treated using disinfectant impregnated towels or dipped in disinfectant solution, which must be effectively removed before teat cups are attached (*Hristov et al., 1995; Ruegg, 2006; Anon., 2013*).

Hygiene of cow milking has direct influence on the milk quality and indirect on udder health condition. Hygiene of milking personnel, milking parlour or barns, milking machines, milk tanks and other equipment are very important. Our research has shown that the most frequent failures come from inadequate milking personnel hygiene, hygiene of a milking parlour and pre-milking procedures.

Cow milking is one of the most important and most complex operations in milk production, since milk is an ideal medium for bacterial multiplication.

The opportunities for bacterial multiplication are numerous: during milking, storage, and distribution; adequate and hygienically correct milking should prevent the contamination of milk from environmental microorganisms (*Hristov et al., 2002; Hristov et al., 2016a; Hristov et al.,2016b*).

Streptococcae and staphylococcae, as the most frequent mastitis causing pathogens, are mostly spread through milking machines (*Hristov et al., 1997; Hristov et al., 1998*). Technical adjustment of milking machines may significantly influence milking efficacy and udder health, as well as economy of milk production. Inadequate use of milking machines is the main factor for increasing subclinical mastitis occurrence rate (*Hristov and Relić, 2003*), and also clinical mastitis occurrence rate (*Nakov et al., 2014*). Decreasing of new udder infections rate implies the disinfection of teats after each milking, maintenance of milking machines, teat cups disinfection, udder washing with warm running water and other hygienic-sanitary measures (*Hristov et al., 2016*a,b,c).

Very important aspect of preserving good quality of milk is necessity to observe milk from each teat prior to every milking. Milk from each cow must be checked for any physical, chemical and organoleptic abnormalities. In the most situations, this is most effectively done by taking premilking or by a combination of premilking and another suitable method. Milking personnel should always be aware that premilking assists early detection of mastitis and removes potentially contaminated milk from the teat canal and stimulates milk let down (*Anon., 2013*; *Hristov et al., 2016c*).

Milk is good medium for bacteria, including pathogenic organisms, especially if it is produced and stored under non hygienic conditions. When identified, abnormal milk must be stored separately and not used for human consumption. Continuous application of hygienic and sanitary measures in prevention of the mastitis occurrence and control is necessary (*Hristov et al., 1996; Hristov et al., 2000*). Animals producing milk that is unfit for human consumption must be clearly identified. The recommendations for excluding abnormal milk are: milk affected cows last (with a full sanitizer cleaning routine after each milking) and milk into a dump bucket or dump line (with a clean, well maintained separate teat cups and milk tube). A good milking procedure helps to ensure no transfer of udder infection to other cows within the herd; it was proved that bacteria from infected udder may be spread up to eight cows through successive teat cups use. Pre and post milking disinfectants must be used in accordance with the manufacturer's instructions in order to maintain effectiveness. Dipping cups and spray devices should be kept visually clean (*Hristov et al., 1995; Hristov et al., 1997; Hristov et al., 2003).*

Wherever cows are not in good health, especially when genital discharge occurs, or enteritis with diarrhoea, fever or udder infection, the milk must not be used for human consumption, as well as milk that origins of cows positive for brucellosis or tuberculosis must not be used for human consumption. This means that milk for human consumption must come from animals that are in good health with no udder lesions that may contaminate the milk, especially coliform mastitis causing germs (*Hristov, 1996*), streptococcal mastitis (*Hristov et al., 1997*) and staphylococcal mastitis (*Hristov et al., 1998)*. Special attention should be paid to subclinical forms of mastitis and their prevention (*Hristov and Relić, 2013; Nakov et al., 2014*).

Milking equipment must be kept clean all the time. Milk contact surfaces must be appropriately cleaned and disinfected immediately after milking. All equipment must be kept clean and in good condition. Any dirt on milking equipment must be washed off before teat cups reattachment. Dung has to be removed from the floors and stall work as soon as practicable, and before another animal or group enters the milking area (*Anon., 2009; Hristov et al., 2016c*).

Milking environment is very important. The movement of dusty feeds or bedding materials should not be carried out close to the milking area immediately before or during milking. The level of dust on overhead pipes must be kept to a minimum.

Personnel who carry out milking and/or handling raw milk must wear suitable clean clothes and maintain a high degree of personal cleanliness. Suitable facilities must be available near the milking place, enabling staff performing milking and handling raw milk to wash their hands and arms. Hands must be cleaned before milking and kept clean during milking and milk handling. Exposed skin wounds must be hygienically covered (*Chirlaque, 2011; Hristov et al., 2016c*).

A jet of sufficient volume and pressure to wash equipment and cow standings thoroughly during and after milking is recommended. Warm running water, preferably containing a suitable disinfectant should be available to rinse hands, protective clothing, udders and equipment whenever they become soiled. Paper towels should be available. A suitable bin should be available for the disposal of used towels and other waste and emptied after each milking. Within a milking area all floors, walls, fittings and touch points should be cleaned thoroughly after every milking. The upper walls and ceiling should be kept free from dust and cobwebs accumulations. Animals may have access up to the entrance and exit of the milking area between two milkings, but dung, slurry and other noxious materials must not accumulate on floors, walls and fittings in these areas (*Anon., 2013; Hristov et al., 2016a,b,c).*

In our country, it is especially important to pay attention to milk contamination prevention during storage, cooling and transport. It is generally recommended for milk to be cooled to an acceptable temperature within a few hours after milking and stored at 4°C or below to minimise bacteria multiplication. Primary cooling might be required to avoid the blend temperature of the milk in the bulk tank, exceeding a certain level. Cooling of milk shortly after milking is also important for minimising lipolytic activity. The latter process becomes more important the higher the milking frequency is, and also where intervals between milking occasions vary for individual cows. Bulk tanks must be cleaned and disinfected after each milk collection and kept in good condition (*Anon., 2013; Hristov et al., 2016c*).

Besides good design and management of the housing, there are several measures which can be implemented to improve animal cleanliness: trim or clip tails at housing and turn-out (cows with a clean, trimmed tail will attract fewer flies), flaming of udders and/or clipping of flanks, bellies and udders reduces the amount of soil or faeces which can adhere to these areas, remove dirt manually and encourage grooming with cow brushes; clean animals are more likely to remain disease free and at milking time, are less likely to contaminate the milk with harmful bacteria and consider altering diets to minimise loose dung (*Ruegg, 2006; Chirlaque, 2011; Anon., 2013*).

Housing must be managed to avoid soiling of the animals. Quality bedding helps to minimise soiling and improves animal comfort. All passageways and loafing areas should be kept free of accumulations of dung, slurry and stale feed. Animals must have clean teats, udders and adjacent parts (flanks, hindquarters, tails and abdomen) before milking (*Hristov, 2002; Ward et al., 2002*).

Conclusion

Total number of microorganisms and somatic cell count in the production of the raw cow's milk have very important role. Poor milking hygiene, inadequate cooling and transportation of milk increase their value. These aspects are closely linked, which is especially obvious not only in small and medium-sized family dairy farms, but high capacity, intensive production farms as well. Success in the production and processing of milk in practice depend on the adequate implementation of hygiene measures during milking and milk procedures immediately after the milking.

Therefore, the knowledge of the basic aspects of hygiene of cows milking and procedures during milk cooling and transport to the processing of milk has exceptional importance.

Najznačajniji propusti u održavanju higijene muže, sistema hlađenja i transporta kravljeg mleka

Slavča Hristov, Jelena Miočinović, Branislav Stanković, Zorica Radulović, Dušica Ostojić - Andrić, Zvonko Zlatanović

Rezime

Povremena povećanja broja somatskih ćelija i ukupnog broja mikroorganizama u sirovom mleku ukazuju na potrebu za sveobuhvatnim pregledom najvažnijih propusta u održavanju higijene muže, sistema za hlađenje i transporta mleka u našoj zemlji. U cilju sagledavanja stanja, izvedena su istraživanja u okviru projekta finansiranog od strane nacionalnog Ministarstva poljoprivrede, šumarstva i vodoprivrede, kroz detaljno sagledavanje u okviru upitnika sa svim učesnicima u proizvodnji kravljeg mleka. Sagledavanje trenutne situacije je pokazalo da se najznačajniji propusti odnose na uslove smeštaja u staji, higijenu prostirke, primenu higijenskih mera pre i posle muže, što se posebno odnosi na pranje, brisanje i dezinfekciju sisa. Sezonski poljski radovi imaju negativan uticaj na higijenu ruku radnika, vimena i aparata za mužu. Pored toga, propusti se odnose i na hlađenje mleka i održavanje higijene u prostoriji za smeštaj tanka za hlađenje. U cilju prevazilaženja navedenih problema, kao jedan od ciljeva projekta je napravljen priručnik koji sadrži preporuke za unapređenje efikasnosti postupaka muže, hlađenja i transporta mleka.

Ukupan broj mikroorganizama i broj somatskih ćelija ima vrlo važnu ulogu u proizvodnji mleka. Nizak nivo higijene, nepravilno hlađenje i transport povećavaju ove vrednosti. Ovi aspekti su u bliskoj vezi, što je očigledno ne samo na malim i srednjim porodičnim farmama, već i na farmama velikog kapaciteta sa intenzivnom proizvodnjom. Uspeh u proizvodnji mleka zavisi od adekvatne primene higijenskih mera tokom i odmah posle muže. U tom smislu, poznavanje osnovnih principa higijene muže i postupaka u vezi hlađenja i transporta mleka imaju izuzetan značaj.

Ključne reči: sirovo mleko, higijena, muža, hlađenje, transport

Aknowledgement

This paper was financed by Ministry of Science and Technological Development of Republic of Serbia, TR 31086

References

ANON. (2004): European Commission. Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004: Laying Down Specific Hygiene Rules for Food of Animal Origin. European Commission; Luxembourg City, Luxembourg: 2004. p. L139.
ANON. (2009): Pravilnik o kvalitetu sirovog mleka („Sl. glasnik RS“, br. 21/2009)
ANON. (2013): Food Standards Agency (2013) A Practical Guide for Milk Producers to: The Food Safety and Hygiene (England) Regulations 2013, The Food Hygiene (Wales) Regulations 2006, Incorporating EC Regulations 852/853/854 – 2004.
BARRETT D. (2002): High somatic cell counts – a persistent problem. Irish Vet. J., 55, 173-178.

BAŠIĆ Z., BOŽANIĆ R., KONJAČIĆ M., ĐERMADI J., ANTUNAC N., VOLARIĆ V. (2012): Kemijska i higijenska kvaliteta mlijeka na farmama mliječnih krava u tri hrvatske regije, Mljekarstvo 62 (4), 251-260.

CHIRLAQUE R. (2011): Factors influencing raw milk quality and dairy products. Universidad Politecnica de Valencia.

CHYE F., ABDULLAHB A., AYOBB M., K. (2004): Bacteriological quality and safety of raw milk in Malaysia, Food Microbiology 21, 535–541.

GODIČ TORKAR, K., GOLC TEGER, S. (2008): The microbiological quality of raw milk after introducing the two days milk collecting system, Acta agriculturae Slovenica, 92, 1, 61–74.

HEESCHEN W.H. (1997): Bestimmungsfaktoren der hygienisch-qualitativen Wertigkeit von Milch. Arch. Tierz., Dummerstorf 40, Sonderheft, 155-162.

HILL B., SMYTHE B., LINDSAY D., SHEPHERD J. (2012): Microbiology of raw milk in New Zealand. International Journal of Food Microbiology, 157(2), 305 – 308.

HRISTOV S. (1996): Koliformni mastitis krava. Vet. glasnik, 50, 5-6, 329-340.

HRISTOV S. (2002): Zoohigijena. Poljoprivredni fakultet, Beograd.

HRISTOV S. (2002a): Najznačajniji aspekti utvrđivanja broja somatskih ćelija u mleku krava. Zbornik radova jugoslovenskog mlekarskog simpozijuma Savremeni trendovi u mlekarstvu, Vrnjačka Banja, 32-38.

HRISTOV S. (2002b): Najznačajniji aspekti utvrđivanja ukupnog broja mikroorganizama u svežem mleku krava. Mlekarstvo, 208 – 216.

HRISTOV S., RADULOVIĆ Z., MIOČINOVIĆ J., VAKANJAC S., STANKOVIĆ B., DAVIDOVIĆ V., PETRUJKIĆ B., (2016a): Studija o analizi rezultata projekta "Higijena muže krava u laktaciji, korišćenja zatvorenih sistema za transport mleka i uređaja za hlađenje mleka", Projekat Uprave za agrarna plaćanja, Ministarstvo poljoprivrede i zaštite životne sredine Republike Srbije i Univerzitet u Beogradu – Poljoprivredni fakultet, Beograd.

HRISTOV S., RADULOVIĆ Z., MIOČINOVIĆ J., VAKANJAC S., STANKOVIĆ B., DAVIDOVIĆ V., PETRUJKIĆ B. (2016b): Studija o analizi higijenskih, zootehničkih i veterinarskih mera od značaja za ekonomičnost proizvodnje mleka. Projekat Uprave za agrarna plaćanja, Ministarstvo poljoprivrede i zaštite životne sredine Republike Srbije i Univerzitet u Beogradu – Poljoprivredni fakultet, Beograd.

HRISTOV S., RADULOVIĆ Z., MIOČINOVIĆ J., VAKANJAC S., STANKOVIĆ B., DAVIDOVIĆ V., PETRUJKIĆ B., (2016c): Priručnik za primenu higijenskih mera u cilju poboljšanja kvaliteta mleka. Projekat Uprave za agrarna pladanja, Ministarstvo poljoprivrede i zaštite životne sredine Republike Srbije., Univerzitet u Beogradu – Poljoprivredni fakultet, Beograd.

HRISTOV S., LAZAREVIĆ N., RADOVANOVIĆ M., JOŽEF I. (1998): Stafilokokni mastitis krava. Zbornik naučnih radova XII Savetovanja agronoma, veterinara i tehnologa, Aranđelovac, 4, 1, 411422.

HRISTOV S., LAZAREVIĆ N., RADOVANOVIĆ M., PAVLOVIĆ M. (1997): Streptokokni mastitis krava. Zbornik naučnih radova XI Savetovanja agronoma i tehnologa sa međunarodnim učešćem, Aranđelovac, 3, 1, 415-424.

HRISTOV S., RELIĆ R. (2003): Subklinički mastitis i program preventive u intenzivnim uslovima gajenja krava. Mlekarstvo, 23, 792-797.

HRISTOV S., RELIĆ R., STANKOVIĆ B. (2002): Failures in conveying hygienic procedures during milking of cows. Journal of Agricultural Sciences. Vol. 47, No 2, 233 – 240.

HRISTOV S., STANKOVIĆ B., RELIĆ R. (2003): Ispitivanje učinka Jodogala na površinama sisnih čašica, kože vimena i ruku muzača. Zbornik radova XIV Savetovanje iz dezinfekcije, dezinsekcije i deratizacije u zaštiti životne sredine, sa međunarodnim učešćem, Subotica, 6570.

HRISTOV S., STOJANOVIĆ Z., STANKOVIĆ B. (1996): Najznačajnije higijensko-sanitarne mere za sprečavanje pojave i suzbijanje mastitisa krava. Zbornik radova VII Savetovanje DDD u zaštiti životne sredine, Sutomore-Bar, 62-67.

HRISTOV S., TODOROVIĆ M., NEŠIĆ K. (2000): Najznačajniji aspekti programa sprečavanja pojave i suzbijanja mastitisa krava. Arh. Poljopr. nauke, 61, 1-2, 135 – 146.

HRISTOV S., TRBOJEVIĆ R., ANOJČIĆ B., VUČINIĆ M., HRISTOV-PRENKIĆ M. (1995): Dezinfekcija vimena i aparata za mužu. Zbornik radova VI Simpozijuma iz DDD u zaštiti životne sredine, Donji Milanovac, 48-52.

HRISTOV S., VUČINIĆ M., JOŽEF I. (1997): Dezinfekcija vimena krava pre i posle muže. Zbornik radova VIII Savetovanja iz DDD u zaštiti životne sredine, Subotica, 15-21.

IBTISAM E., EL ZUBEIR M., AHMED MAHBORA I.A. (2007): The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. Research Journal of Microbiology, 2, 988–991.

KALMUS P., KRAMARENKO T., ROASTO M., MEREMÄE K., VILTROP A. (2015): Quality of raw milk intended for direct consumption in Estonia, Food Control, 51, 135-139.

KATIĆ V., STOJANOVIĆ L. (2003): Može li se proizvesti mleko za trzište Evrope? Simpozijum mlekarske industrije Srbije i Crne Gore „Savremeni trendovi u mlekarstvu“, Zbornik radova, 15–21, Zlatibor.

NAKOV D., HRISTOV S., ANDONOV S., TRAJCHEV M. (2014): Udder-related risk factors for clinical mastitis in dairy cows. Vet. arhiv 84, 111-127.

PANKEY J.W. (1989): Hygiene at milking time in the prevention of bovine mastitis. Br. Vet. J. 145, 401-409.
PANKEY J.W.; DRECHSLER P.A. (1993): Evolution of udder hygiene. Premilking teat sanitation. Vet. Clin. North Am.: Food Anim. Pract. 9, 519-530.
PETROVIĆ M. D., PETROVIĆ M.M., NENADOVIĆ G., KURĆUBIĆ V., MARINKOV G. (2006): Hemijski i mikrobiološki parametri kvaliteta sirovog kravljeg mleka, Biotechnology in Animal Husbandry 22 (5-6), 109 – 119.
REINEMANN D., COOK N. (2007): A Tool Box for Assessing Cow, Udder, Teat Hygiene. 46th Annual Meeting, January 21 – 24, San Antonio, Texas.
RELIĆ R., HRISTOV S. (2016): Praktikum iz zoohigijene. Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.
RUANGWITTAYANUSORN K., PROMKET D., CHANTIRATIKUL A. (2016): Monitoring the Hygiene of Raw Milk from Farms to Milk Retailers. Agriculture and Agricultural Science Procedia 11 95 - 99.
RUEGG P. (2006): The Role of Hygiene in Efficient Milking. WCDS Advances in Dairy Technology, Volume 18: 285-293.
SMIGIC, N., DJEKIC, I., TOMASEVIC, I., MIOCINOVIC, J., GVOZDENOVIC, R. (2012): Implication of food safety measures on microbiological quality of raw and pasteurized milk, Food Control, 25, 728-731
STANKOVIĆ B., HRISTOV S., ZLATANOVIĆ Z. (2013): Benefits of biosecurity plans application in dairy farm production. Proceedings of the First International Symposium on Agricultural Engineering, 4th-6th October 2013, Belgrade – Zemun, Serbia, II – 19-28.
TUCOVIĆ N., NAKIĆ M. (2002): Podizanje nivoa kvaliteta sirovine i proizvoda primenom politike ISO standarda sistema kvaliteta u mlekarama AD „Imlek“-a. Mlekarstvo, 1(2): 61–63, Beograd.
WARD W.R., HUGHES H.W., FAULL W.B., CRIPPS P.J., SUTHERLAND J.P., SUTHERST J.E. (2002): Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. Vet Rec 151:199-206.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

RELATIONS OF UDDER HYGIENE WITH QUALITY AND QUANTITY PARAMETERS OF MILK IN HOLSTEIN COWS

Edit Mikóné Jónás[1], Savaş Atasever[2], Szabina Szilagyi[1]

[1] Department of Animal Husbandry, Faculty of Agriculture, University of Szeged, 6800, Szeged, Hungary
[2] Department of Animal Science, Faculty of Agriculture, University of Ondokuz Mayis, 55139-Samsun, Turkey
Corresponding author: Savaş Atasever, satasev@omu.edu.tr
Original scientific paper

Abstract: The objective of this study was to determine the associations of udder hygiene with bovine milk quality and quantity parameters. In total, 1783 Holstein cows reared at a private farm in Szegvár, South- Hungary, were examined. To obtain udder hygiene scores (UHS) a five point system (1 indicates absolutely clean and 5 indicates very dirty) was used. While daily milk yield (dMY) was used to be quantity parameter, fat, protein, lactose and somatic cell count (SCC) were used to be quality ingredients. As non-genetic factors, parity and stage of lactation affected all parameters except for protein and dMY, respectively (P<0.05). The parameters changed by UHS groups (P<0.05) and UHS had negative, but insignificant correlation coefficients with fat, protein and lactose. It is advised that UHS values should closely be observed in dairy herds to achieve eligible raw milk.

Key words: cow, udder hygiene, milk composition, somatic cell count.

Introduction

A bonus or punishment system for milk quality has currently been applied in many countries. In spite of marked increments have been achieved in milk production level of culture dairy cow breeds, high quality, clean and safe raw milk is widely demanded by consumers. In addition to genetic merit of cows, environmental factors such as barn conditions, feeding regime, season or hygienic status of animals affect the milk amount and composition. Especially, keeping dairy herd within a sanitary condition throughout production period plays a major role to manage this target. Barning milking cows in unsuitable ambiance my expose animals for a high microorganism load. Moreover, this case may also cause some acute or chronic disorders in the herds. *Schreiner and Ruegg (2003)* pointed out that dirty teats and udders are the principal sources of microorganisms in cow milk. Today, some indirect markers have been used to assess milk quality in many countries. Of these, somatic cell count (SCC) is the most reliable parameter to evaluate the quality level. A negative correlation between SCC and body condition score (BCS) of cows was reported by *Stadnik and Atasever (2016)*. Moreover, the authors indicated that SCC played an important role on milk protein level. In an investigation that conducted earlier *(Memisi et al., 2011*), increment in SCC from $50x10^3$ cells/ml to $800x10^3$ cells/ml reduced milk production by 6.3% in first parity cows and 9.6% in cows with third or later lactation orders. Also, many investigations have been conducted to emphasize the importance of hygiene for dairy enterprises *(Atasever et al., 2012; Devries et al., 2012; Schreiner and Ruegg, 2013; Neja et al., 2016)*. But, detailed studies including the associations of hygiene with quality and quantity markers are still moderately. Revealing the relations between these parameters may be seen to be a critical approach to prevent milk losses in dairy herds.

The aim of the present study was to indicate the associations of udder hygiene status with milk quality and quantity parameters in Holstein cows.

Materials and Methods

For examination, Holstein cows, 1783, reared at a private farm in Szegvár, South- Hungary, were used to be the study material. All cows were clinically healthy and kept in similar conditions by feeding and milking management during the experiment period. Milking was applied two times in a day. To collect udder hygiene scores (UHS) a five point system (1 indicates absolutely clean and 5 indicates very dirty) was used. Daily milk yield (dMY) was used to be quantity parameter, and fat, protein, lactose and somatic cell count (SCC) were chosen to be quality items. The cows were evaluated by five parity groups (cows with >5 parities were combined to 5^{th} parity) and three stage of lactation groups (SL1=11-99d; SL2=100-199d and SL3=≥200d) to determine the effects of environmental factors on the parameters. Milk quality parameters (fat, protein, lactose and SCC) were tested by a spectrometer and a milk analyzer. Due to high variation among SCC numbers, the values were transformed to logarithmic scale ($\log_{10}$) to ensure homogeneity. The data were examined by one-way analysis of variance and means were compared with Duncan’s multiple comparison test. The mathematical model was as follows:

$$Y_{ij}=\mu+\alpha_i+e_{ij}$$

where: Y_{ijk}: is dependable variable *(parameters),*
μ: population mean,
α_i: effect of environmental factors and
e_{ij}: random residual.

Correlations among the investigated parameters were estimated by Kendall's tau-b phenotypic correlation analysis. All statistical works were performed using SPSS 17.0 for Windows.

Results and Discussion

In this study, effects of parity and SL on selected parameters are shown in Table 1. As seen, parity significantly (P<0.05) affected all parameters. While the lowest mean for UHS was calculated from the first parity cows, this level tended to elevate with later parities. This case may be commented with giving more attention to primiparous cows in the herds. Normally, cows with advanced parities are expected to give more milk yield *(Mikóné Jonás et al., 2016).* However, dMY means according to the parity groups showed an alternating distribution in the present investigation (Table 1). Such that, the lowest means were obtained both first and the latest parities. Actually, the means of the parity groups have not a big variation and nearby to overall mean (35.06 kg/d). As expected, the lowest and the highest logSCC means were calculated in the first and 5^{th} parity groups, respectively. This finding was found as harmonic with many study results *(Erdem et al., 2007; Atasever and Erdem, 2008; Mikóné Jonás et al., 2016).* Erosion in the udder glands of cows with advanced lactation might be the major reason for elevated SCC in that period. Fat and protein percentages showed a similar distribution and both markers reached to higher levels in the later parities. However, obtained fat and protein means might be assumed in acceptable thresholds for Holstein Fresian cows. In contrast, lactose percentage decreased with the advancing parity. Also, the lactose content of milk samples was found as suitable for Holstein cows.

It is clear that SL also affected all parameters except for UHS (Table 1). In spite of relatively lower UHS mean had been calculated from the first SL group, there was no significant difference among the groups. Attractively, similar results were obtained in dMY and logSCC by SL groups. Really, highest means were found for both parameters in the first SL. Really, the findings of *Mikóné Jonás et al. (2016),* who conducted an investigation in Hungarian conditions, supported these findings.

Table 1. Effects of environmental factors on the investigated parameters

Parity	n	UHS (point)	dMY (kg)	SCC (log10)	Fat (%)	Protein (%)	Lactose (%)
1	694	2.68±1.37[a]	33.70±7.44[a]	4.84±0.52[a]	3.93±0.63[a]	3.39±0.32[a]	5.06±1.17[c]
2	544	3.09±1.35[b]	36.02±11.38[b]	5.00±0.60[b]	3.95±0.72[a]	3.40±0.36[a]	4.93±0.20[b]
3	305	3.27±1.38[bc]	37.05±12.00[b]	5.11±0.60[c]	3.88±0.69[a]	3.38±0.31[a]	4.91±0.21[b]
4	127	3.38±1.35[c]	35.60±10.10[b]	5.16±0.55[c]	4.09±0.69[b]	3.44±0.34[b]	4.87±0.21[a]
5	113	3.47±1.38[c]	32.83±11.00[a]	5.30±0.58[d]	3.97±0.62[ab]	3.39±0.29[b]	4.86±0.28[a]
SL							
1	337	2.92±1.41	42.11±9.93[c]	5.05±0.19[a]	3.67±0.65[a]	3.10±0.25[a]	5.05±0.19[a]
2	564	3.06±1.40	38.45±8.62[bc]	4.99±0.19[b]	3.90±0.67[b]	3.33±0.29[b]	4.99±0.19[b]
3	882	3.01±1.38	30.19±8.48[a]	4.92±0.22[c]	4.06±0.65[c]	3.55±0.29[c]	4.92±0.22[c]
Total	1783	3.01±1.39	35.06±10.12	4.99±0.58	3.94±0.67	3.39±0.33	4.97±0.21

(UHS: udder hygiene score, dMY: daily milk yield, SCC: somatic cell count, SL: stage of lactation: 1= 11-99d, 2=100-199d and 3= ≥200d)

As expected, a lactating cow reach to peak milk production in the early lactation period and this level normally dropped within lactation persistency. At this period, higher SCC could be assumed as a normal physiologic case in dairy enterprises. In other words, postpartum stress and excessive attritions in the udder might be seen the main reasons for high SCC. In this study, fat, protein and lactose percentages had similar trends and levels of both parameters increased with later SL. Also, this result was found to be parallel to findings on three parameters by parity groups.

Table 2. Effects of UHS on milk parameters

UHS	n	dMY (kg)	SCC(log10)	Fat (%)	Protein (%)	Lactose (%)
1	286	33.59±10.49[a]	4.87±0.59[a]	4.06±0.77[c]	3.44±0.34[b]	4.99±0.22[b]
2	477	35.86±10.55[b]	4.94±0.57[ab]	3.99±0.69[bc]	3.41±0.33[b]	4.95±0.23[a]
3	345	35.26±10.15[b]	5.08±0.61[c]	3.93±0.68[b]	3.42±0.32[b]	4.96±0.22[ab]
4	278	33.56±9.57[a]	5.00±0.55[bc]	3.94±0.64[b]	3.41±0.35[b]	4.97±0.19[ab]
5	397	36.04±9.45[b]	5.04±0.57[c]	3.79±0.62[a]	3.31±0.31[a]	4.97±0.20[ab]
Total	1783	35.06±10.12	4.99±0.58	3.94±0.67	3.39±0.33	4.97±0.21

(UHS: udder hygiene score, dMY: daily milk yield, SCC: somatic cell count)

As seen from Table 2 that UHS affected ($P<0.05$) milk yield and composition. It was found that dMY showed a dispersed character. Also, a narrow variation among the means might be observed when compared to the overall mean (35.06 kg/d). However, the lowest mean was calculated from the first UHS group and the highest one was obtained from the latest UHS group. Actually, this finding apparently supported that cows with clean udder had lower SCC when compared to dirty ones. *Mitev et al. (2013)* emphasized that dairy cows with UHS 1 (clean udder) had the minimal SCC and these cows were assumed to be related to lower risk of mastitis disease. At this point, showing more attention to cow cleanness may especially be advised to dairy owners to achieve quality raw milk. In the study, relatively high fat, protein and lactose percentages were obtained from the UHS groups. This finding also points out to the importance of udder hygiene in lactating cows.

Table 3. Correlation coefficients among all parameters

Parameter	dMY	SCC	Fat	Protein	Lactose
UHS	0.029	0.078	-0.095	-0.097	-0.002
dMY		-0.237	-0.221	-0.353	0.147
SCC			0.077	0.162	-0.255
Fat				0.382	-0.087
Protein					-0.120

(UHS: udder hygiene score, dMY: daily milk yield, SCC: somatic cell count)

In this study, negative correlation coefficients of dMY with SCC, fat and protein were attractive (Table 3). Really, a negative association between dMY and fat or protein could be assumed to be a normal result. Nevertheless, keeping SCC within the minimal threshold should be seen a gold step to gain more and quality milk in dairy herds. Also, estimated negative (*r=-0.255*) between SCC and lactose supports this comment.

Conclusion

Finally, the present investigation shown that udder hygiene plays an important role on milk yield and composition ingredients.

Regarding multiple milk composition parameters and giving more effort to cow cleanness may be seen the major approaches for achieving quality and quantity milk and also selecting elite cows.

Odnos higijene vimena sa parametrima kvaliteta i kvantiteta mleka holštajn krava

Edit Mikóné Jónás, Savaş Atasever, Szabina Szilagyi

Rezime

Cilj ove studije bio je utvrđivanje veze između higijene vimena s parametrima kvaliteta i kvantiteta kravljeg mleka. Ukupno 1783 holštajn krave odgajane na privatnoj farmi u Segžvaru, u južnoj Mađarskoj, su bile muključene u ovo istraživanje. Da bi se dobil ocene higijene vimena (UHS) korišćen je sistem pet tačaka (1 označava apsolutno čisto a 5 označava veoma prljavo). Dok je dnevni prinos mleka (dMY) bio korišćen kao kvantitativni parametar, masti, proteini, laktoze i broj somatskih ćelija (SCC) su korišćeni kao kvalitetni parametri. Kao ne-genetski faktori, paritet i stadijum laktacije su uticali na sve parametre osim proteina i dMY, respektivno (P<0,05). Parametri su se razlikovali u UHS grupama (P<0.05) i UHS su imali negativne, ali nesignifikantne korelacione koeficijente sa mlečnom mastima, proteinima i laktozom. Savetuje se da se vrijednosti UHS prate pažljivo u mlečnim zapatima kako bi se dobilo odgovarajuće sirovo mlijeko.

Ključne reči: krava, higijena vimena, sastav mleka, broj somatskih ćelija.

References

ATASEVER S., ERDEM H. (2008): An investigation on the determination of mastitis risk levels and milk production traits in Holstein cows. Journal of Applied Animal Research, 34, 13–16.

ATASEVER S., ERDEM H., DEMİRYÜREK K. (2012): Association of some milking parameters with milk quality of smallholder dairy farms in Samsun region, Turkey. Journal of Environmental Biology, 33, 1, 123-126.

DEVRIES T.J., AARMOUDSE M.G., BARKEMA H.W., LESLIE K.E., von KEYSERLINGK M.A. (2012): Associations of dairy cow behavior, barn hygiene, cow hygiene, and risk of elevated somatic cell count. Journal of Dairy Science, 95, 10, 5730-5739.

ERDEM, H., ATASEVER, S., KUL, E. (2007): Some environmental factors affecting somatic cell count of Holstein cows. Journal of Applied Animal Research, 32, 173–176.

MEMISI N, BOGDANOVIC V, TOMIC Z, KASALICA A, ZUJOVIC M, STANISIC N, DELIC N (2011): Variability and correlation between basic quality parameters of raw cow milk. Biotechology in Animal Husbandry, 27, 959-967.

MÍKÓNE JÓNÁS E., ATASEVER S., GRÁFF M., ERDEM H: (2016): Non-genetic factors affecting milk yield, composition and somatic cell count in Hungarian Holstein cows. Kafkas Universitesi Veteriner Fakultesi Dergisi, 22, 3, 361-366.

MITEV J., GERGOVSKA Z., MITEVA T., VASILEV N., UZUNOVA K., PENEV T. (2013): Effect of the degree of udder contamination in dairy cows on the somatic cell count in milk. Journal of the Faculty of Veterinary Medicine Istanbul University, 39, 1, 76-83.

NEJA W., BOGOCKI M., JANKOWSKA M., SAWA A. (2016): Effect of cow cleanliness in different housing systems on somatic cell count in milk. Acta Veterinaria Brno, 85, 55-61.

SCHREINER D.A., RUEGG P.L. (2003): Relationship between udder and leg hygiene scores and subclinical mastitis. Jornal Dairy Science, 86, 11, 3460-3465.

STADNIK K., ATASEVER S. (2016): Influence of somatic cell count and body condition score on reproduction traits and milk composition of Czech Holstein cows. Indian Journal of Animal Research, (in press), DOI: 10.18805/ijar.11469

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

PARASITES CONTAMINANT OF GOAT MEAT

Ivan Pavlović[1], Snežana Ivanović[1], Boris Pisinov[1], Zsolt Becskei[2], Mila Savić[2], Danica Todorović[1]

[1] Scientific Veterinary Institute of Serbia, V.Toze 14, 11000, Belgrade, Republic Serbia
[2] Faculty of Veterinary Medicine, Bulevar oslobodjenja 18, 11000 Belgrade, Republic Serbia
Corresponding author: dripavlovic58@gmail.com
Review paper

Abstract. Goat meat consumed in all parts of the world because of its distinctive taste and desired chemical composition. This meat is rich in protein, vitamins and minerals; it contains very little fat, especially cholesterol and is healthier alternative compared to other types of red meat. The quality of the meat of goats is influenced by breed, gender, productivity and adaptability to stress, environment, management, nutrition, body weight at slaughter and health status as well as the slaughter and procedures with the body after slaughter. In addition, quality also affected by parasitic, bacterial and viral etiology contaminants, which may condition that the meat of these animals is unusable for human consumption and therefore should take care of them. In muscle and edible internal organs of small ruminants, we found adult and developmental forms of the parasite. In the muscles and organs of the most common encountered cyst of zoonotic protozoa *Toxoplasma gondii*. In the liver, we found *Fasciola hepatica* and *Dicrocelium dendriticum*. In the brain occurred *Coenuris cerebralis* (cyst form of *Multiceps multiceps*) Hydatid cysts (cyst stage of *Echinococcus granulosus*) we encountered by the liver, lungs, kidneys, muscles and other internal organs. Cysticercosis caused by *Taenia ovis* and *T.hydatigena* luck of the liver, omentum and muscle.

Their presence leads to a change in the organoleptic characteristics of the food and hygiene failure causing elimination of food from the market and therefore leads to major economic losses.

Key words: goats, meat, parasites, contamination, zoonoses

Introduction

Goats play an important role in many part of Serbia in providing animal protein for diet, especially for those people who live in village at mountains part of this area. Goats are milked and they produce the bulk milk supply, together with a large proportion of the meat that is consumed (*Ivanović and Pavlović, 2015*).

The quality of the meat of goats is influenced by breed, gender, productivity and adaptability to stress, environment, management, nutrition, body weight at slaughter and health status as well as the slaughter and procedures with the body after slaughter. In addition, quality also affected by parasitic, bacterial and viral etiology contaminants, which may condition that the meat of these animals is unusable for human consumption and therefore should take care of them.

Way of breeding of goats had prerequisite to many infections including parasitoses. They are usually kept under extensive conditions and graze or brows on any land that is not being cultivated (*Vlassoff et al., 2001, Ash and Truong, 2003, Pavlović et al.,2012a,b*). After harvesting, the animals are turned onto wheat and barley stubble from which they obtained nourishment. At last decade urban and peri-urban livestock, some households in cities around the world have hailed keeping as a source of livelihood. With the limited grazing spaces, urban farmers have opted for animals that require less space such as small ruminants and pigs. Further, that kind of the breeding of small ruminants (goats and sheep) increased during last decade worldwide. Pasture breeding make possible contact goat within eggs, larvae stages and intermediate host of parasites or ectoparasites like mange or ticks (*Truong and Baker, 1998; Truong et al., 2000; Pavlović et al., 2003a, b; 2012c*)

Those induce that there are no one goats without parasites. Their presence leads to a change in the organoleptic characteristics of the food and hygiene failure causing elimination of food from the market and therefore leads to major economic losses (*Jovanović et al., 2012*).

In our paper, we present the most important parasite species whose presence affects the quality of meat goats and leads to rejection of infected organs on the slaughter line, which affects the profitability of goat production. Besides, some parasitic disease gives rise to human diseases.

TOXOPLASMOSIS

Toxoplasmosis is parasitic zoonosis caused by protozoa *Toxoplasma gondii*. Primarily hosts was animals from familly Felidae and like intermediate hosts perzistant at 280 species of mammals, birds and reptile (*Acha and Szyfres,1989*). During human infection *T.gondii* was clasified with citomegolviruses and rubeola virus like most dengerous and most usually infection of pregnat women (*Pavlović and Ivanović,2005,2006a*).

Infection with *T.gondi* occured by ingestion of infected tisues or oocists of *T.gondii* (fecal contamination) and by congenital way. At intermediate hosts we have not enteral cystiforms part of development. Route of infection and free of sporozoites which intracelural replicated in enteral epithelium and belonging limphonoduls where formed tachizoites which via blood transported to tisues (brain,muscles,liver) where formet cysts which contants thousends of cystizoites and bradizoites. Those tisues was infective to definitive and intermediate hosts (*Kapperud et al.,1996*)

T.gondii was recognized as a singnificant cause of abortion of small ruminats. Abortion, stillbirths and neonatal mortality occur when susceptible goats are infected during pregnacy. Infection in early gestation leads to death and reasorption of the fetus and can be mistake to fertility (*Dubey,1990; Oréfice and Bonfiol, 2000*). Infection in midle gestation more likely to cause abortion and birth of weak kids, while infection in late gestation results in the birth of live, infected and imune kids (*Pavlović and Ivanović,2005*).At necropsy, macro pathological changes were most significiance at placenta – focal inflamation and necrosis of fetal cotyledon. In fetus we found changes in brain – focal chronically inflamation, unpurulent encephalitis and central calcification. At liver we found focal granulomatosis, at lung necrosis and intestinal miocarditis (*Dubey and Kirkbride, 1990; Tenter an et al., 2000*).

Today we have several methods to diagnosis T.gondii. Serological detection of antibodies in goats sera. High level of IgG occured immediatela afther infection and they persis several months. Necropsy and patholgical changes present reliable signs of toxoplasmosis and use in all suspected cases of infection. Finally, goats meat was examined by digestive methods to presence of cyst of toxoplasma (*Butko and Kostenko, 1983; Pavlović and Ivanović, 2006a).*

Helminths are the largest group of parasites of goats. Although infections with planthelminths are less frequent, related to gastrointestinal helminths, they can also cause serious health problems. This group presented fluke - fasciolosis, distomatosis and paramphistomiasis and larval forms of canidas tapeworm who generating cysts the internal organs of goats.

FASCIOLOSIS

Fasciola hepatica (liver fluke) is leaf-shaped trematoda measured 30 × 2–12 mm. The common liver fluke occurs worldwide but is particularly abundant in humid regions with temperate climate where it can be endemic (*Boray, 2007*). Adult flukes produce eggs in the biliary ducts of their hosts. Eggs are passed in the feces, and miracidia develop within in as little as 9–10 days. Hatching only occurs in water, and miracidia are short-lived and must infected lymnaeid snails at next 2-3 hour. In snail, started asexual development and multiplication occur through the stages of sporocysts, rediae, daughter rediae, and cercariae. Cercariae emerge from snails, encyst on aquatic vegetation, and become metacercariae (*Maingi et al., 1997*).

Metacercariae may remain viable for many months unless they become desiccated. The incidence of fasciolosis is highest in years when rainfall is above average during May–July (*Hurtrez-Bousses et al., 2001*). The epidemiology of liver fluke is often viewed as the result of two distinct cycles of snail infection and pasture contamination (*Mayberry and Casey, 2001*). After ingestion by the host, usually with herbage, young flukes excyst in the duodenum, penetrate the intestinal wall, and enter the peritoneal cavity, migrating through the liver tissues and crossing the wall of the bile ducts cause the major harm (*Pavlović et al., 2011a*) This process destroys the tissues and causes bleeding. Once in the biliary ducts they complete their development to adult flukes and start producing eggs.

Liver fluke disease in goats occurs in three main clinical forms – acute, subacute and chronic fasciolosis. Which form occurs depends on the numbers of infective metaceriae ingested and the period of time over which they are ingested. There are no typical and easily recognizable symptoms of a liver fluke infection. The major symptoms are related with the inflammation of the liver **(**hepatitis**)** and of the bile ducts **(**cholangitis**)** that can be also due to other disorders (*Hurtrez-Bousses et al., 2001; Mayberry and Casey, 2001*).

Acute fasciolosis caused by the sudden migration of many immature flukes through the liver, which leads to complete organ failure. It can develop in healthy animals that may be killed in a few days. Chronic fasciolosis is develops along the gradual establishment of adult flukes in the bile ducts. It is characterized by the progressive development of such symptoms as anemia edema often as "bottle jaw", digestive disturbances and cachexia**.** Goats do not appear to develop resistance to infection, and chronic liver damage is cumulative over several years (*Kagira and Kanyari, 2001, Pavlović et al., 2011a*).

DICROCELIOSIS

Dicrocoelium dendriticum, the lancet liver fluke, is slender 6–10 mm long × 1.5–2.5 mm wide parasites. They have an_indirect life cycle with two intermediate hosts, **a** snail **(***Theba* spp, *Zebrina* spp, or *Cionella* spp**)** and an ant. The eggs shed by adult flukes reach the host's gut with the bile and are expelled with the feces (*Kagira and Kayari, 2001; Manga-Gonzàlez et al., 2003*). Once outside the host terrestrial snails ingest the eggs. Inside the snails young miracidia hatch out of the eggs, develop to sporocysts**,** which on their turn multiply asexually, producing daughter sporocysts which can produce up to cercariae that it remains inside the snail. The snail encysts these cercariae and expels them in the form of sticky slime balls that adhere to the vegetation. Ant (species *Formica* spp or *Lasius* spp) second intermediate host**,** ingested slimeballs of cercariae (*Alunda and Rojo-Vàzquez, 1983; Sotiraki et al. 2007; Pavlović et al., 2012d*). Inside the ants, most cercariae continue their development to metacercariae in the abdominal cavity. One or two metacercariae in the subesophageal ganglion of the ant cause abnormal behavior in which the ants climb up and remain on the tips of the herbage where they attach themselves, which increases the probability of ingestion by the definitive host.

Once ingested, the metacercariae excyst in the small intestine, migrate up the main bile duct, and then on to smaller ducts (*Mayberry and Casey, 2001; Palić, 2001*). Severe pathologic changes occur within the liver and bile ducts, including cirrhosis, abscesses, and granulomas. Clinical signs are not obvious but may be seen in massive infections (*Pavlović et al., 2012d; Ivanović and Pavlović, 2015*). *D.dendriticum* is part of a group of flukes that can infect the bile ducts of humans.

COENUROSIS

Coenurosis is a disease of the brain and spinal cord caused by the intermediate stage of *Taenia multiceps.* Adults tapeworms occur in the small intestine of dogs and wild carnivores, and can reach lengths of up to 1 metre. (*Pavlović, 1994, Pavlović et al., 2008; Cirović et al., 2015a,b*).

When the proglottides burst, the eggs are disseminated in the environment contaminating pastures and water supplies. The intermediate host (sheep) ingests eggs expelled with dog faeces. The *larvae* hatch in the intestine and pass with the blood stream towards different organs. The larvae, which reach the brain and spinal cord, grow to the *coenurid* stage. Coenurus cerebralis will further mature in the brain and spinal cord (*Welchman and Bekr-Ochir, 2006; Oryan et al., 2014*). Only those parasites, which reach the nervous system, will develop into a fully developed Coenurus in 7 to 8 months. The definitive host (dog) gets infected by eating infected sheep tissue.

At acute infection in lambs may occur meningoencephalitis because of migration of large numbers of immature stages of this parasite. The chronic stages develop because of increased destruction of brain and spinal cord tissue as the Coenurus grows. The neurological clinical signs are recognised as "gid" or "staggers" and are dependent on the location of the cyst in the central nervous system (*Giadinis et al., 2005; Nourani and Kheirabadi, 2009; Polizopoulou et al., 2016*). Sheep at the chronic stage of the infection may show circular movements, jerky movements or staggering gait (*Pavlović et al., 2017*).

HYDATIDOSIS

Hydatid disease is an infection caused by tapeworms of the genus *Echinococcus*, a tiny tapeworm just a few millimeters long. They reproduce releasing eggs into the environment in the faeces of the host animal (*Constantine et al., 1993*). Like all tapeworms, the life cycle involves two animals. A carnivore is the definitive host – where the adult worms live in the intestines – and almost any mammal, including humans, can be the intermediate host - where the worms form cysts in various organs (*Pavlović and Ivanović, 2006b; Pavlović et al., 2011b).*

The intermediate host ingests the eggs incidentally while grazing, foraging or drinking. The eggs hatch in the small intestine, become larvae, which penetrate the gut wall, and are carried in the circulatory system to various organs. There the cysts, called hydatid cysts or metacestodes, are formed (*Thompson and Lymbery, 1995*). The disease symptoms are caused by the cysts, which are slow growing fluid-filled structures that contain the larvae and are most often located in the liver or lungs. Called hydatid cysts, for *E. granulosus*, they act like tumours that can disrupt the function of the organ where they are found, cause poor growth, reduced production of milk and meat, and rejection of organs at meat inspection (*Jiménez et al.,2002; Roming, 2005*).

However, the cysts grow slowly so that many infected animals are slaughtered before the cysts ever cause disease problems. There may however be multiple cysts of *E. granulosus* which can also occur in the brain, kidneys, bones,or testes causing more severe illness. Without control measures, infection rates can be very high in livestock and dogs, with associated signifi cant incidence in humans (*Pavlović and Ivanović, 2006b; Brunetti and Filice, 2008*).

CISTICERCOSIS

Cysticercosis is a disease caused by the larval forms of tapeworm species of which commonly encountered by *Taenia hydatigena* and *T.ovis*.

Taenia hydatigena

This tapeworm lives in the small intestine of dogs, foxes and other carnivores and was long 1.5-5 m (*Pavlovic, 1994; Pavlovic et al., 2008).* In mature targument are eggs that excreted into the environment. Infection occurs with parasite eggs. In the gastro-intestinal tract of intermediate host oncospheres eggs, penetrate the intestinal wall and get into the peritoneal cavity (*Pathak et al., 1982*). Then occur bleeding on the surface of the liver and then in the parenchyma. In these channels leads to the destruction of the liver parenchyma, the accumulation of fibrin and red blood cells and the developing worm-like larvae that for a couple of weeks coming to the surface of the liver. Hence the switch to omentum and mesentery, where end development. In addition to liver and mesentery, larvae encountered other organs, most in lungs (*Manfredi et al., 2006*). There the larvae are fixed and form cysts known as *Cysticercus tenuicollis*. These cysts are long neck, retracted in a vesicle diameter of about 3 cm (and larger). Harmful operation ovh cyst is the most common mechanical nature - caused by damage to blood vessels during migration through the liver and the resulting inflammation that can spread to the peritoneum and cause peritonitis. In the abdominal cavity found bloody content. In acute inflammation, the liver are present fibrin deposits and at the intersection to see the ducts (*Blazek et al., 1985*). The lungs are lucky emphysema and bleeding in the lung parenchyma. The thoracic cavity is serofibrinozna fluid and pleural edema. The disease is usually chronic course.

Taenia ovis

T. ovis lives in the small intestine of dogs, foxes and other carnivores and the debt is up to 5m (*Soulsby, 1977*). After infection, the eggs of parasites in the intestines of small ruminants are released embryos that penetrate the intestinal wall and the bloodstream to muscle tissue where they develop cystic formations (*Gregory 1976*). Most often infected myocardium, diaphragm, skeletal muscles and chewing. (*Coman and Rickard, 1975; DeWolf et al., 2013*). The cysts are developing larvae for 46 days become infectious for real hosts. Pregnant animal is possible intrauterine infection of the fetus.

Over time, the cysts of the muscles degenerate, and then calcifying caseous nodules formed structure. This is the stage of the disease is in small ruminants also known as "chicken pox" because it looks like pure muscle that is similar to pustular changes with smallpox (although nothing to do with real uzročnicma goddess - pox virus) (*Ericson, 2011*). T. ovis not pathogenic for small ruminants and does not constitute a risk to human health, but these calcified cysts have unpleasant looks as mentioned above and lead to the rejection of large quantities of sheep meat especially in Britain, Australia and New Zealand where disease are most present.

Conclusion

Parasitic infections produced great losses to goat production. In addition to direct damaging through the operation of reduced growth, milk yield, and the concept of these diseases affect the quality of meat of the skin as well as the rejection of the infected organs from slaughter line.

However, some forms of food borne parasite development (*Toxoplasma gondii*) are retained in the animal tissue and cause infection in humans who eat inadequately cooked meat and organs of infected animals.

Their presence leads to a change in the organoleptic characteristics of the food and hygiene failure causing elimination of food from the market and therefore leads to major economic losses.

Parazitska kontaminiranost mesa koza

Ivan Pavlović, Snežana Ivanović, Boris Pisinov, Zsolt Becskei, Mila Savić, Danica Todorović

Rezime

Meso koza se konzumira u svim delovima sveta zbog svog jedinstvenog ukusa i poželjnog hemijskog sastava. Ovo meso je bogato proteinima, vitaminima i mineralima, sadrži veoma malo masti pogotovuholesterola i mnogo ej zdravijeg sastava od većine drugih vrsta mesa. Kvalitet mesa zavisi od načina držanja, pola životinja, produktivnog i adaptibilnog stresa, životnih uslova i načina držanja, ishrane, težine i zdravstvenog stanja. Na kvalitet mesa utiče i način klanaj i postupak sa mesom po klanju. Na kvalitet uiče i prisustvo parazitskih, bakterijskih i virusnih kontaminenata koje mogu učiniti meso koza neupotrebljivim za ljudsku ishranu.

U mišićima i jestivim organima malih preživara može se naći ciste zoonotske protozoe *Toxoplasma gondii*. U jetri, mogu se naći odrasli i razvojni oblici *Fasciola hepatica* i *Dicrocelium dendriticum*. U mozgu se mogu naći ciste *Coenuris cerebralis* (cistični oblik *Multiceps multiceps*). Hidatidne (ehinokokne) ciste se sreću po jetri, plućima, bubrezima, u mišićima i po drugim organima. Konačno tu su i cistični oblici *Taenia ovis T.hydatigena* koje nalazimo na omentumu i jetri.

Nalaz ovih parazita menja organoleptička svojstva mesa i organa čineći ih neupotrebljim za ishranu i preradu što dovodi do značajnih ekonomskih gubitaka.

Ključne reči: koze, meso, paraziti, kontaminacija, zoonoze

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053 and TR 31085

References

ACHA P., SZYFRES B. (1989): Zoonoses et malades transmissible communes a l homme et animaux, OIE, Paris. pp39.
ALUNDA J., ROJO-VÀZQUEZ F. (1983): Survival and infectivity of Dicrocoelium dendriticum eggs under field conditions in NW Spain. Veterinary Parasitology 13, 245-249.
ASH R., TRUONG P. (2003): The use of vetiver grass wetland for sewerage treatment in Australia. Proceeding of the International Conference on Vetiver and Exhibition, October 6-9, Guangzhou, 20.
BENNER C., CARABIN H, SÁNCHEZ-SERRANO L.P, BUDKE C.M., CARMENA D. (2010): Analysis of the economic impact of cystic echinococcosis in Spain. Bulletin of the World Health Organization 88, 49-57.
BLAZEK K, SCHRAMLOVA J, HULINSKA D. (1985): Pathology of the migration phase of *Taenia hydatigena* (Palas 1766) larvae. Folia Parasitologica 32, 127–137.
BORAY J.C. (2007): Liver fluke disease in sheep and cattle. Primefact 446.
BUDKE CM, DEPLAZES P, TORGERSON PR. (2006): Global socioeconomic impact of cystic echinococcosis. Emerging Infectiouse Disease 12, 296-303.
BUTKO, M.P.,KOSTENKO J.B (1983): Rukovodstvo po veterinarskoj ekspertizi mjesnih produkov. Legkaja pišćeva promišlenost, Moskva, pp.56.
COMAN B.J., RICKARD M.D. (1975): The location of Taenia pisiformis, Taenia ovis and Taenia hydatigena in the gut of the dog and its effect on net environmental contamination with ova. Zanderblat fur Parasitekund 47, 237-248.
CONSTANTINE C.C., THOMPSON R.C.A., JENKINS D.J., HOBBS R.P., LYMBERY A.J. (1993): Morphological characterisation of adult Echinococcus granulosus as means of determinating transsmition paterns. Journal of Parasitology 79, 57-61.
CIROVIC D.S., PAVLOVIC I.N., PENEZIC A.Z., KULISIC Z,B., SELAKOVIC S. (2015a): Levels of infection of intestinal helminths species in the golden jackal Canis aureus from Serbia. Journal of Helminthology 89 (1), 28-33.
ĆIROVIC, D., PAVLOVIĆ, I., PENEZIĆ A. (2015b): Intestinal helminth parasites of the grey wolf (Canis lupus L.) in Serbia. Acta Veterinaria Hungarica 63(2),189-198.
DeWOLF D.B., POLJAK Z., ANDREW S. PEREGRINE S.A., JONES-BITTON A., JANSEN T.J., MENZIESP.I. (2013): Development of a Taenia ovis transmission model and an assessment of control strategies. Veterinary Parasitol 198 (1-2), 127-135.

DUBEY, J.P., KIRKBRIDE C.A. (1990): Toxoplasmosis and other causes of abortion in sheep from North central United States. Journal of American Veterinary Medicine Association 196, 287 -290.

ERICKSON A. (2011): Taenia ovis (Sheep measles) infection in sheep Department of Agriculture and Food, Kesington,Western Australia, pp1-2.

GIADINIS N.D., PANOUSIS N.K., POLIZOPOULOU Z.S., KARATZIAS H. (2005): Neurological examination of small ruminants. Jpurnal of Hellen Veterinary Medicine Society 57, 11-18.

GREGORY G.G. (1976): Fecundity and proglottid release of Taenia ovis and T. Hydatigena.Australian Veterinary Journal 52 (6), 277-279.

HURTREZ-BOUSSES S., MEIJNIER C., DURAND P., RENAUD F. (2001): Dynamics of host-parasite interactions: the example of population biology of the liver fluke (Fasciola hepatica). Microbes and Infections 3, 841-849.

IVANOVIĆ S., PAVLOVIĆ I. (2015): Meso koza-bezbedna namirnica. Naučni institut za veterinarstvo Srbije,Beograd, pp.75-131, 186-194.

JIMÉNEZ S, PÉREZ A, GIL H, SCHANTZ P, RAMALLE E, JUSTE R. (2002): Progress in control of cystic echinococcosis in La Rioja, Spain: decline in infection prevalences in human and animal hosts and economic costs and benefits. Acta Tropica 83, 213-221.

JOVANOVIĆ L., PEŠIĆ-MIKULEC D., PAVLOVIĆ I, (2012): Granski standardi kvaliteta i bezbednosti u prehrambenoj industriji i primarnoj proizvodnji : upravljanje rizicima u proizvodnji i distribuciji hrane. Naučno-stručno društvo za zaštitu životne sredine Srbije "Ecologica", Beograd, pp.137.

KAGIRA J.M., KANYARI P.W.N. (2001): The role of parasitic diseases in causing mortalities in small ruminants in a highly productive area of Central Province, Kenya. Journal of South Africa Veterinary Association 72, 147-149.

KAPPERUD G, JENUM P.A.,STRAY-PEDERSEN B.,MELBY K.K.,ESKILD A., ENG J. (1996): Risk factors for Toxoplasma gondii infection in pregnancy. Results of a prospective case-control study in Norway. American Journal of Epidemiology 144, 405–412.

MAINGI N., GICHOHI V.M., MUNYUA W.K., GATHUMA J.M., THUMSBORG S.M. (1997): The epidemiology of nematode and liver fluke infections in sheep in Nyandarua district of Kenya. Bulletin of Animal Health and Production 45: 27-34.

MAYBERRY C., CASEY R. (2001): Stockguardsheep and goats strategic assessment –liverfluke. Department of Agriculture and Food, Kesington,Western Australia, pp1-13.

MANGA-GONZALEZ, M., GONZÀLEZ-LANZA C., CABANAS E., CAMPO R. (2003): Contributions to and review of dicrocoeliosis, with special reference to the intermediate hosts of Dicrocoelium dendriticum. Parasitology 123, 91-114.

MAS-COMA S. (2005): Epidemiology of fascioliasis in human endemic areas. Journal of Helminthology 79, 207-216.

MANFREDINI M.T., GHIRARDELLI R., ZANZANI S.(2006): Cysticercus tenuicollis infection in a goat farm. Parassitologia 48(3), 433-436.

NOURANI H., KHEIRABADI K.P. (2009): Cerebral coenurosis in a goat: pathological findings and literature review. Compared and Clinical Pathology 18, 85–87;

ORÉFICE F., BONFIOL A.A. (2000): Toxoplasmose In: Oréfice F, editor. Uveíte clínica e cirúrgica- Rio de Janeiro: Editora Cultura Médica; pp 619–680.

ORYAN A., AKBARI M., MOAZENI M., AMRABADI O.R. (2014): Cerebral and non-cerebral coenurosis in small ruminants. Tropical Biomedicine 31(1),1-16.

PALIC D. (2001): Bolesti koza, Grafos internacional,Pančevo, pp.122.

PATHAK K.M.L., GAUR S.N.S., SHARMA S.N. (1982): The pathology of Cysticercus tenuicollis infection in goats. Veterinary Parasitology 11(2-3),131-139.

PAVLOVIĆ, I. (1994): Helmintofauna lisica (Vulpes vulpes L.) ulovljenih na području Beograda u periodu 1988-1992.godine. Doktorska disertacija. Univerzitet u Beogradu Fakultet veterinarske medicine.

PAVLOVIĆ I., IVANOVIĆ S. (2005): Toxoplasmosis of goats and its role and importance of goat production. Biotechnology in Animal Husbandry 21 (5-6), 123-126.

PAVLOVIĆ I., IVANOVIĆ S. (2006a): Toksoplazmoza.Naučni institut za veterinarstvo Srbije i Ministarstvo poljoprivrede, šumarstva i vodoprivrede, Beograd, pp33.

PAVLOVIĆ I., IVANOVIĆ S. (2006b): Ehinokokoza/hidatidoza,bolest životinja i ljudi, izd. Naučni institut za veterinarstvo Srbije i Ministarstvo poljoprivrede, šumarstva i vodoprivrede, Beograd, pp33.

PAVLOVIĆ I., TAMBUR Z.,DODER R., KULIŠIĆ Z.,JAKIĆ-DIMIĆ, D. (2008) The cestodes of the foxes (Vulpes vulpes L.) catched in Serbia in the paeriod 1994-2006. Veterinaria 57 (1-2), 100-108.

PAVLOVIĆ I., IVANOVIC S., ŽUJOVIĆ M.,TOMIĆ Z., MEMIŠI N. (2011a): Fascioloza koza u Srbiji. Zbornik naučnih radova Instituta PKB Agroekonomik 17 (3-4),163-169.

PAVLOVIĆ, I., HADŽIĆ I., ŽUGIĆ G., ANĐELIĆ-BUZANIĆ G.,VAIĆ D., JOVČEVSKI SR. (2011b): Hidatidoza aktuelni problem stočarske proizvodnje. Zbornik naučnih radova Instituta PKB Agroekonomik 17 (3-4), 133-139.

PAVLOVIĆ I., IVANOVIĆ S., ŽUGIĆ G., JOVČIĆ D., BOJKOVSKI J, PAJIĆ M. (2012a): Season distribution of gastrointestinal. helminths of small ruminants in spread Belgrade area Lucrări Ştiinţifice Medicină Veterinară Timişoara XLV(3), 155-160.

PAVLOVIĆ I., IVANOVIĆ S., JOVIČIĆ D., ŽUGIĆ G., JOVČEVSKI SR., JOVCEVSKI ST. (2012b): Gastointestinal helmints of goats breeding at Stara Plana area(Serbia). Book of Abstracts V International Symposium of Livestock Production, September 5-7, Skopje, Macedonia,144.

PAVLOVIĆ I., IVANOVIĆ S., ŽUJOVIĆ M., TOMIĆ Z. (2012c): Influence of gastrointestinal helminths to goat health and production. Proceedings of 6th Central European Congress on Food, May 23-26, Novi Sad, Serbia, 1605-1607.

PAVLOVIĆ I., IVANOVIĆ S., SAVIĆ B., BOJKOVSKI J., HADŽIĆ I., ANĐELIĆ-BUZADŽIĆ G., JOVČEVSKI SR., ŽUGIĆ G., JOVČEVSKI ST.(2012d): Značaj dikrocelioze u zdravstvenoj problematici koza i ovaca**.** Zbornik naučnih radova Instituta PKB Agroekonomik 18 (3-4), 163-169.

PAVLOVIĆ I. IVANOVIĆ S. BOJKOVSK J. JOVČEVSKI SR. JOVČEVSKI ST. ROGOŽARSKI D. DOBROSAVLJEVIĆ I. HADŽIĆ I. MINIĆ S. (2017): Cenuroza malih preživara. Zbornik naučnih radova Institut PKB Agroekonimk 23 (3-4), 51-57.

POLIZOPOULOU S.Z., GIADINIS. D.N., PAPAHRISTOU. A..PAPAIOANNOU. N. (2016)**:** Neurological diseases of small ruminants in Greece: a retrospective study in 114 flocks. Acta Veterinaria-Beograd, 66 (2), 160-171.

SOTIRAKI S., LEONTIDES L., HIMONAS C. (2007): The effect of transportation and confinement stress on egg production by Dicrocoelium dendriticum in sheep. Journal of Helminthology 73, 337-339.

SOULSBY E.J.L. (1977): Helminth, Arthropod and Protozoa of Domesticated Animals. Bailler,Tindall and Cassell, London, pp.237.

TENTER A.M., HECKEROTH A.R.,.WEISS L.M (2000): Toxoplasma gondii: from animals to humans. International Journal of Parasitology 30, 1217–1258.

THOMPSON R.C.A:, LYMBERY A.J (1995): Echinococcus and hidatid disease. CAB International, Biddles Ltd. Guildford, pp75.

TRUONG, P.N., BAKER, D. (1998): Vetiver grass system for environmental protection. Technical Bulletin N0. 1998/1. Pacific Rim Vetiver Network. Royal Development Projects Board, Bangkok, Thailand, 45.

TRUONG P.N., MASON F., WATERS, D., MOODY P. (2000): Application of vetiver Grass Technology in off-site pollution control. I. Trapping agrochemicals and nutrients in agricultural lands. Proceeding of Second International Vetiver Conference, Januar 18-22, Bangkok, Thailand, 20.

VLASSOFF A., LEATHWICK D.M., HEATH A.G.C. (2001): The epidemiology of nematode infections of sheep. New Zeland Veterinary Journal 49, 213-221.

WELCHMAN, D,DE B., BEKR-OCHIR, G. (2006): Spinal coenurosis causing posterior paralysis in a goat in Mongolia. Veterinary Record, 158, 238–239.

POSTER SECTION I

EFFECT OF BODY CONDITION AT CALVING ON LACTATION CURVE OF HOLSTEIN COWS

Savaş Atasever[1], Szabina Szilágyi[2], Myrtill Gráff[2], Edit Mikóné Jónás[2]

[1]Department of Animal Science, Faculty of Agriculture, University of Ondokuz Mayis, 55139-Samsun, Turkey
[2]Department of Animal Husbandry, Faculty of Agriculture, University of Szeged, 6800, Szeged, Hungary
Corresponding author: Edit Mikóné Jónás mikone@mgk.u-szeged.hu
Original scientific paper

Abstract: The objective of this study was to present the role of body condition scoring system in dairy management. The main focus was the impact of calving BCS on milk production. We collected 5197 test day milking data. The cows were grouped according to the BCS at the calving ($BCSc_{2.0}$, $BCSc_{2.5}$ $BCSc_{3.0}$ and $BCSc_{>3.0}$). The data of different BCS grouped cows was analysed using Wood-function. The least amount of milk was produced by the cows with $BCSc_{2.0}$. At the beginning of lactation the highest amount of milk was predicted by cows with the largest BCS at calving (32.617 kg). However, after the first month of lactation the production of this group decreased spectacularly. The higher coefficient of determination (R^2=0.322) was predicted the cows with $BCSc_{>3.0.}$ Considering all the results we could conclude that at the beginning of the lactation the cows used up the accumulated fat reserves for the production needs, but later as these fat reserves disappear and the milk production decreases. It is demonstrated that the integration of the regular body condition scoring into the technology is a useful solution.

Key words: Body Condition, lactation curve estimation, milk production

Introduction

Professional feeding of dairy cows is a very important target based on the economic and animal health reasons. Feed intake is characterized as dry matter intake (DMI) to compare diets of variable moisture concentrations. DMI is affected by both animal and feed factors. The weight of cows, the milk production, and the stage of lactation or gestation are the major animal factors.

In practice, the grouping of cows in nutritional groups is based on milk production, reproductive cycle and the days spent in milking. The cows are usually grouped monthly, after milk recording. The using of body condition scoring system is more useful than measurement of body weight (BW) in the feeding. The value of BCS is much less dependent on the size of animal than BW. The body weight in cows is affected by the size of animal, the status of pregnancy stage of lactation and the fed up. The variance of BW is higher than variance of BCS, *Halasz and Jonas (2014*) studied 2572 body weight data of Holstein cows the extreme values varied between 450 and 915 kg The results of the relationship examination between body condition and milk production (*Domecq et al., 1997; Dillon et al., 2003; Wathes et al., 2007*) are different. A number of studies did not confirm relationship between body condition and milk production (*Markusfeld et al., 1997; Heuer et al., 1999; Berry et al., 2002). Kadarmideen (2004)* found a negative (range, –0.50 to –0.39) genetic correlations of BCS with milk, fat and protein yield. Several authors (*Waltner et al., 1993; Ruegg and Milton, 1995; Bewley and Schutz, 2008*) refer to the research by *Frood and Croxton (1978*). Their publications confirmed that the cows lean at calving (with BCS <2) produced below their potential milk yield whereas those calving with BCS above 2.5 produced according to their expected potential milk yield. According to *Domecq et al. (1997)* an increase in BCS during the dry period resulted in an increase in milk yield and the milk -yield –acceleration after calving. According to *Akbar et al. (2016)* the calving body condition score is an important determinant of early-lactation dry matter intake, milk yield, and disease incidence. Body condition score at calving and nadir as well as loss of body condition score between calving and nadir has significant effects on milk production, which also reflects in body weight change (*Roche et al., 2007*). According to the study of *Pedron et al. (1993)* and *Ryan et al. (2003)* the cows with the higher BCS at calving had a higher milk yield in early lactation than those with the lower BCS. *Samarütel et al. (2006)* divided the cows into three groups based on their BCS at calving (thin: BCS <3.0; moderate= BCS 3.25-3.5 and fat: BCS >3.75). In they study it was found that the fat cow group had higher fat corrected milk (FCM) production during the 305-day lactation period. *Markusfeld et al. (1997)* report that the effect of BCS at calving on milk fat content is most pronounced in the first 90 days of lactation.

The BCS and the changes in BCS during the dry period also affect milk yield. *Contreras et al. (2004*) estimated the BCS at the end of the dry period, and they found that the cows with BCS ≤3.0 produce more milk than cows with BCS ≥3.5 during early lactation. *Stockdale (2005)* also observed in his experiment that the milk production was better and the milk fat content was higher in case of the cows with higher BCS at calving. The increased body fat reserves mobilisation in the lactation results in decreasing BCS, which is typically accompanied by high milk production (*Roesch et al., 2005; Bines, 1976; Hart et al., 1979).* It is generally observed that the cows with high milk yield have a greater BCS loss during the lactation than the lower producers (*Gallo et al., 1996; Cutullic et al., 2009).* This process may be related to the fact that there are differences in energy intake and milk production during the lactation.

The objective of this study was to present the role of body condition scoring system in dairy management. The main focus was the impact of calving BCS on milk production. The data of different BCS grouped cows was analysed using Wood-function, which is the most favorable model to fitting the observed daily milk yield. According to *Jeretina et al. (2013)* the Wood function provides good flexibility in fitting the estimated curve to the measured values. This opinion agrees with that of *Boujenane et al. (2012*) who fitted four mathematical functions (Wood, Wilmilk, Gao and Swalve and Ali and Scheffer). They concluded that the Wood function was observed to be the most suitable.

Materials and methods

The study was conducted in a private dairy farm in South- Hungary. A total of 5197 records of Holstein cows from 1 to 10 lactation were evaluated. The cows were monthly recorded by BCS using a 5-grade scoring system, which describes 1 point is emaciated and 5 points refer to an obese cow, and to achieve more sensitivity, 0.50 points were also used. BCS was determined the weeks of milk recording by the same person using a scale according to Edmonson et al. (1989). Additionally, cow test day milk volume was recorded all month on the farm. A total of 5197 data milk test record from 585 cow were collect to the analysis. Monthly milk yield was calculated order in the test day recording during the first 12 months of lactation. The data of measurements was recorded by dairy farm management software.

When making the groups according to the BSC at calving (BCS_c) we made 5 groups ($BCS_{c2.0}$; $BCS_{c2.5}$;. $BCS_{c3.0}$; and $BCS_{c>3.0}$).

The statistical analyses were performed using SPSS 18.0 for Windows. The data were tested by the non linear regression using the Wood incomplete gamma function (Wood, 1967) with the following equation:

$$Y_t=at^b exp^{-ct}.$$

where Y_t is the average daily milk yield in the t month of lactation, a is the initial milk yield after calving, b is the ascending slope parameter up to the peak yield, and c is the descending slope parameter.

The differences between the milk yield of the BCS*c* groups were tested by analysis of variance (One-Way ANOVA). The means were compared by Duncan's multiple range test based on the 0.05 level of probability and all statistical analyses were performed using SPSS 18.0 for Windows.

Results and discussion

The descriptive statistics for test day milk production are presented by BCSc in Table 1. Mean daily milk production per cow was 24.39 kg (S.D. = 8.07 kg, maximum 50.8 kg, minimum 4 kg). The least amount of milk was produced by group of $BCS_{c2.0}$ the average daily milk production of $BCS_{c2.0}$ was significantly less than for all other group ($P < 0.005$). Based on the BCS at calving the two moderate BCS groups produced the most.

Table1. Summary statistic for test day milk yield (DMY) according to BCS at calving

BCSc	$BCS_{c2.0}$	$BCS_{c\ 2.5}$	$BCS_{c\ 3.0}$	$BCS_{c\ >3.0}$
N	212	3330	1225	430
Mean	20.97^{a}	24.62^{b}	24.52^{b}	23.83^{b}
Median	21.30	25.20	24.80	23.60
Std. Deviation	7.82	7.90	8.42	8.17
Range	40.10	42.70	45.50	43.20
Minimum	5.50	5.30	5.30	4.00
Maximum	45.60	48.00	50.80	47.20

a,b letters with the same superscripts in rows do not differ significantly ($p < 0.005$)

Our results are in agreement with previously reported studies. Despite of that *Jílek et al. (2008)* demonstrated no significant relationship between BCS before calving and milk yield, cows with BCS lower than 3.5 points in the first month of lactation showed the highest milk yield during the first 5 months of lactation. In fact, in a previous study, *Samarütel et al. (2006)* reported that cows calved in BCS classified thin could not realize their genetic potential for milk production due to lack of body reserves.

In the Table 2. the lactation curve parameters are presented, with parameters of Wood's curve. The peak milk yield was observed in the $BCS_{c>3.0}$ (32.617) while minimum yield was observed in the group of $BCS_{c2.0}$. Lactation curve parameter up to peak yield (b) was similar in three group ($BCS_{c2.0}$, $BCS_{c2.5}$ and $BCS_{c>3.0}$)

Table2. Wood function parameters for test day milk yield according to BCS at calving

BCS at calving	Wood function parameters		
	a	b	c
$BCS_{c2.0}$	27.117	0.105	0.067
$BCS_{c2.5}$	29.896	0.186	0.085
$BCS_{c3.0}$	32.189	0.111	0.076
$BCS_{c>3.0}$	32.617	0.119	0.066

Table3. Phenotypic correlation between lactation curve parameters (Pearson coefficiens)

$\mathbf{BCS_{c2.0}}$	**b**	**c**	$\mathbf{BCS_{c2.5}}$	**b**	**c**
a	-0.541	-0.251	**a**	-0.487	-0.196
b		0.932	**b**		0.936
$\mathbf{BCS_{c3.0}}$	**b**	**c**	$\mathbf{BCS_{c>3.0}}$	**b**	**c**
a	-0.485	-0.189	**a**	-0.492	-0.199
b		0.933	**b**		0.933

Correlation coefficients between lactation curve parameters in different calving BCS are presented in Table 3. The results agree with the findings *of Gradiz et al. (2009)* the negative correlation between *a* and *b* factors it means that higher yields at the beginning of lactation is associated with lower rate of increase to peak yield. The positive, strong correlation between *b* and *c* indicated that cows which reach the peak of lactation more rapidly also have a more rapidly decline from the peak. In the present study the strongest correlation was observed between *a* and *b* parameters the case of $BCS_{c2.0}$ (-0.541), however the values were quite in same all cases.

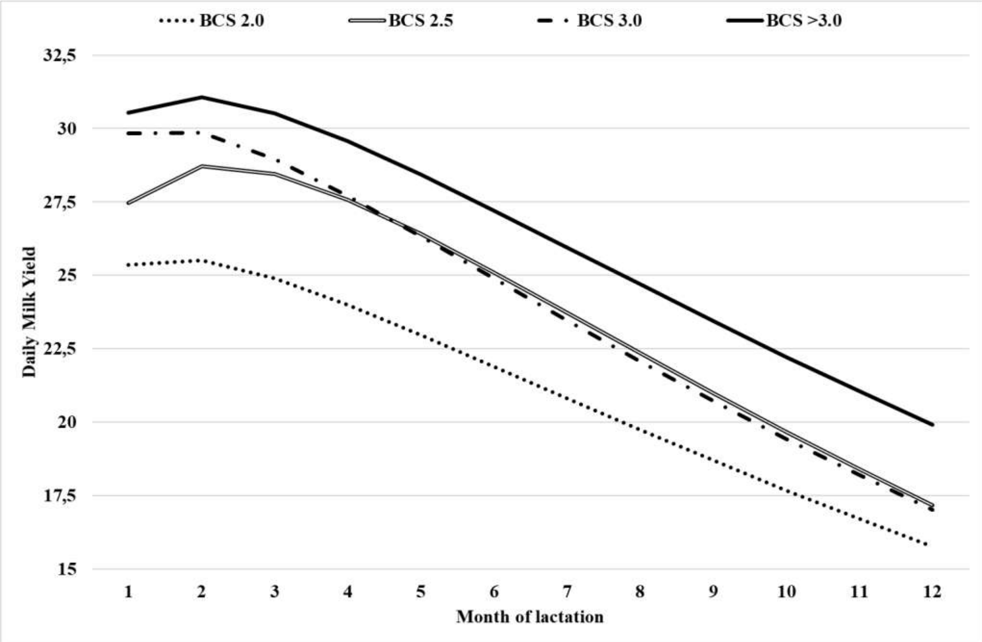


Figure 1. The fitted lactation curves (by Wood function) of cows with different BCS at calving.

The calving BCS wise estimated lactation curve (Fig1.) parameters indicated higher average initial milk yield (a) in cows with $BCS_{c3.0}$ and $BCS_{c>3.0}$ as $BCS_{c2.0}$ and $BCS_{c2.5}$. The peak milk yield was observed in the group $BCS_{c>3.0}$ while minimum milk production was observed in cases of BCS c2.0 (27.117 kg). Lactation curve parameters ascending slope parameter up to peak yield (b) was similar in two groups ($BCS_{c3.0}$ and $BCS_{c>3.0}$), declined the $BCS_{c2.0}$ (0.105) and increased in the BCSc2.5. The descending slope parameter (c) was lower in case of $BCS_{c2.5}$ and $BCS_{c>3.0}$ (0.066 and 0.067) and higher in the $BCS_{c3.0}$ (0.076) and maximum in the $BCS_{c2.5}$(0.085).

The coefficients of determination (R^2) ranged from 0.23 to 0.32, the average value was 0.27. The level of accuracy was different as reported in previous studies *Gradiz et al. (2009)* and *Jingar et al. (2014)* where were characterized a high value of determination coefficient (0.85 and 0.98).

Present study conclusion was also consistent with the results presented by *Roche et al. (2007)* that the highest lactation curve, greatest peak, least persistency and greatest 60-d and 270-d milk yield were obtained from cows calving at a BCS of 6.0 to 6.5 units (10-point scale; approximately 3.5 in the 5-point scale)

Conclusion

Result from the present study indicated a significant association between BCS at calving and daily milk yield. Cows with lower than 3.0 BCS at calving showed lower milk production. The highest lactation curve, greatest peak and least persistency were obtained from cows calving at BCS more than 3.5 unit.

Our study demonstrated the usefulness of regular checked BCS in the dairy management.

Uticaj telesne kondicije na laktacijsku krivu krava holštajn rase

Savaş Atasever, Szabina Szilágyi, Myrtill Gráff, Edit Mikóné Jónás

Rezime

Cilj ove studije bio je predstavljanje uloge sistema ocene telesne kondicije u upravljanju mlečnim govedarstvom. Glavni fokus bio je uticaj BCS (ocena telesne kondicije) na telenju na proizvodnju mleka. Sakupili smo u kontroli mlečnosti podatake 5197 test dana. Krave su grupisane prema BCS (ocena telesne kondicije) na teljenju ($BCSc_{2.0}$, $BCSc_{2.5}$ $BCSc_{3.0}$ i $BCSc_{>3.0}$). Podaci krava grupisanih prema različitim BCS analizirani su pomoću Wood funkcije. Najmanju količinu mleka proizvele su krave sa $BCSc_{2.0}$. Na početku laktacije, najveća količina mleka predviđena je za krave sa najvišom BCS ocenom na telenju (32.617 kg). Međutim, posle prvog meseca laktacije, proizvodnja ove grupe pala je spektakularno. Veći koeficijent determinacije (R^2 = 0,322) je predviđen za krave sa $BCSc_{>3.0.}$ Uzimajući u obzir sve rezultate, mogli bismo zaključiti da su na početku laktacije krave iskoristile akumulirane rezerve masti za potrebe proizvodnje, ali kasnije, kako nestaju ove rezerve, smanjuje se proizvodnja mleka. Pokazalo se da je integracija redovnog ocenjivanja telesne kondicije krava u tehnologiju korisno rešenje.

Ključne reči: telesna kondicija, procena krive laktacije, proizvodnja mleka

References

AKBAR, H. T.M. GRALA, M. VAILATI RIBONI, F.C. CARDOSO, G. VERKERK, J. MCGOWAN, K. MACDONALD, J. WEBSTER, K. SCHUTZ, S. MEIER, L. MATTHEWS, J.R. ROCHE, J.J. LOOR (2015): Body Condition Score At Calving Affects Systemic And Hepatic Transcriptome Indicators Of Inflammation And Nutrient Metabolism In Grazing Dairy Cows, Journal Of Dairy Science, Volume 98, Issue 2, 2015, 1019-1032.

BERRY, D. P.- BUCKLEY, F.- DILLON, P.- EVANS, R. D.- RATH, M. - VEERKAMP, R. F. (2002): Genetic parameters for level and change of body condition score and body weight in dairy cows. J. Dairy Sci. 85. 8. 2030-2039.

BEWLEY, J. M.- SCHUTZ, M. M. (2008): Review: An Interdisciplinary review of body condition scoring for dairy cattle. Professional Animal Scientist. 24 6 507-529.

BINES, J. A. (1976): Regulation of food intake in dairy cows in relation to milk production. Livest. Prod. Sci. 3 2 115-128.

BOUJENANE I., HILAL B (2012) Genetic and non genetic effects for lactation curve traits in Holstein-Friesian cows Archiv Tierzucht 55 (2012) 5, 450-457.

CONTRERAS, L. L.- RYAN, C. M. - OVERTON, T. R. (2004): Effects of dry cow grouping strategy and prepartum body condition score on performance and health of transition dairy cows. J. Dairy Sci. 87 2 517-523.

CUTULLIC, E.- DELABY, L.- CAUSEUR, D.- MICHEL, G. - DISENHAUS, C. (2009): Hierarchy of factors affecting behavioural signs used for oestrus detection of Holstein and Normande dairy cows in a seasonal calving system. Anim. Reprod. Sci. 113 1-4 22-37.

DILLON, P.- BUCKLEY, F.- O’CONNOR, P.- HEGARTY, D. - RATH, M. (2003): A comparison of different dairy cow breeds on a seasonal grass-based system of milk production: 1. Milk production, live weight, body condition score and DM intake. Livest. Prod. Sci. 83 1 21-33.

DOMECQ, J. J.- SKIDMORE, A. L.- LLOYD, J. W. - KANEENE, J. B. (1997): Relationship between body condition scores and milk yield in a large dairy herd of high yielding holstein cows. J. Dairy Sci. 80 1 101-112.

GALLO, L.- CARNIER, P.- CASSANDRO, M.- MANTOVANI, R.- BAILONI, L.- CONTIERO, B. - BITTANTE, G. (1996): Change in body condition score of holstein cows as affected by parity and mature equivalent milk yield. J. Dairy Sci. 79 6 1009-1015.

GRADIZ L., L. ALVARADO, A.K. KAHI, H. HIROOKA (2009): Fit of Wood's function to daily milk records and estimation of environmental and additive and non-additive genetic effects on lactation curve and lactation parameters of crossbred dual purpose cattle, Livestock Science, Volume 124, Issue 1, Pages 321-329.

HALASZ, A.- E. JONAS (2014) Optimised dairy cow feeding economy in Hungary, APSTRACT - APPLIED STUDIES IN AGRIBUSINESS AND COMMERCE 8:(2-3) pp. 69-72.

HART, I. C.- BINES, J. A. - MORANT, S. V. (1979): Endocrine control of energy metabolism in the cow: correlations of hormones and metabolites in high and low yielding cows for stages of lactation. J. Dairy Sci. 62 2 270-277.

HEUER, C.- SCHUKKEN, Y. H. - DOBBELAAR, P. (1999): Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J. Dairy Sci. 82 295-304.

JERETINA, J., BABNIK, D., & ŠKORJANC, D. (2013). Modeling lactation curve standards for test-day milk yield in Holstein, Brown Swiss and Simmental cows. The J Anim and Plant Sci, 233, 754-EDMONSON, A. J.- LEAN, I. J.- WEAVER, L. D.- FARVER, T. - WEBSTER, G. (1989): A body condition scoring chart for holstein dairy cows. J. Dairy Sci. 72 1 68-78.

JÍLEK, F., PYTLOUN, P., KUBEŠOVÁ, M., ŠTÍPKOVÁ, M., BOUŠKA, J., VOLEK, J., ... & RAJMON, R. (2008). Relationships among body condition score, milk yield and reproduction in Czech Fleckvieh cows. Czech Journal of Animal Science, 53(9), 357-367.

JINGAR, S., MEHLA, R. K., SINGH, M., & ROY, A. K. (2014). Lactation curve pattern and prediction of milk production performance in crossbred cows. Journal of veterinary medicine, 2014.

KADARMIDEEN, H. N. (2004): Genetic correlations among body condition score, somatic cell score, milk production, fertility and conformation traits in dairy cows. Anim. Sci. 79 191-201.

MARKUSFELD, O.- GALON, N. - EZRA, E. (1997): Body condition score, health, yield and fertility in dairy cows. Vet. Rec. 141 3 67-72.

PEDRON, O.- CHELI, F.- SENATORE, E.- BAROLI, D. - RIZZI, R. (1993): Effect of body condition score at calving on performance, some blood parameters, and milk fatty acid composition in dairy cows. J. Dairy Sci. 76, 9, 2528-2535.

ROCHE, J. R.- LEE, J. M.- MACDONALD, K. A. - BERRY, D. P. (2007): Relationships among body condition score, body weight, and milk production variables in pasture-based dairy cows. J. Dairy Sci. 90, 8, 3802-3815.

ROESCH, M.- DOHERR, M. G. - BLUM, J. W. (2005): Performance of dairy cows on swiss farms with organic and integrated production. J. Dairy Sci. 88, 7, 2462-2475.

RUEGG, P. L.- MILTON, R. L. (1995): Body condition scores of holstein cows on prince edward island, canada: relationships with yield, reproductive performance, and disease. J. Dairy Sci. 78, 3, 552-564.

RYAN, G.- MURPHY, J. J.- CROSSE, S. - RATH, M. (2003): The effect of pre-calving diet on post-calving cow performance. Livest. Prod. Sci. 79, 1, 61-71.
SAMARÜTEL, J.- LING, K.- JAAKSON, H.- KAART, T. - KART, O. (2006): Effect of body condition score at parturition on the production performance, fertility and culling in primiparous Estonian Holstein cows. Veterinarija ir Zootechnika, 36, 69-74.
STOCKDALE, C. (2005): Investigating the interaction between body condition at calving and pre-calving energy and protein nutrition on the early lactation performance of dairy cows. Australian Journal of Experimental Agriculture, 45, 12, 1507-1518.
WALTNER, S. S.- MCNAMARA, J. P. - HILLERS, J. K. (1993): Relationships of body condition score to production variables in high producing holstein dairy cattle. J. Dairy Sci. 76, 11, 3410-3419.
Wathes, D. C.- Cheng, Z.- Bourne, N.- Taylor, V. J.- Coffey, M. P. - Brotherstone, S. (2007): Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domest. Anim. Endocrinol., 33, 2, 203-225.
WOOD P. D. P. (1967): Algebraic Model of the Lactation Curve in Cattle Nature 216, 164 – 165.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EFFECT OF FIXED AND CONTINUOUS ENVIRONMENTAL FACTORS ON MILK PRODUCTION IN THE FIRST THREE STANDARD LACTATIONS IN SIMMENTAL COWS

Milun D. Petrović[1], Milan M. Petrović[2], Vladan Bogdanović[3], Radica Đedović[3], Simeon Rakonjac[1], Vladimir Dosković[1], Miloš Ž. Petrović[1]

[1]University of Kragujevac, Faculty of Agronomy, Čačak.
[2]Institute for Animal Husbandry, Belgrade-Zemun
[3]University of Belgrade, Faculty of Agriculture, Belgrade-Zemun.
Corresponding author: Milun D. Petrović, Faculty of Agronomy, e-mail: milunp@kg.ac.rs
Original scientific paper

Abstract: The effect of non-genetic factors on milk and milk fat production in first three standard lactations was examined in 241 Simmental cows. Milk performance traits in standard lactations (milk and milk fat production and milk fat content) were evaluated in terms of the effect of farm, lactation number, season of birth, season of calving, their interactions and age at first fertilisation.

The mathematical and statistical analysis of data, involving calculation of least squares means, standard errors, analysis of variance and coefficients of determination, was performed according to the general linear model procedure. The effect of farm, lactation group and year of birth on milk and milk fat production was very highly significant ($p<0.001$). The interaction between year of birth and season of birth and the interaction among year of birth, season of birth and season of calving had a significant effect on the variability of milk and milk fat production, whereas season of birth, season of calving and age at first fertilisation did not significantly affect ($P>0.05$) milk and milk fat production. Milk fat content in standard lactations was very highly significantly affected ($P<0.001$) by breeding area and the interaction among year of birth, season of birth and season of calving; significantly affected by year of birth, season of birth and age at first fertilisation; and non-significantly affected by lactation number and season of calving. The coefficient of determination (R^2) was very highly significant ($p<0.001$), ranging from 0.419 for milk fat content to 0.659 and 0.641 for the production of milk and milk fat, respectively, in standard lactations.

Key words: Simmental breed, milk production, milk fat content, season of calving, age at first fertilisation.

Introduction

The cattle breeding procedures used today most commonly employ linear methods and models that combine fixed parameters (year, farm, season, lactation) and random variables (age at first fertilisation or calving, genetic effect of sire, genetic effect of an individual animal, etc.), which can be interdependent (interrelated) or independent, with or without interactions, depending on the trait analysed. The model selected results, essentially, in the breeding value of an individual animal (*Bogdanović et al., 2003*).

Apart from being induced by the effect of genotype, differences in production parameters are also due to the effects of nutrition, care and management, housing system and the human factor. The interrelationship of these factors is characteristic of each individual farm. It is for this reason that differences occur among herds or farms even when animals have similar genetic potential for a particular production level. The classification into regions and breeding areas as a method used in studying the effect of non-genetic factors on either productive, reproductive or functional traits has been employed in numerous experiments mostly showing significant and very significant effects of this systematic non-genetic factor on milk performance traits. *Perišić (1998), Petrović D.M. et al. (2009a, 2009b, 2010, 2012, 2015, 2016, 2017), Petrović M.M. et al. (2009a* and *2009b)* and *Bogdanović et al. (2012)* evaluated the effect of region on milk and milk fat production and milk fat content in Domestic Spotted and Simmental cows, and found significant variations ($P<0.01$) in milk and milk fat yield as induced by the effect of region.

Depending on the level of production, sample size, and mathematical and statistical model, non-genetic discontinuous factors (year, farm, season, lactation) can account for more than 50% of the total variance in milk production *(Stojić et al., 1996).* The same authors, *Stojić et al. (1995),* reported that farm, year and season of calving explained 35.7% of the total variance in milk production. A somewhat lower proportion (22.7%) of the total variance in performance traits attributable to non-genetic factors (farm, year, calving season and lactation number) was found by *Jovanovac* (1987), whereas *Hansen et al. (1983)* determined a considerably higher percentage of about 45% (in terms of the farm-year-season effect).

The effect of season of birth and calving i.e. beginning of lactation as a systematic factor on milk performance traits is reflected through various climatic circumstances and nutrition throughout the year; therefore, it is included in models for the evaluation of the breeding value of dairy animals. As indicated by most authors, milk and milk fat production is maximum during the winter and spring seasons, and minimum during summer and autumn (*Perišić, 1998; Kučera et al., 1999; Cilek and Tekin, 2005; Petrović D.M. et al., 2005* and *2006; Panić 2005;*

Petrović M.M. et al., 2006; Petrović M.M. et al., 2009a; Petrović M.M. et al., 2009b; Lazarević et al., 2013; Nikšić et al., 2013).

Most domestic and foreign authors indicate significant and highly significant effects of lactation number i.e. lactation group on the expression of production traits. Namely, maximum milk production is dependent on the intensity of breeding in the period from the third until the fifth lactation (*Mchau and Syrstad, 1991; Tarkowski et al., 1994; Gaydarska et al., 2001; Đurđević, 2001; Pantelić et al., 2005; Petrović D.M. et al., 2005, 2006 and 2010).* Milk production is lowest in the first lactation due to insufficient body development of animals, whereas a drop in production after the third i.e. fifth lactation often occurs due to health disorders.

There is general agreement among most researchers that milk and milk fat yield in standard lactations increases with increasing age at first fertilisation *(Fiss and Wilton, 1989; Michel et al., 1989; Perišić, 2002; Petrović D.M. et al., 2005 and 2006).*

Material and methods

The effect of non-genetic factors on milk performance traits in the first three standard lactations was evaluated in 241 Simmental cows born between 1998 and 2010, and raised in three breeding areas (Kraljevo, Jagodina and Niš).
Milk and milk fat production in standard lactations was examined in terms of the effect of the following environmental factors:

- ***Breeding area*** i.e. the effect of three locations (1 – Kraljevo, 2 – Jagodina and 3 – Niš).
- ***Lactation number*** i.e. the effect of the first three lactations.
- ***Year of birth.*** To equalise the number of animals across different years of birth as much as possible and reduce variability, cows were grouped according to year of birth (group I – cows born between 1999 and 2004; group II – cows born between 2005 and 2006; group III – cows born in 2007; group IV – cows born between 2008 and 2010)
- ***Season of birth*** i.e. the effect of four seasons (I-spring, II-summer, III-autumn, IV-winter).
- ***Season of calving*** i.e. beginning of lactation (I-spring, II-summer, III-autumn, IV-winter).
- ***Interaction between year of birth*** (cows that calved between 1999 and 2010) ***and season of birth*** (I-spring, II-summer, III-autumn and IV-winter)***)***.
- ***Interaction among year of birth*** (4 groups) ***season of birth*** (4 seasons) ***and season of calving*** (4 seasons).
- ***Age at first fertilisation.***

The mathematical and statistical analysis of data, involving calculation of least squares means (LSM), standard error of the mean (SE_{LSM}), analysis of variance (F_{exp}) and coefficient of determination (R^2), was performed following the general linear model:

$\mathbf{y}_{ijklm} = \boldsymbol{\mu} + \mathbf{B}_i + \mathbf{L}_j + \mathbf{Y}_k + \mathbf{Sb}_l + \mathbf{Sc}_m + \mathbf{SbY}_{\mathbf{lk}} + \mathbf{SbYS}_{\mathbf{Clkm}} + \mathbf{b_1(x_1}\text{-}\bar{x}_{\mathbf{1}}\mathbf{)} + \mathbf{e}_{ijklm}$, where:

$\mathbf{y}_{ijklm}$– an individual animal of $\mathbf{i}^{th}$ breeding area, $\mathbf{j}^{th}$ lactation group, $\mathbf{k}^{th}$ year of birth, $\mathbf{l}^{th}$ season of birth and $\mathbf{m}^{th}$ season of calving.
$\boldsymbol{\mu}$ – population mean at equal proportions of all classes of effects (B, L,Y, Sb, Sc,SbY, SbYSc),
$\mathbf{B}_i$ – fixed effect of i^{th} breeding area (1-3),
$\mathbf{L}_j$ – fixed effect of j^{th} lactation (1-3),
$\mathbf{Y}_k$–fixed effect of k^{th} year of birth (1-4),
$\mathbf{Sb}_l$ –fixed effect of l^{th} season of birth (1-4),
$\mathbf{Sc}_m$ –fixed effect of m^{th} season of calving (1-4),
$\mathbf{SbY}_{\mathbf{lk}}$–fixed effect of the interaction between l^{th} season of birth and k^{th} year of birth (1-16)
$\mathbf{SbYSc}_{\mathbf{lkm}}$ –fixed effect of the interaction among l^{th} season of birth, k^{th} year of birth and m^{th} season of calving (1-64),
$\mathbf{b}_1$– linear regression coefficient of the effect of age at first fertilisation, and
$\mathbf{e}_{ijklm}$– other undetermined effects.

Results and discussion

The results of the analysis of the effects of systematic environment-related factors on milk performance traits in the first three standard lactations i.e. least squares means (LSM), standard errors of the means (SE_{LSM}), significance of the effects and coefficients of determination (R^2) are presented in Table 1.

Breeding area had a very highly significant effect (P<0.001) on milk performance traits in standard lactations due to the effect of nutrition, care and management, housing method and the human factor, which justifies the correction of the productive traits for the effect of this factor. Milk and milk fat production was very highly significantly lower in the Niš area i.e. 3771 kg milk and 150.5 kg milk fat.

Lactation number had a very highly significant effect (P<0.001) on milk and milk fat production in standard lactations. The production of milk and milk fat showed a steadily increasing tendency across lactations, the lowest in the first lactation (4121 and 161.7 kg, respectively), and the highest in the third standard lactation (4784 and 189.1 kg, respectively), which is in agreement with almost all researchers who evaluated the breeding value of cows *(Mchau and Syrstad, 1991;*

Tarkowski et al., 1994; Gaydarska et al., 2001; Đurđević, 2001; Pantelić et al., 2005; Petrović D.M. et al., 2005, 2006, 2010). Milk fat content showed no statistically significant differences across lactations (P>0.05), and exhibited a downward trend, as opposed to milk and milk fat content.

The effect of *year of birth*, as evidenced through different climatic circumstances, feed quality and quantity, and improvement in breeding technology, was very highly significant (P<0.001), showing no pronounced tendency to increase or decrease across years. Most authors reported significant and very significant effects of year of birth on milk and milk fat yield as an individual systematic factor, whereas *Perišić (1998)* found a non-significant effect (P>0.05) of year of calving on productive traits in Simmental cows, low fluctuations across years and a weak positive genetic trend.

Season of birth and season of calving had no significant effect (P>0.05) on milk performance traits in the first three standard lactations, whereas the *season of birth x year of birth interaction* and the *season of birth x year of birth x season of calving interaction* significantly affected (P < 0.05) milk and milk fat production, and had a very highly significant effect (P<0.001) on milk fat content in the first three standard lactations. There is disagreement among scientists as to the effect of season on productive traits. Naturally, its effect decreases when mono diets are used for dairy cows.

Age at first fertilisation had no significant effect (P>0.05) on the production of either milk or milk fat, whereas its effect on milk fat content in the first three standard lactations was significant (P>0.05).

Table 1. Least squares means, standard errors of the means and significance of the effects of systematic factors and age at first fertilisation on milk performance traits in cows over standard lactations

Systematic effects		MPSL (kg)		MFCSL (%)		MFPSL (kg)	
Farm	**N**	**LSM**	**SE_{LSM}**	**LSM**	**SE_{LSM}**	**LSM**	**SE_{LSM}**
Kraljevo	201	4809.15	80.47	3.935	0.008	188.58	2.990
Jagodina	195	4852.59	50.47	3.910	0.012	189.86	2.106
Niš	207	3771.22	41.20	3.994	0.020	150.48	1.805
F_{exp}		72.485***		10.18***		59.498***	
Lactation							
I	201	4121.23	66.77	3.934	0.013	161.73	2.564
II	201	4495.15	68.18	3.947	0.016	176.92	2.591
III	201	4784.28	65.25	3.960	0.016	189.10	2.545
F_{exp}		**45.211*** **		**0.22ns**		**47.868*** **	
Year of birth							
I	135	4352.31	76.56	3.939	0.025	171.06	3.071
II	60	4764.31	120.31	3.942	0.026	187.15	4.538
III	90	4703.46	105.08	3.908	0.016	183.88	4.196
IV	318	4392.46	55.53	3.963	0.010	173.60	2.153
F_{exp}		**9.398*** **		**3.03***		**7.451*** **	
Season of birth							
I	126	4374.88	79.22	3.939	0.020	172.24	3.215
II	150	4423.90	86.15	3.973	0.017	175.21	3.284
III	177	4585.23	77.05	3.910	0.017	178.90	2.991
IV	150	4447.53	75.73	3.971	0.015	176.18	2.870
F_{exp}		**1.401ns**		**3.09***		**1.652ns**	
Season of calving							
I	150	4419.00	83.226	3.953	0.018	174.21	3.218
II	148	4507.38	81.034	3.960	0.019	177.89	3.083
III	165	4411.92	75.576	3.938	0.016	173.41	2.937
IV	140	4540.17	81.019	3.937	0.015	178.61	3.168
F_{exp}		**0.531ns**		**1.04ns**		**0.659ns**	
Season of birth x year of birth							
F_{exp}		**2.316***		**3.88*** **		**2.271***	
Season of birth x year of birth x season of calving							
F_{exp}		**1.656***		**2.09** **		**1.700***	
Age at 1st fert.							
F_{exp}		**1.249ns**		**4.44***		**0.362ns**	
R^2		**0.659*** **		**0.419*** **		**0.641*** **	

N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001; MPSL(kg) –milk production in standard lactations; MFCSL(%) –milk fat content in standard lactations; MFPSL(kg) –milk fat production in standard lactations, in kg

The *coefficients of determination* (R^2) of 0.659, 0.641 and 0.419 for milk production, milk fat production and milk fat content, respectively, suggest that most (about 65%) of the total variance in productive traits across standard lactations was explained by the environmental factors analysed, and the rest of the variance (to reach 100%) by different factors which were not evaluated in this study and which can be dealt with in further research. Considerably lower coefficients of determination were determined by *Petrović D.M. et al. (2009 and 2015)* in their studies on the effect of non-genetic factors on milk and milk fat production in whole and standard lactations in Simmental cows.

Conclusion

Based on the model used for the correction of the effect of fixed and continuous environmental factors (breeding area, lactation number, season of birth, season of calving, their interactions, and age at first fertilisation) on milk performance traits in the first three standard lactations, the following conclusions can be drawn:

- The effects of breeding area (farm), lactation group and year of birth were very highly significant ($P<0.001$).
- Season of birth and season of calving had no significant effect ($P>0.05$) on milk and milk fat production in standard lactations.
- The *season of birth x year of birth* interaction and the *season of birth x year of birth x season of calving* interaction significantly affected ($P<0.05$) milk and milk fat production, whereas their effect on milk fat content was very highly significant ($P<0.001$) and highly significant ($P<0.01$), respectively.
- Age at first fertilisation had a significant ($P<0.05$) effect only on milk fat content in the first three standard lactations.
- Coefficient of determination (R^2) was very highly significant ($P<0.001$) and ranged from 0.419 for milk fat content to 0.659 and 0.641 for the production of milk and milk fat, respectively, in standard lactations.

Uticaj fiksnih i kontinuelnih ambijentalnih faktora na proizvodnju mleka u prve tri standardne laktacije kod krava simentalske rase

Milun D. Petrović, Milan M. Petrović, Vladan Bogdanović, Radica Đedović, Simeon Rakonjac, Vladimir Dosković, Miloš Ž. Petrović

Rezime

Uticaj paragenetskih faktora na proizvodnju mleka i mlečne masti u prve tri standardne laktacije ispitivan je na uzorku od 241 krave simentalske rase.

Na ispoljenost osobina mle;nosti u standardnim laktacijama (proizvodnja mleka i mlečne masti i sadržaj mlečne masti) ispitivan je uticaj farme, broja laktacija, sezone rođenja i telenja, njihovih interakcija i uzrasta pri prvoj oplodnji.

Matematičko-statistička analiza podataka, odnosno sve potrebne veličine, kao što su sredine najmanjih kvadrata, standardne greške, analiza varijanse i koeficijenti determinacije izračunate su u programskoj proceduri Opšti linearni model (GLM procedura). Uticaj farme, grupe laktacija i godine rođenja na proizvodnju mleka i mlečne masti bio je vrlo visoko značajan ($p<0,001$). Interakcija između godine i sezone rođenja, kao i između godine i sezone rođenja i sezone telenja značajno je uticala na ispoljenu varijabilnost u proizvodnji mleka i mlečne masti, dok sezona rođenja i telenja kao i uzrast pri prvoj oplodnji nisu statistički značajno uticali ($P>0,05$) na proizvodnju mleka i mlečne masti. Statistički vrlo visoko značajan uticaj ($P<0,001$) na sadržaj mlečne masi u standardnim laktacijama imalo je odgajivačko područje i interakcija godine i sezone rođenja i sezone telenja. Značajan uticaj na sadržaj mlečne masti imala je godina i sezona rođenja kao i uzrast krava pri prvoj oplodnji, dok je uticaj laktacije po redu i sezone telenja bio nesignifikantan. Koeficijenta determinacije (R^2) bio je vrlo visoko značajan ($p<0,001$) i kretao se od 0,419 kod sdržaja mlečne masti do 0,659 i 0,641 kod proizvodnje mleka i mlečne masti u standardnim laktacijama.

Ključne reči: simentalska rasa, proizvodnja mleka, sadržaj mlečne masti, sezona telenja, uzrast pr prvoj oplodnji.

Acknowledgement

This work was financed by the Ministry of Education and Science, Republic of Serbia, Project TR 31086.

References

BOGDANOVIĆ V., ĐEDOVIĆ R. (2003): Testiranje i metode procene priplodne vrednosti simentalskih bikova. Savremeni trendovi u mlekarstvu, Zlatibor, Zbornik radova, 46-50.

BOGDANOVIĆ V., ĐEDOVIĆ R., STANOJEVIĆ D., PETROVIĆ D.M., BESKOROVAJNI R., RUŽIĆ-MUSLIĆ D., PANTELIĆ V.(2012): Regional

Differences in Expression of Milk Production Traits in Simmental Cows. Proceedings of the First International Symposium on Animal Science. November 8-10th , Belgrade, Serbia. Book I, 223-230.

ĐURĐEVIĆ R. (2001): Genetička analiza mlečnosti i reprodukcijskih svojstava krava simentalske rase. Doktorska disertacija. Poljoprivredni fakultet Novi Sad.

FISS F.C., WILTON J.W. (1989): Effects of breeding system, cow weight and milk yield on reproductive performance in beef cattle. Journal of Animal Science, Vol. 67, Num. 7, 1714-1721.

GAYDARSKA V., KRUSTEV K., SIMEONOVA S., IVANOV M. (2001): Influence of environmental and genetic factors on the milk yield and phenotypic and genotypic parameters of milk production in Black and White dairy cows in Bulgaria. Biotechnology in Animal Husbandry 17 (1-2), 11-15.

HANSEN L., FREEMAN A.E., BERGER P.J. (1983): Variances, Repeatabilites and Age Adjustmens of Yield and Fertility in Dairy Cattle. Journal of Dairy Science, 66, 281-292.

JOVANOVAC S. (1987): Utjecaj sistematskih faktora okoline na mliječnost krava Holstein-Friesian pasmine. Znanost i praksa u poljoprivrednoj i prehrambenoj tehnologiji 17(3-4), 303-314.

KUCERA J., HYANEK J., MIKSIK J., CERMAK V. (1999): The influence of the season of parurition on milk performance in Czech Pied cattle. Czech Journal of Animal Science, 44: 8, 343-350.

LAZAREVIĆ M., PETROVIĆ M.M., PANTELIĆ V., RUŽIĆ-MUSLIĆ D., BOGDANOVIĆ V., ĐEDOVIĆ R., PETROVIĆ D.M. (2013): Study of the Variabiliti of Milk Traits in the Population of Holstein Friesian Cattle in Central Serbia. Proceedings of the 10th International Symposium Modern Trends in Livestock Production. Belgrade, Serbia, October 2-4, 2013.,543-549.

MCHAU K.W., SYRSTAD O. (1991): Production characteristics of Mpwapwa cattle. 2. Shape of the lactation curve. World Anim. Rev., 66: 49-54.

MICHEL A., LEUENBERGER H., KUNZI N. (1989): Optimales erstkalbealter fur gealpte rinder unterschiedlicher zuchtrichtung. Simentaler Fleckvieh 6, 15-19.

NIKŠIĆ D., PETROVIĆ M.M., PANTELIĆ V., OSTOJIĆ-ANDRIĆ D., CARO-PETROVIĆ V., PERIŠIĆ P., PETROVIĆ D.M. (2013): Variability of Milk Traits in the Population of Simmental Cattle in Serbia. Proceedings of the 10th International Symposium Modern Trends in Livestock Production. Belgrade, Serbia, October 2-4, 536-542.

PANIĆ J. (2005): Kvantitativno–genetička analiza svojstava mlečnosti krava simentalske rase. Poljoprivredni fakultet, Univerzitet u Novom Sadu (Magistarska teza).

PANTELIĆ V., SKALICKI Z., LATINOVIĆ D., PETROVIĆ M.M., KUČEVIĆ D. (2005): Ispitivanje dejstva pojedinih paragenetskih faktora na osobine plodnosti bikovskih majki simentalske rase. Biotehnologija u stočarstvu, vol. 21 (3-4), 35-41.

PERISIC P., SKALICKI Z., PETROVIC M.M., MEKIC C., ĐEDOVIC-VIDIC RADICA (2002): Uticaj uzrasta pri prvoj oplodnji na proizvodne osobine krava simentalske rase. Savremena poljoprivreda, vol. 51, 3-4, 97-99.
PERIŠIĆ P. (1998): Reproduktivne i proizvodne osobine različitih genotipova krava simentalske rase. Magistarska teza, Poljoprivredni fakultet, Beograd-Zemun.
PETROVIĆ D.M., SKALICKI Z., PETROVIĆ M.M., BOGDANOVIĆ V.(2009a): The Effect of Systematic Factors on Milk Yield in Simmental Cows Over Complete Lactations. Biotechnology in Animal Husbandry 25(1-2), 61-71.
PETROVIĆ D.M., BOGDANOVIĆ V., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., ĐOKOVIĆ R., PETROVIĆ M. (2016): Uticaj fiksnih i kontinuelnih ambijentalnih faktora na proizvodnju 4% mast-korigovanog mleka u prve tri laktacije kod krava simentalske rase. XXI Savetovanje o Biotehnologiji, Čačak, 11-12. Mart 2016. Zbornik radova, Vol. 21.(24), 525-533.
PETROVIĆ D.M., BOGDANOVIĆ V., ĐEDOVIĆ R., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., ĐOKOVIĆ R., PETROVIĆ M. (2017): Uticaj paragenetskih faktora na intenzitet plodnosti kod krava simentalske rase. XXII Savetovanje o Biotehnologiji, Čačak, 10-11. mart 2017. Zbornik radova, Vol. 22, 711-717.
PETROVIĆ D.M., BOGDANOVIĆ V., PETROVIĆ M.M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., ĐOKOVIĆ R., ĐEDOVIĆ R., RAKONJAC S. (2015): Effect of Non’genetic Factors on Standard Lactation Milk Performance traits in Simmental Cows. Ann. Anim. Sci. Vol. 15, No. 1(2015), 211-220.
PETROVIĆ D.M., BOGDANOVIĆ V., PETROVIĆ M.M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S. (2010): Uticaj paragenetskih faktora na proizvodnju 4% mast-korigovanog mleka u celim i standardnim laktacijama. XV savetovanje o biotehnologiji. Zbornik radova Vol.15(17), 585-590.
PETROVIĆ D.M., ĐOKOVIĆ R., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., KURĆUBIĆ V. (2006): Uticaj paragenetskih faktora na proizvodne osobine standardnih laktacija kod krava simentalske rase. Savremena poljoprivreda, Vol.55, 1-2,138-143.
PETROVIĆ D.M., SKALICKI Z., BOGDANOVIĆ V., PETROVIĆ M.M., KURĆUBIĆ V. (2005): The Effect of Paragenetic Factors on Performance Traits in Complete Lactations in Simmental Cows. 8th International Symposium Modern Trends In Livestock Production. Belgrade Zemun, Serbia and Montenegro, 5.-8.10.2005. Biotechnology in Animal Husbrandy 21 (5-6), 7-12.
PETROVIĆ D.M., Z. SKALICKI, V. BOGDANOVIĆ, M.M. PETROVIĆ, R. ĐEDOVIĆ, P. PERIŠIĆ, R. ĐOKOVIĆ, V. DOSKOVIĆ (2009b): The Effect of Systematic Factors of the Service Period in Simmental Cows. Journal of Mountain agriculture on the Balkans, vol. 12, 4, 662-675.
PETROVIĆ D.M., Z. SKALICKI, V. BOGDANOVIĆ, M.M. PETROVIĆ, S. BOGOSAVLJEVIĆ-BOŠKOVIĆ, R. ĐOKOVIĆ, S. RAKONJAC (2012): The Effect

of Geographical Region on Lifetime Milk Yield in Simmental Cows. Proceedings of the First International Symposium on Animal Science. November 8-10th , Belgrade, Serbia. Book I, 103-110.

PETROVIĆ M.M., SRETENOVIĆ LJ., ALEKSIĆ S., PANTELIĆ V., NOVAKOVIĆ Ž., PERIŠIĆ P., PETROVIĆ D.M. (2009b): Investigation of the Heritability of Phenotypes of Fertility and Milk Performance of Simmental Cattle Breed in Serbia. 9th International Symposium Modern Trends in Livestock Production (7-9.10.2009). Biotehnology in Animal Husbandry, 25(5-6), 285-292.

PETROVIĆ M.M., SRETENOVIĆ LJ., BOGDANOVIĆ V., PERIŠIĆ P., ALEKSIĆ S., PANTELIĆ V., PETROVIĆ D.M., NOVAKOVIĆ Ž.(2009a): Quantitative Analysis of Genetic Impromevent of Milk production Phenotypes in Simmental Cows. Biotechnology in Animal Husbandry 25(1-2), 45-51.

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

STATSOFT INC. STATISTICA FOR WINDOWS, Version 6.0, Computer program manual. Tulsa,: StatSoft Jnc., 1995.

STOJIĆ P. (1996): Faktori korekcije osobina mlečnosti i njihov doprinos oceni priplodne vrednosti bikova i krava. Poljoprivredni fakultet, Univerzitet u Beogradu, Doktorska disertacija.

STOJIĆ P., KATIĆ M., LAZAREVIĆ LJ., LATINOVIĆ D., TRIFUNOVIĆ G., BESKOROVAJNI R., BRKIĆ N. (1995): Ponovljivost dnevnih prinosa mleka tokom laktacije crno belih krava. Prvi Simpozijum za oplemenjivanje organizama sa međunarodnim učešćem. Vrnjačka Banja.

TARKOWSKI J., TRAUTMAN J., JAMROZ D. (1994): Evalution of changes in milk yield and fertility of Simmental cows at the Pakoszowka State Animal Breeding Centre. Annales Universitatis Marie Curie Sklodowska. Sectio EE Zootechnia, 12:46-53.

VARIABILITY OF MILK TRAITS IN PROGENY TESTING OF THE SIMMENTAL BULLS

Marina Lazarević[1], Vlada Pantelić[1], Dragan Nikšić[1], Nevena Maksimović[1], Miloš Marinković[1], Nenad Mićić[1], Dragan Stanojević[2]

[1] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, Belgrade-Zemun, Republic of Serbia
Corresponding author: marinaplazarevic@gmail.com
Original scientific paper

Abstract: The data of the Institute for Animal Husbandry, Belgrade-Zemun have been used to investigate the variability of the properties that are observed in the progeny testing of Simmental bulls. The analysis is based on a sample of 7,845 first-calving heifers in the period 2007-2010. The tests included the following milk properties in the standard first lactation (305 days): milk yield (kg), milk fat content (%) and milk fat yield (kg). An analysis of the production traits of the first-calving heifers under control showed that their average milk yield was 4346.49 kg milk with 3.93% milk fat and yield of 171.11 kg of milk fat. Sires, years and calving season exhibited a highly significant impact ($p<0.01$) on all the milk properties observed in the progeny testing. Based on the BLUP solution, the rank of bulls for each trait was formed, and then the correlation coefficients of the rank, ranging from 0.402 (relationship between the yield of milk and milk fat content) to 0.989 (the relationship between the yield of milk and the yield of milk fat) were established.

Key words: Simmental breed, first – calving heifers, milk properties, variability of traits, progeny test.

Introduction

The Simental breed in Serbia represents the dominant population of cattle. In the total stock of cattle it makes more than 85%, i.e. cattle of the Simmental type, with the majority of the most represented local Simmental cattle, and to a lesser extent, animals imported from West European countries (Germany, Austria, Switzerland). As a breed of combined abilities, Simmental cattle enable the production of milk and meat, however, it is mainly selected to improve milk quality traits. The improvement of milk quality traits has so far been the most important selection route in the Simmental breeding program. One of the important preconditions for long-term provision of high-yielding populations is the systematic work on genetic improvement and rearing of heifers, which will be used as the first generation of dairy herds.

From the aspect of the genetic potential of dairy cows, the most important measure for increasing the yield of milk is the implementation of systematic breeding and selection work in the registered population. The genetic advancement of each herd equally depends on the breeding values of the male and female animals. The choice of parental pairs of future generations of dairy herds, selection criteria for heifers and their proper breeding in terms of nutrition and conditions of keeping should be given a primary importance. The choice of bull sires should be given the highest attention when it comes to the results of the progeny testing on milk performance and body development. The selection based on the results of the progeny testing is of particular importance for the features that are gender specific-limiting, such as milk performance and fertility, i.e. features that are only expressed in female animals.

The results of the study of the reproductive and production properties of Simmental cows, the heritability values and values of phenotypic and genetic correlations of these characteristics, are presented in the studies of *Pantelić, (2006), Pantelić et al., (2007), Petrović et al., (1999, 2006, 2009), Perišić et al. (1999a, 1999b), Miščević et al., (1995), Đurđević and Vidović, (1994), Romčević et al, (1992).* According to the results of previous studies, it can be concluded that the Simmental breed of cattle in Serbia is characterized by a marked variation in terms of production and reproductive characteristics.

The aim of this paper is to determine the average manifestation of milk properties, their variation and determine the influence of certain genetic and non-genetic factors on milk performance traits and determine the breeding value of bulls-sires.

Material and Methods

The data of the Institute for Animal Husbandry, Belgrade-Zemun have been used to investigate the variability of milk performance properties that are controlled in the progeny testing of Simmental bulls. The analysis is based on a sample of 7,845 first-calving heifers in the period 2007-2010. The study included the following milk performance properties in standard first lactation (305 days):

- Milk yield (kg);
- Milk fat content (%);
- Milk fat yield (kg).

In each year, two seasons were observed:

1. Summer: it included heifers calving for the first-time in May, June, July, August, September and October (3819 lactations),
2. Winter: it included heifers calving for the first-time in November, December, January, February, March and April (4026 lactations).

The first-calving heifers under control originated from 81 bull-sires, with the smallest number of daughters in the bull test being 40 and the maximum of 435. Distribution of the first-calving heifers to bull-sires is shown in Table 1.

Most of them originally came from family farms and fewer from commercial farms in Central Serbia. Milk control was performed using the reference A4 method and calculation of lactation Test interval method (*ICAR, 2009).*

Table 1: Distribution of first-calving heifers to bull-sires

Sire's HB	Number of daughters	Sire's HB	Number of daughters	Sire's HB	Number of daughters
B20	82	1224	69	1410	124
B21	89	1227	74	1416	57
B54	43	1230	79	1419	77
B121	70	1243	40	1423	49
B137	63	1261	56	1424	74
788	113	1264	47	1437	89
800	75	1267	109	1438	66
859	302	1279	53	1440	435
923	95	1280	58	1442	73
925	85	1286	217	1445	248
969	53	1287	81	1447	204
977	92	1294	60	1450	54
1012	78	1298	124	1452	84
1019	50	1302	44	1453	149
1029	70	1318	46	1457	83
1031	94	1319	48	1464	100
1036	112	1329	238	1465	66
1084	86	1347	111	1479	80
1091	51	1364	60	1491	55
1102	103	1376	97	1492	189
1109	55	1377	66	1499	63
1124	50	1380	55	1501	56
1171	67	1382	42	1506	155
1185	60	1383	111	1509	128
1187	58	1388	121	1512	189
1197	97	1390	40	B138	59
1206	266	1394	192	V028	42

In order to determine the influence of certain genetic and non-genetic factors on the investigated milk performance properties of the first calving heifers of Simmental breed, a fixed model of the least squares of *LSMLMW* was used (*Harvey, 1987*). The non-genetic factors included in the study were the fixed impact of the year and the calving season, while the genetic factor included in the examination was the fixed impact of the bull-sire.
A fixed model for analyzing different impacts on variability of traits in progeny testing:

$Y_{ijkl} = \mu + O_i + G_j + S_k + e_{ijkl}$
where:

- Y_{ijkl}: observed trait,
- μ: population average for given trait,
- O_i: fixed effect of i sire (i=1,…,81),
- G_j: fixed effect of j calving year (j=1, 2, 3, 4),
- S_k: fixed effect of k calving season (k=1, 2),
- e_{ijkl}: random error with characteristics N $(0,\sigma^2)$.

A mixed model of the least squares of L*SMLMW* adapted by *Harvey (1990)* for application in the process of breeding of farm animals was used to estimate the breeding value of bull-sires in the progeny test. The model used is identical to the model already mentioned, but in this case the bull-sire is seen as a random factor.

Results and Discussion

Table 2 presents descriptive statistical indicators and the variability of milk performance traits in the progeny testing of Simmental bulls.

Tabele 2. Descriptive statistical indicators of milk performance traits in the first standard lactation

Trait	*X*	*Min*	*Max*	*SD*	*CV (%)*
Milk yield, kg	4346.49	1675	8303	658.21	15.14
Milk fat content, %	3.93	3.29	4.65	0.10	2.51
Milk fat yield, kg	171.11	64	320	27.16	15.87

In the period from 2007 to 2010, in the standard lactation, they achieved the average milk yield of 4346.49 kg with a pronounced variation interval of 1675 and 8303 kg, with a standard deviation of 658.21 kg.
Majority of other authors *(Romčević et al., 1992, Đurđević and Vidović, 1994, Perišić, 1998*) emphasize the lower milk yield of controlled cattle of the Simental breed. Thus, *Petrović (2000)* in his research states that cows in standard lactations have an average milk yield of 4282 kg, varying from 1786 to 7721 kg and a standard deviation of 1121.68 kg. According to the data of the *Institute for Animal Husbandry (2008),* the milk yield of registered cows in the Simmental population in the period from 2003 to 2007 ranged from 3986 to 4440 kg.
Comparing their results with the results shown, it is obvious that the milk performance of the first-calving heifers of Simmental breed in Serbia has a positive trend. The variability of milk performance traits in the progeny test by years of calving is shown in Table 3.

Observing the average milk yield of the first-calving heifers of Simmental breed in standard lactation in the four-year period, there is a positive trend except in 2009 when the drop in the production of milk was recorded (4238.83kg). The highest milk production was achieved in 2010 (4420.34) with a pronounced coefficient of variation (17.20). However, according to data of *ICAR (2011),* the Simmental population of the first-calving heifers in the developed countries (Germany, Austria) achieved significantly higher production (5000-6000 kg).

Table 3. Mean values and variability of milk performance traits in progeny test by years (standard lactation)

Trait		Calving year			
		2007	2008	2009	2010
Milk yield, kg	*X*	4319.24	4400.51	4238.83	4420.34
	Min	1847	2663	1675	2233
	Max	7637	7745	8303	7207
	SD	628.93	630.23	574.03	760.12
	CV (%)	14.56	14.32	13.54	17.20
Milk fat content, %	*X*	3.91	3.93	3.95	3.94
	Min	3.33	3.29	3.40	3.34
	Max	4.64	4.38	4.65	4.65
	SD	0.11	0.08	0.11	0.09
	CV (%)	2.81	2.04	2.78	2.28
Milk fat yield, kg	*X*	169.02	172.85	167.29	174.73
	Min	64.00	103.32	65.85	88.90
	Max	312.20	316.00	320.00	292.54
	SD	25.21	26.53	23.20	31.76
	CV (%)	14.92	15.35	13.87	18.18

The content of milk fat in relation to the milk and milk fat yield is largely influenced by the genotype in relation to the effect of the external factor *(Pantelić, 2006)*. The average milk fat content was 3.93% with a standard deviation of 0.10 and a coefficient of variation of 2.51%. Approximately the same value is stated by *Pantelić (2006), Petrović et al. (2006).* Observed by years, the percentage of milk fat varied slightly, and the highest value was achieved in 2009 when the lowest milk yield was achieved.

Milk fat yield in standard lactation depends on milk yield and milk fat content. The average yield of milk fat in first-calving heifers under control in a four-year period was 171.11 kg with a standard deviation of 27.16 kg and a coefficient of variation of 15.87%. By 2008, the yield of milk fat increased to drop sharply in 2009 (167.29 kg) and then increase again, when the highest production of 174.73 kg with the highest variability (CV = 18.18%) was achieved in 2010.

Table 4 shows the effect of genetic and non-genetic sources of variability on milk performance traits.

Bull-sires exhibited significant influence (P<0.01) on all milk performance traits in the first standard lactation. *Stojić (1996)* has established in his research a high significance (P<0.01) of the bull-sire on the traits of milk yield, yield of milk fat and 4% MCM in the first standard lactation, while the milk fat content did not show significant effect (P>0.05). According to *Pantelić (2006),* in the first-calving heifers of Simmental breed, bull-sires had a very significant influence (P<0.01) on all the studied milk performance traits. The year of calving showed a highly significant effect (p<0.01) on all milk performance properties observed in the progeny test. The same results are reported by *Đurđević et al (1994). Perišić et al. (1999a), Pantelić (2006)* report insignificant impact of the year on the production results for the first-calving heifers in their researches. The calving season showed a high significance (p<0.01) on the yield of milk and milk fat while it significantly influenced (p<0.05) the percfentage of milk fat.

Table 4. The general averages of the leastsquares (μ) and their errors (Se) for milk performance traits, F-test of the tested influences

Trait	μ	Se	F values of tested influences		
			Sires	*Year*	*Season*
			df1=80	*df1=3*	*df1=1*
			df2=7760	*df2=7760*	*df2=7760*
Milk, kg	4345.88	35.11	17.62^{**}	6.64^{**}	24.68^{**}
Milk fat, %	3.93	0.004	13.04^{**}	15.91^{**}	3.99^{*}
Milk fat, kg	171.04	1.51	19.41^{**}	5.94^{**}	23.47^{**}

$p>0.05^{NS}$, $p<0.05^{*}$, $p<0.01^{**}$

The impact of the year on the yield of milk and milk fat is mainly reflected in the quality and quantity of food available. The effect of the year will be more pronounced in those years when vegetation is more extensive and better than in the average years.

Table 5 shows BLUP solutions and 15 best ranked bulls for milk yield in the progeny test.

The best ranked bull for milk yield is HB 1197, and it is in the first place also in regard to the yield of milk fat, while it is ranked 13 according to the content of milk fat.

Table 5. Evaluation of the Breeding value of bull-sires (BLUP) and rank of 15 best bulls for milk yield in the progeny test

Sire's HB	Number of daughters	Milk, kg	Rank milk, kg	Milk fat, %	Rank milk fat, %	Milk fat, kg	Rank milk fat, kg
1197	97	795.04	1	0.034	13	32.92	1
V028	42	634.18	2	0.015	28	25.24	2
1410	124	502.06	3	0.022	23	20.97	5
1171	67	497.42	4	0.083	1	23.52	3
800	75	452.84	5	0.071	2	21.28	4
1383	111	374.33	6	0.028	17	16.20	6
B54	43	351.92	7	0.021	26	14.46	9
859	302	347.67	8	0.031	14	15.19	8
1382	42	340.50	9	0.009	37	13.38	11
1479	80	340.14	10	0.044	5	16.16	7
788	113	335.61	11	0.012	33	13.75	10
1206	266	316.01	12	0.021	25	13.32	12
1465	66	313.59	13	0.011	35	12.49	14
1031	94	287.16	14	0.040	8	13.05	13
1491	55	270.28	15	-0.026	63	9.37	17

Observing the 15 best ranked bulls for milk yield, it is also noted that they are within the 15 best bulls for milk fat yield while being poorly ranked for milk fat content. Bull HB 1171 is found to be the first in regard to the milk fat content, ranked 4th in regard to the milk yield, and third in regard to the yield of milk fat.

Considering that the ranking of bulls for the three milk performance traits was determined, the degree of agreement between the obtained ranges was tested using the Spirman coefficient of correlation of rank, Table 6.

The determined coefficients of the rank correlations indicate that there was a medium to complete positive interdependence between the ranks of the bulls. The rank correlation of bulls of 0.989 was established between the yield of milk and the yield of milk fat in standard lactation. The medium correlation was determined between the content and the yield of milk fat of 0.494. The medium correlation of 0.402 was determined between the yield of milk and the content of milk fat.

Table 6. The rank correlation of bulls

Trait	Milk yield, kg	Milk fat content, %	Milk fat yield, kg
Milk yield, kg	—	0.402^{**}	0.989^{**}
Milk fat content, %	—	—	0.494^{**}
Milk fat yield, kg	—	—	—

$p>0.05^{NS}$, $p<0.05^{*}$, $p<0.01^{**}$

Ranking of bulls and determination of rank correlations among the examined milk performance traits will enable more efficient use of bulls in artificial insemination programs.

Conclusion

Looking at the obtained results, it is evident that a lot of selection work is needed to reach the level of production of the Simmental population in the more advanced countries (Germany, Austria). It is encouraging that a positive trend is observed over a four-year period, and the established variability provides enough room for improving the quality of milk performance. From the aspect of the genetic potential of dairy cows, the most important measure for increasing the yield of milk in the following period is the implementation of systematic breeding and selection work in the main population. The choice of parental pairs of future generations of dairy herds, selection criteria for heifers and their proper rearing in terms of nutrition and housing and keeping conditions should be given a primary importance.

Varijabilnost osobina mlečnosti praćenih u progenom testu bikova simentalske rase

Marina Lazarević, Vlada Pantelić, Dragan Nikšić, Nevena Maksimović, Miloš Marinković, Nenad Mićić, Dragan Stanojević

Rezime

Za ispitivanje varijabilnosti osobina koje se kontrolišu u progenom testu bikova simentalske rase iskorišćeni su podaci Instituta za stočarstvo iz Zemuna.

Analiza je zasnovana na uzorku od 7.845 prvotelki oteljenih u periodu 2007-2010 godine. Ispitivanja su obuhvatila sledeće osobine mlečnosti u standardnoj prvoj laktaciji (305 dana): prinos mleka (kg), sadržaj mlečne masti (%) i prinos mlečne masti (kg). Analiza proizvodnih karakteristika kontrolisanih prvotelki pokazala je da je njihova prosečna mlečnost na nivou od 4346,49 kg mleka sa 3.93% mlečne masti i prinosom od 171,11 kg mlečne masti. Očevi, godina i sezona teljenja ispoljili su visoko signifikantan uticaj ($p<0,01$) na sve osobine mlečnosti praćene u progenom testu. Na osnovu BLUP rešenja formiran je rang bikova za svaku osobinu a zatim utvrđeni koeficijenti korelacija ranga koji kreću se od 0,402 (povezanost ranga između prinosa mleka i sadržaja mlečne masti) do 0,989 (povezanost ranga između prinosa mleka i prinosa mlečne masti).

Ključne reči: simentalska rasa, prvotelke, osobine mlečnosti, varijabilnost osobina, progeni test.

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053.

References

ĐURĐEVIĆ, R., VIDOVIĆ, V. (1994): Ocena genetskih i fenotipskih parametara osobina mlečnosti krava simentalske rase. Savremena poljoprivreda, Novi Sad, 43, 3, 49-54.

GOO (2010): Glavna odgajivačka organizacija-Institut za stočarstvo: Stručni izveštaj i rezultati obavljenih poslova kontrole mera za sprovođenje odgajivačkog programa u 2008. godini. Beograd-Zemun

ICAR(2011): International Committee for Animal Recording.Milk recording database.www.icar.org

PANTELIĆ P., (2006): Fenotipska i genotipska varijabilnost proizvodnih osobina prvotelki simentalske rase u različitim regionima Srbije. Doktorska disertacija Univerzitet u Beogradu, Poljoprivredni fakultet.

PANTELIĆ V., SKALICKI Z., PETROVIĆ M.M., ALEKSIĆ S., MIŠČEVIĆ B., OSTOJIČ-ANDRIĆ D. (2007): Fenotipske korelacije proizvodnih i reproduktivnih osobina krava simentalske rase. Biotechnology in Animal Husbandry, 23 (3-4), 11 - 17

PERIŠIĆ, P. (1998): Reproduktivne i proizvodne osobine različitih genotipova krava simentalske rase. Magistarska teza. Univerzitet u Beogradu, Poljoprivredni fakultet.

PERIŠIĆ P., SKALICKI Z., LATINOVIĆ D., PETROVIĆ M.M., RUŽIĆ D.(1999a): Uticaj uzrasta pri prvoj oplodnji na neke reproduktivne i proizvodne osobine prvotelki simentalske rase. Savremena poljoprivreda, 48, 1-2, 155 - 159.

PERIŠIĆ P., SKALICKI Z., PETROVIĆ M. M., MIŠČEVIĆ B., RUŽIĆ DRAGANA, DELIĆ N. (1999b): Uticaj genetskih i nekih paragenetskih faktora na prinos mleka krava simentalske rase. Biotechnology in Animal Husbandry, 15 (1-2), 29 - 37

PETROVIĆ, D.M. (2000): Ispitivanje dugovečnosti, proizvodnje mleka i mlečne masti krava simentalske rase. Magistarska teza. Univerzitet u Beogradu, Poljoprivredni fakultet.

PETROVIC M.M., LAZAREVIC LJ., ALEKSIC S., MISCEVIC B., PERKOVIC S., ILIC Z. (1999): Comparative investigation of the heritability of milkiness phenotypes in the daughters of Simmental and Holstein-Friesian bull-sires in Serbia. Biotechnology in Animal Husbandry 15 (5-6), 125 - 132.

PETROVIĆ, M.M., SRETENOVIĆ, LJ., PANTELIĆ, V., ALEKSIĆ, S., MIŠČEVIĆ, B., BOG
DANOVIĆ, V., OSTOJIĆ - ANDRIĆ, D., PETROVIĆ,M.(2006): Results of the Application of the Technology of Genetic Improvement of Simmental Cattle Population in Serbia. Biotechnology in Animal Husbandry, 22 (1-2), 1 - 8.

PETROVIĆ M.M., SRETENOVIĆ LJ., BOGDANOVIĆ V., PERIŠIĆ P., ALEKSIĆ S., PANTELIĆ V., PETROVIĆ M.D., NOVAKOVIĆ Ž. (2009): Quantitative analysis of genetic improvement of milk production phenotypes in simmental cows. Biotechnology in Animal Husbandry 25 (1-2), 45 - 51

ROMČEVIĆ, LJ., STOJIĆ, P., AZANJAC, N., BULJ, M., BERISAVLJEVIĆ, B. (1992): Mlečnost simentalskih krava u dva proizvodna područja. Poljoprivreda, Beograd, 362-363, 8-11.

STOJIĆ, P. (1996): Faktori korekekcije osobina mlečnosti i njihov doprinos oceni priplodne vrednosti bikova i krava. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE EFFECT OF COLLECTION SEASONS ON THE SEMEN QUALITY OF HOLSTEIN-FRIESIAN BULLS

Miloš Marinković[1], Predrag Perišić[2], Milan M. Petrović[1], Vlada Pantelić[1], Marina Lazarević[1], Nenad Mićić[1], Vladimir Živković[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: Miloš Marinković majlo1989@gmail.com
Original scientific paper

Abstract: The study, which included 9 Holstein-Friesian bulls from the Livestock Center of the PKB Corporation, analyzed the impact of the ejaculate collection season on certain semen properties (ejaculate volume, sperm concentration, sperm motility in native ejaculate, sperm dilution, number of doses of one ejaculate and mobility of spermatozoa after thawing). For the purposes of this analysis, data for the three-year (2011-2013) and one-year period (2014) were used. In the three-year period, based on 621 ejaculates of three bulls, the average values for examined properties were calculated and the season's impact on these properties analyzed. The results show that the ejaculate collection season had a very significant effect ($p<0.001$) on the sperm motility in native ejaculate, and a significant effect ($p<0.05$) on the level of dilution of ejaculate and the mobility of spermatozoa after thawing. In the single-year period, based on 326 ejaculates from 9 bulls, the average values of the studied properties were calculated, and the influence of the season was also analyzed. The ejaculate collecting season showed a very high impact ($p<0.001$) on the mobility of spermatozoa after thawing, but no significant effect was found on other properties ($p> 0.05$).

Keywords: bulls, season, spermatozoa, ejaculate, Holstein-Friesian breed

Introduction

The production and quality of bull semen are strongly influenced by genetic factors such as breed and individual properties. However, the production and quality of sperm is conditioned by environmental factors such as diet, way of keeping and nursing, temperature and humidity, ejaculation frequency, and seed collecting skills. One of the most common factors that significantly impairs spermatogenesis is elevated ambient temperature. High daily temperatures (over 28^{o}C) can cause a spermatogenesis disorder, especially if they last for a long time. The spermatozoa in the first stage of spermatogenesis, when they are still located in the semen canals, are most sensitive to the effects of high temperatures. Subsequently, spermatozoids are wrapped in membranes that should enable them to better survive higher temperatures. Therefore, it can be said that the quality of the ejaculate depends on the temperature affecting the spermatozoa 45-60 days ago (*Stančić, 2014*).

The influence of the season on the semen properties was the subject of many studies. *Mathevon et al. (1998)* state that the season significantly affects all the properties of ejaculate (volume, concentration, mobility, total sperm count and total movable sperm count) in young bulls and most of the properties in older bulls (it does not significantly affect the volume of ejaculate and mobility of spermatozoa). Furthermore, the same author states that the concentration and total sperm count were higher during the winter and spring compared to summer and autumn.

Schwab et al. (1987) and *Menendez-Buxadera et al. (1984)* state that the highest values of the volume of ejaculate, concentration, and number of spermatozoa were also achieved during the winter. These results are contrary to the values established by *Fuente et al. (1984)* who have established the lowest results in the winter.

Orgal and Roth (2008) have analyzed the impact of the collecting season on the quality of bull semen. Their results show that there is a progressive decrease in the motility of spermatozoa after thawing during the summer months, although the significant effect of the season on motility has not been confirmed in the native ejaculate. Also, the percentage of spermatozoa in doses after thawing with defective acrosomes is higher in summer months (54.2 ± 3.5%) compared to winter months (51.4 ± 1.9%). The results obtained suggest that the ejaculates collected during the summer are less able to survive the cryoconservation process precisely because of the lower vitality of sperm recorded after thawing.

Vilakazi and Webb (2004), have examined the effect of the season on the presence of normally developed spermatozoa in the ejaculate. The percentage of normal spermatozoa in the ejaculate was higher during the spring (84.4 ± 2.4%) and winter (82.5 ± 2.4%) compared to the summer (72.8 ± 1.6%) and autumn (79.4 ± 2.2%). Based on these results, it can be again concluded that summer months, or high temperatures, have an adverse effect on the vitality of spermatozoa.

Söderquist et al. (1996) have found significant seasonal variations in the sperm counts with head damage and with total defects. However, the percentage of spermatozoa with damage was significantly higher during spring and summer compared to colder months of autumn and winter.

Artificial insemination is a process that has significantly improved livestock breeding, and especially cattle breeding. The use of this method has led to more effective use of the bulls, the intensity of selection has increased, the cost of keeping male animals is reduced, the safety of people and animals has increased and the risk of transmission of sexually transmitted diseases is reduced. For this reason, obtaining high quality doses of semen for insemination is an important process in any program of artificial insemination.

Every season is characterized by specific weather conditions that can affect the bull and its production to a lesser or greater extent. The aim of this study is to determine if the ejaculate collection season significantly influences the quality of the doses obtained.

Material and Methods

Research for the purpose of this study was carried out at the Livestock Centre of the PKB Corporation in Belgrade. At the time that data used in the present study was collected, 12 Holstein-Friesian bulls were used in the artificial insemination program in this Centre, and our research included the results of 9 bulls. The entire process of semen production at the Centre was monitored, starting with the collection of bulls' ejaculate, to the final stages of checking of semen quality after thawing.

Taking of the ejaculate in most bulls is done twice a week, most often on Mondays and Fridays. Only one ejaculate is taken from a bull once a day, rarely twice a day. The collection of the ejaculate is performed by artificial vagina, while the second bull is used to induce a full reflex, or jump.

A general overview of sperm involves assessing colour, odour and consistency. Ejaculates that are dirty or with traces of blood/puss are discarded, but are registered in the record for the current year. If the sperm passes the general examination, an estimate of the volume of ejaculate, density and motility of spermatozoa is done. The volume of the ejaculate is determined volumetrically in a graduated sperm collector. The density of the ejaculate (the number of spermatozoa in 1 ml of ejaculate) is measured using a photometer while the motility is estimated based on the number of spermatozoa that exhibit progressive penetration. Observation is performed using a microscope (magnification of 20 - 40x) and score/estimates are given for motility from 1 to 5.

The ejaculate, which satisfies the basic criteria, is then diluted and divided into a number of doses, which are packaged in the form of straws. The most common is the median dilution rate (1:10 - 1:15) and the doses of up to 20 million sperm count. The most commonly used diluent is AndroMed, and diluted semen is packed in vacuums in straws of a volume of 0.22ml, and then closed with ultrasound. Subsequently, a conventional method of deep freezing of the semen dose is applied. The first control of deep-frozen semen is carried out 24 hours after freezing when the percentage of progressively moving spermatozoa is determined, which should be at least 50% in the dose.

The analysis is based on the examination of the impact of the season of semen collection, and semen quality of bulls in the three-year period (2011 - 2013) and the one-year period (2014).

For the three-year period (2011 - 2013), data was collected from 3 bulls who produced between them 621 ejaculates, and for the purpose of the present analysis the year was divided into four seasons:

1) Winter (January, February, March)
2) Spring (April, May, June)
3) Summer (July, August, September)
4) Autumn (October, November, December)

For the one-year period (2014), data was collected from 9 bulls, which produced between them 326 ejaculates, and due to limited data, the year was divided into three seasons:

1) Winter (January, February)
2) Transitional period (March, April, May)
3) Summer (June, July)

The following properties of semen quality were analysed: 1) the volume of the ejaculate (in ml), 2) the concentration of spermatozoa (in 10^6/ml), 3) the motility of spermatozoa in the native ejaculate (grade 1 to 5), 4) the level of sperm dilution (sperm ratio: thinner), 5) number of doses of single ejaculate, 6) sperm motility after thawing (%). For these properties, the basic parameters of descriptive statistics (average, minimum, maximum, standard deviation) were calculated. By the method of variance analysis (F test), the influence of the ejaculate collection season on the semen quality was examined, and the significance of differences in the average values of the observed properties between the individual levels of observation was tested, by the least significant difference test (LSD - test). The number of ejaculates, whose quality parameters were analysed, was not the same for all investigated traits. The minimum values that the semen had to meet in order to be used for artificial insemination are: volume of ejaculate - 2 ml, concentration of spermatozoa - 800 x 10^6/ml, motility score – 4 - (75-80% of progressively moving spermatozoa), motility after thawing - 50 %. This decrease in the total number of ejaculates, which are finally included in the analysis, is about 50% of the total number of ejaculate collected, and there are huge differences between the bulls. Statistical processing of the received data was performed with the program "STATISTICA 6.0 StatSoft, 2001".

Results and Discussion

Table 1 shows the values of the examined properties for three bulls over a three-year period. During this period, the average volume of the ejaculate for these three bulls was 4.9 ml, the minimum ejaculate was 0.5 and the maximum 11 ml. The lowest concentration of spermatozoa was 100 x10^6/ml, and maximum 2780x10^6/ml, but the average was 1118.7x10^6/ml. Scores for the motility of spermatozoa in native ejaculate were from 1 to 5, while the average score was 3.5. The average dilution rate was 14, the minimum was 5 and the maximum level of dilution 27. The number of doses per ejaculate was on average 298.5; the lowest number of doses were 68, and the maximum of 730. The average motility of spermatozoa after thawing was 52.8%, minimum 10 and maximum motility 75%.

Table 1. Parameters of descriptive statistics for studied quality traits of bull semen in a three-year period

Trait	N	Average	Minimum	Maximum	Std.Dev.
Ejaculate volume, ml	621	4.9	0.5	11.0	1.58
Concentration (10^6/ml)	620	1118.7	100.0	2780.0	423.64
Motility, score	620	3.5	1.0	5.0	1.06
Dilution (semen : thinner)	386	14.0	5.0	27.0	3.41
Single ejaculate doses, number	386	298.5	68.0	730.0	122.77
Post-thawing sperm motility	374	52.8	10.0	75.0	11.92

Tables 2 and 3 show the average values and errors of the average for the tested traits depending on the season of the year. It is determined that the season significantly influences the following traits: the motility of spermatozoa in native ejaculate, the level of dilution and the motility of spermatozoa after thawing. The remaining three properties (volume of ejaculate, sperm concentration and number of doses) were not under the significant impact of the season.

Table 2. The impact of the season on the quality of bull semen in the three-year period (2011-2013)

Season	Ejaculate volume, average	Ejaculate volume, std. error	Concentration (10^6/ml), average	Concentration (10^6/ml), Std. error	Motility, average	Motility, std. error	N
1	4.7	0.117	1105.7	31.41	3.5	0.078	181
2	5.0	0.108	1170.6	29.23	3.7	0.073	209
3	4.9	0.179	1124.5	48.15	3.4	0.120	77
4	4.9	0.127	1060.4	34.16	3.3	0.085	153
F exp.	0.857 n.s.		2.085 n.s.		5.931***		620

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Table 3. The impact of the season on the quality of bull semen in the three-year period (2011-2013)

Season	Level of dilution, average	Level of dilution std. error	Number of doses, average	Number of doses, std. error	N	Post-thawing motility, average (%)	Post-thawing motility, std. error (%)	N
1	14.4	0.316	310.0	11.384	115	54.4	1.120	111
2	13.3	0.275	283.5	9.902	152	53.7	0.970	148
3	14.2	0.529	277.4	19.066	41	48.3	1.865	40
4	14.4	0.384	321.7	13.823	78	50.8	1.362	75
F exp.	2.949*		2.449 n.s		386	3.692*		374

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

The motility of spermatozoids in native ejaculate is the only studied trait on which the season had a very significant effect (p<0.001) as determined by the application of F test. The best average score of motility was achieved during the 2nd season (3.7), while the lowest score was recorded in the 4th season (3.3). After a significant seasonal effect on the mobility of spermatozoa was identified, LSD test was also conducted to determine the significance of the differences between the seasons. The results of the conducted test indicate that the values during the 2nd season were significantly different compared to the other seasons, while between the 1st and the 4th season there was no significant difference.

The average level of dilution was the best during the 1st and 4th season (14.4) while the worst was during the 2nd season (13.3). The analysis of the data showed a significant influence (p<0.05) of the season on the level of dilution. The LSD test was carried out which examined the significance of the differences between the seasons. The results of this test show that the 2nd season significantly differed ($p<0.05$) from the 1st and 4th season, while in other cases of mutual comparisons of the seasons, no significant difference was found.

By analysing the collected data, a significant influence ($p<0.05$) on the motility of sperm count after thawing was established, similar to results presented by *Orgal and Roth (2008)* and *Vilakazi and Webb (2004).* The average values for this trait were the highest in the 1st season (54.4%), and at lowest in the 3rd season (48.3%). After having determined the significant impact of the season, the LSD test was performed in order to check the significance of the differences between the seasons. The results of this test show that the 3rd season was very different ($p<0.01$) from the 1st and 2nd seasons, and that the 4th did not differ significantly. Season 4 was significantly different from the 1st season ($p<0.05$), while in all the remaining cases of mutual comparison there was no significant difference. The influence of the season on the operation of the PKB Corporation's Livestock Centre is reflected in the number of collected ejaculates per season, which is significantly higher during the spring compared to the summer (Table 2). Reduction in the number of collected ejaculates during the summer is carried out with the aim of minimizing the impact of heat stress on animals.

Table 4 shows the values of the examined properties for nine bulls, which they achieved in 2014. These data show that the average volume of the ejaculate was 5.1 ml, with the lowest volume being 1ml, and a maximum of 14 ml. *Rasbech (1983)* states that the bull's ejaculate volume varies from 2 to 10 ml. The average concentration of spermatozoa was 1067.8×10^6/ml of ejaculate, the lowest was 30, and the highest as high as 2600×10^6/ml. The data reported by *Miljković (1995),* which show an average concentration of spermatozoa of 1200×10^6/ml, are slightly higher than the results obtained in this analysis of the examined quality traits of bull semen in 2014Motility scores of spermatozoa in native ejaculate ranged from 1 to maximal 5, but the average score was 3.4. The level of sperm dilution was on average 16.3, but the variation of this property was remarkable. The lowest level of dilution was only 9, and the highest was 27. The number of doses ranged from 95 to 920, with an average of 368.8 doses per ejaculate. As stated by *Stančić (2014),* the modern dose of semen contains 12 - 15 $\times 10^6$ progressively motile spermatozoa corresponding to the results in this paper. The average motility of spermatozoa after thawing was 51%, the minimum was 10% and the maximum 75%. It can be concluded that sperm tolerated well the deep freezing, which justifies the use of artificial insemination in cattle breeding.

Table 4. Parameters of descriptive statistics for studied quality traits of bull semen in year 2014

Trait	N	Average	Minimum	Maximum	Std.Dev.
Ejaculate volume, ml	326	5.1	1.0	14.0	1.95
Concentration (10^6/ml)	326	1067.8	30.0	2600.0	490.73
Motility, score	326	3.4	1.0	5.0	0.87
Dilution (semen : thinner)	158	16.3	9.0	27.0	3.44
Single ejaculate doses, number	157	368.8	95.0	920.0	145.60
Post-thawing sperm motility, %	153	51.0	10.0	75.0	11.01

Table 5. The impact of the collection season on the quality of bull semen (2014)

Season	Ejaculate volume, average	Ejaculate volume, std. error	Concentration (10^6/ml), average	Concentration (10^6/ml), Std. error	Motility, average	Motility, std. error	N
1	5.4	0.238	1089.9	60.113	3.3	0.106	67
2	5.2	0.144	1068.3	36.473	3.4	0.064	182
3	4.8	0.222	1047.5	56.074	3.4	0.099	77
F exp.	$1.438^{n.s.}$		$0.133^{n.s.}$		$0.016^{n.s.}$		326

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Table 6. The impact of the collection season on the quality of bull semen (2014)

Season	Level of dilution, average	Level of dilution std. error	N	Number of doses, average	Number of doses, std. error	N	Post-thawing motility, average (%)	Post-thawing motility, std. error (%)	N
1	15.9	0.635	29	394.6	26.833	29	48.3	1.894	29
2	16.8	0.369	86	379.1	15.582	86	48.6	1.107	85
3	15.7	0.521	43	330.0	22.297	42	58.3	1.634	39
F exp.	$1.869^{n.s.}$		158	$2.1967^{n.s.}$		157	13.485***		153

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Tables 5 and 6 show the average values and standard errors for the examined traits depending on the season. Of all the examined traits, the only significant influence of the season was registered on the mobility of spermatozoa after thawing. In the other examined traits, the average values of the ejaculate properties were somewhat better in the first two seasons compared to the third season, but F test showed that these differences were not statistically significant (p>0.05). A very significant influence (p<0.001) of the season on the motility of spermatozoa after thawing was determined, which enabled the LSD test to determine the significance of the differences between the seasons. The results of the test showed that the 3rd season was very significantly different ($p<0.001$) from the 1st and 2nd seasons, while between the 1st and 2nd season there was no significant difference. The results of this analysis were probably affected by the shortening of the season in 2014 due to the reduced number of data, however, it is certainly interesting that the motility of spermatozoa after thawing during January and February (season 1) was below the average and amounted to 48.3% and during June and July (season 3) was as high as 58.3% (Table 6). Climatic conditions that define a season can be unpredictable as well as their effect on bulls, therefore testing the impact of the season on semen quality should include many years to make the available data relevant and comparable to other surveys.

Conclusion

Based on the obtained results of the research carried out at the Livestock Centre of the PKB Corporation on the influence of different factors on production and the quality of bull sperm, the following conclusions can be drawn:

- by analysing the data obtained during the three-year and one-year period, it was found that ejaculates on average met basic criteria with a higher or lower variation of properties,

- ejaculate collecting season exhibited a very high impact ($p<0.001$) on sperm motility in native ejaculate and a significant influence ($p<0.05$) on the motility of spermatozoa after thawing in a three year period; especially the summer season is highlighted by its negative impact on the motility of spermatozoa.

- in a one-year period, a very significant effect ($p<0.001$) of the season on the motility of spermatozoa after thawing was recorded; the summer season has shown a positive effect on this property; the justification for such a result probably lies in the specific effect of the climate in the year of testing,

- future studies could analyse the impact of the semen doses from different seasons on the success of the insemination of cows.

Uticaj sezone kolekcionisanja na kvalitet semena bikova holštajn-frizijske rase

Miloš Marinković, Predrag Perišić, Milan M. Petrović, Vlada Pantelić, Marina Lazarević, Nenad Mićić, Vladimir Živković

Rezime

Ovim istraživanjem je obuhvaćeno 9 bikova holštajn-frizijske rase iz Centra za stočarstvo PKB Korporacije i analiziran je uticaj sezone kolekcionisanja ejakulata na određene osobine semena (zapremina ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu, razređenje sperme, broj doza od jednog ejakulata, pokretljivost spermatozoida posle odmrzavanja). Za potrebe analize korišćeni su podaci iz trogodišnjeg (2011-2013.god) i jednogodišnjeg perioda (2014. god). U trogodišnjem periodu su na osnovu 621 ejakulata od tri bika izračunate prosečne vrednosti za ispitivane osobine i analiziran je uticaj sezone na ta svojstva. Rezultati pokazuju da je sezona kolekcionisanja ejakulata imala vrlo visoko značajan uticaj ($p<0,001$) na pokretljivost spermatozoida u nativnom ejakulatu, i značajan uticaj ($p<0,05$) na stepen razređenja ejakulata i pokretljivost spermatozioda posle odmrzavanja. U jednogodišenjem periodu su na osnovu 326 ejakulata od 9 bikova izračunate prosečne vrednosti ispitivanih svojstva i takođe analiziran je uticaj sezone. Sezona kolekcionisanja ejakulata ispoljila je vrlo visoko značajan uticaj ($p<0,001$) na pokretljivost spermatozoida posle odmrzavanja ali na ostala svojstva nije utvrđen značajan uticaj ($p>0,05$).

Ključne reči*:* bikovi, sezona, spermatozoidi, ejakulat, holštajn-frizijska rasa

Acknowledgment

The authors would like to thank the Livestock Centre of the PKB Corporation (Industrijsko naselje bb, 11213 Padinska Skela - Belgrade) for the cooperation with hope that it will continue in the future. Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053.

References

FUENTE L.F., SANCHEZ-GARCIA L., VALLEJO M. (1984): Reproductive characters in Galician Blonds. I. Characters of semen used for artificial insemination. Anal. Fac. Vet. Leon 30: 119–125.

MATHEVON M., BUHR M.M., DEKKERA J.C.M. (1998): Environmental, Management, and Genetic Factors Affecting Semen Production in Holstein Bulls, Journal of Dairy Science Vol. 81, No. 12: 3321 – 3330.

MENENDEZ-BUXADERA A., MORALES J.R., PEREZ A.P., GUERRA Y.D. (1984): Seasonal variation in semen production of Holstein, Zebu and Criollo bulls under artificial insemination conditions in Cuba. Reproduction des ruminants en zone tropicale. Reunion internationale, Pointe a Pitre, Guadeloupe, France, 8–10 June 1983. Les colloques de l'INRA, INRA, Paris, France 20:239–249.

MILJKOVIĆ V. (1995): Veštačko osemenjavanje životinja, udžbenik. Veterinarski fakultet Univerziteta u Beogradu, Beograd.

ORGAL S., ROTH Z. (2008): 13 Seasonal changes in ion concentration, ion-channel expression, acrosome integrity, and bovine sperm quality. Reproduction, Fertility and Development 21(1) 106-107.

RASBECH N. (1983): The Male and Fertility of domestic Animals, INRA, Nouzilly, France.

SCHAWB W., KUPFERSCHMIED H., BACHMANN P. (1987): Factors affecting semen production in bulls. Zuchthygiene 22: 241–246.

SÖDERQUIST L., JANSON L., HÅÅRD M., EINARSSON S. (1996): Influence of season, age, breed and some other factors on the variation in sperm morphological abnormalities in Swedish dairy A.I. bulls. Animal Reproduction Science, Volume 44, Issue 2, Pages 91-98.

STANČIĆ I. (2014): Reprodukcija domaćih životinja, udžbenik. Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad.

VILAKAZI D.M., WEBB E.C. (2004): Effect of age and season on sperm morphology of Friesland bulls at an artificial insemination centre in South Africa. South African Journal of Animal Science, Volume 34, Issue 1, p. 62 – 69.

COMPARISON OF DIFFERENT ENERGY SYSTEMS FOR DETERMINATION OF LUCERNE ENERGETIC VALUE IN DAIRY CATTLE DIET

Bogdan Cekić[1], Aleksa Božičković[2], Dragana Ružić-Muslić[1], Nevena Maksimović[1], Violeta Caro-Petrović[1], Nenad Mićić[1], Vladimir Živković[1]

[1]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: Bogdan Cekić, e-mail address: bogdancekic@gmail.com
Original scientific paper

Abstract: In this paper, samples of lucerne from three harvests were examined, with the aim of determining the net energy value (NEL) based on three different systems. The NRC 2001 system, the Yugoslav energy system and the energy system based on the measurement of In vitro gas production, were processed. Based on the chemical composition of the samples, the energy value for all samples was estimated. The highest nutritive values were recorded in samples of the third harvest. By comparing these three systems, it was concluded that both the highest and the lowest average NEL values were obtained in the NRC 2001 system, depending on the concentration of the total digestible nutrients (TDNconc) and the energy consumption level (DMI) (6.85 MJ/kg and 5.01 MJ/kg, respectively). In the Yugoslav energy system and in the system based on measuring In vitro gas production, there are no significant differences between harvests, as well as between the systems themselves. In addition to comparative testing, which compares systems, it is necessary to implement those that compare estimated energy values of nutrients and real animal performances.

Key words: lucerne, energy systems, net energy, dairy cows

Introduction

Nutrition of domestic animals, as one of the most important paragenetic factors, has great importance in modern and economically profitable breeding of domestic animals. The productivity of animals depends on the energy contained in the foods that these animals consume. Although this problem was known already in the XIV century, from the 1940s, more serious work began to determine the energy value of nutrients, as well as the development of energy systems. In modern ruminant nutrition, these systems can be conditionally divided into systems based on metabolic energy (ME) and net energy (NE).

Lucerne (*Medicago sativa*) is one of the most important forage crops. It is characterized by high productivity potential, excellent plant quality, high protein content, durability, longevity, drought and frost resistance, nitrogen fixation ability, soil desalination ability, the ability to be used by all ruminant species, as well as the possibility of complete mechanization of the production process. In the animal diet, lucerne is used in various ways: fresh, as hay, as silage and heylage, flour, pellets, pasta, Lucerne juice concentrate and others.

In the diet of dairy cattle it is almost irreplaceable due to its high content of proteins and calcium. That is why determining its net energy value is necessary for the optimal meal standardization. The net energy (NE) represents that part of the energy of a nutrient or diet that is retained in the body.

The aim of this research is to use different systems for estimating the net energy value of lucerne in order to compare the differences, both in the different phases of the development and harvest of the lucerne, as well as between the individual systems. In this paper the following systems were studied: NRC (2001), Yugoslav energy system (*Obračević, 1990*) and Energy system based on measurement of In vitro gas production (*Menke and Steingass, 1988*).

Material and Methods

Samples of alfalfa were collected in the fields of PKB Corporation, in the third year of its establishment. The field was founded in 2008, the spring sowing of "Banat" variety, and the lucerne was grown under conditions of dry cropping. Sampling was performed through three harvests, and within each cycle of vegetation, samples from the beginning to the end of the vegetation were included, as follows: the first harvest in the period 07.04.2010. - 15.05.2010, second harvest in the period 10.06.2010. - 18.07.2010 and the third harvest in the period 06.07.2010. - 13.08.2010. From each harvest, ten samples were taken for the calculation of the energy value.

Chemical analyses determined the content of crude protein (SP), crude ash (CA), crude fat (CF), crude fibre (CFi), fibres insoluble in neutral detergent (NDF), fibres insoluble in acid-detergent (ADF), lignin (ADL), crude proteins that insoluble in neutral detergent (NDICP), crude protein insoluble in acid detergent (ADICP). Chemical analyses were done according to *AOAC (2000)* and *ISO (2005; 2008)* standards.

In the paper, the determination of the energy value of lucerne based on 3 energy systems was carried out: 1) the National Research Council (NRC) system from

2001, 2) the Yugoslav system for determining the energy value of nutrients (*Obračević, 1990*) and 3) the energy system based on measurement of In vitro gas production (*Menke and Steingass, 1988).*
The energy value of nutrients, according to these systems summarily, is dependent on the nutritional value of the nutrient, which is most often expressed by the chemical composition, the digestibility associated with the vegetation stage in which the plant is being used, as well as the level of energy consumption and productivity of animals that consume lucerne.

With NRC norms, the energy requirements for maintenance, lactation, gestation and activity of cows are expressed in the form of net energy for lactation - NE_L. Also, the energy value of nutrients and meals is expressed as NE_L. Calculating the net energy value for lactation NE_L is done by calculating the total digestible nutrients in the nutrient (TDN), also through the content of digestible energy in the nutrient (DE_{1x}, and DE_p) and metabolic energy (ME_p). According to the *NRC system (2001),* the energy fractions of nutrients are not fixed, but vary depending on the level of consumption and the composition of the diet. Therefore, it is only possible to talk about the energy of a nutrient at a certain level, as follows: for the level of maintenance needs, the label "1×", i.e. for different fractions of energy $DE_{1\times}$, $ME_{1\times}$, $NEL_{1\times}$ is used. This concept is based on the fact that the increase in consumption reduces digestibility, and that the digestibility of individual nutrients depends on the composition of the meal in which the particular nutrition is used (*Božičković, 2013*). For this reason, when calculating ME and NE, a Discount coefficient is used to make the correction from $DE_{1\times}$ to DE_p. To determine the NEL, depending on whether the sample contains less than 3% CF, or 3% or more, two formulas are used

$$\mathbf{NE_{Lp}\ (Mcal/kg) = [0.703 \times ME_p\ (Mcal/kg)] - 0.19}$$

$$\mathbf{NE_{Lp}\ (Mcal/kg) = 0.703 \times ME_p - 0.19 + ([(0.097 \times ME_p + 0.19)/97] \times [EE - 3])}$$

Since in this norm the energy value is shown in Mcal/kg, for the comparison with other systems, conversion of calories to joules was made based on the following factor: 1 Mcal = 4.184 MJ.

The NEL calculation according to the Yugoslav energy system is characterized by the fact that the content of NEL in nutrients is calculated for a cow weighing 550 kg, which produces 15 kg of milk daily with 4% milk fat. The energy value of nutrients and meals, as well as the energy requirements are expressed in MJ/kg dry matter (DM). NEL is used not only in the nutrition of cows, but also in the nutrition of calves, breeding heifers, and male reproductive animals. The calculation of net energy in nutrients is based on the gross energy content, also the metabolic energy in the nutrient and the coefficient of its utilization in milk production. For the estimation of gross energy it is necessary to have the data on the content of certain groups of nutrients (CP, CF, CFi, Nitrogen Free Extractive Substances - NFE). In order to calculate ME, it is necessary to have data on the value of digestible proteins (DgP), digestible fats (DgF), digestible cellulose (DgC) and digestible nitrogen free extractive substances (DgNFE), expressed in g/kg. To calculate these digestible fractions, it is necessary to dispose with coefficients of digestibility. According to this energy system, there are different digestibility coefficients for the first and later harvests (II-IV), within each group, according to the vegetation stage of the samples, another 4 subgroups (before flowering, beginning to the middle of flowering, full flower, end of flowering). Calculation of the digestibility was carried out using adequate digestibility coefficients according to the vegetation stage of the samples. Based on the ratio of gross and calculated ME, the metabolism of energy is determined, which is necessary for calculating the coefficient of utilization of metabolic energy in milk production (k1), in cows with 15 kg milk and 4% of milk fat. This coefficient serves to calculate the NEL through the form

$$\mathbf{NEL = ME \times k_l}$$

Determination of net energy based on gas production was performed by the method of measuring gas production according to the *Menke and Steingass (1988)* in incubated liquid rumen content. First, it is necessary to calculate the percentage of the digestible organic matter, based on which the NEL is calculated

$$\mathbf{NEL = 0.81 + 0.0816 \times GP + 0.0046 \times XP + 0.0135 \times XL}$$

where NEL is expressed in MJ/kg DM, XP-CP in g/kg DM and XL - CF in g/kg DM. The amount of gas generated for 24 hours expressed in ml/200 mg of DM is indicated as GP.

Statistical analysis

The statistical analysis covered all samples, a total of 30. The test was performed by a single-factor analysis of variance (ANOVA), using software SPSS 20.0.

Results and Discussion

The chemical composition of the samples is shown in Table 1. The chemical parameters determined by the laboratory methods are CP, CA, CFi, NDF, ADF, ADL, NDICP, ADICP, CF, while the structural carbohydrates (NFC) have been calculated.

Within the individual harvest, there is a noticeable linear downward trend in CP, CA, CF, i.e. increase in CFi, NDF, ADF, ADL. Crude fats, as well as NFC, are most drastically decreasing in the 1st harvest. NFC is particularly interesting because, in its case, the trend of decrease of its value in the samples from the 1st and 2nd harvest are observed, while in the 3rd harvest there is almost no trend. In the 1st harvest, a clear linear downward trend for CP and CF was observed, while the CFi, NDF, ADF and ADL values showed an increase. The amount of CFi had the highest values in the second harvest, as well as the NDF. ADL had the highest growth trend in the 1st harvest. As for NDF and ADF associated proteins (NDICP, ADICP), the results are different. The trend of decrease in the NDICP value can be observed in the third harvest, while the values of ADICP show a lack of trend. However, the highest values of ADICP are expressed at the end of the first cut. This is very important because the protein bound to ADF is inaccessible to animals, since this is a lignified protein fraction (*Sniffen et al., 1992).* Lignification affects the digestibility of the cell wall, but not the digestibility of chemical components inside the cell wall *(Van Soest, 1994*). However, it can be concluded that the ADL value increases, while the NDICP value decreases with the process of the vegetation. If the increased amount of CP and NFC, i.e. decreased amount of NDF, ADF and ADL is viewed as a preferred nutrient trait, it can be concluded that the third harvest had a higher nutritional value compared to the first two harvests.

Based on the chemical composition of lucerne samples, the NEL values of these samples were calculated (Table 2), and the values compared between the systems.

Table 1. Chemical parameters of the quality of lucerne (in % dry matter)

Harvest	Sample	CP	CA	CFi	NDF	ADF	ADL	NDICP	ADICP	CF	NSC
I	5	28.74	10.29	15.50	23.43	17.64	2.56	2.93	0.77	4.86	35.62
	7	29.60	11.24	19.00	27.95	21.67	3.25	3.12	0.84	4.62	29.79
	18	21.22	9.64	29.10	38.12	30.80	5.26	2.52	0.77	3.94	29.60
	28	17.78	9.04	32.90	45.98	35.67	7.25	3.29	1.22	3.62	26.87
	43	15.87	8.23	31.10	45.89	37.10	7.03	2.57	1.04	2.59	29.99
	49	16.47	10.46	32.30	45.82	33.93	7.26	3.50	0.99	2.54	28.21
	52	17.37	8.06	33.70	48.49	33.94	7.39	4.50	0.77	2.44	28.15
	61	17.88	7.89	33.70	46.26	35.45	7.78	3.25	1.09	2.58	28.64
	63	18.25	7.55	31.90	46.05	37.15	7.17	2.55	1.50	2.42	28.28
	112	16.07	6.94	41.70	55.81	43.35	9.63	3.06	1.29	2.27	21.96
$\bar{x}$		19.93	8.93	30.09	42.38	32.67	6.46	3.14	1.03	3.18	28.71
Sd		4.84	1.35	7.17	9.32	7.24	2.04	0.56	0.23	0.93	3.18
CV (%)		24.29	15.11	23.84	21.99	22.16	31.61	17.86	23.22	29.32	11.07
II	65	25.96	10.31	18.20	29.84	24.04	3.48	2.28	0.96	4.23	31.94
	69	25.38	11.27	25.80	35.59	28.89	4.40	2.94	0.96	3.41	27.29
	73	21.65	8.26	33.10	43.00	34.73	5.74	3.08	1.04	3.50	26.67
	77	20.57	8.30	32.30	43.07	33.81	5.57	2.88	0.99	3.57	27.37
	84	20.24	7.94	37.40	44.95	36.53	7.15	2.47	1.06	3.54	25.80
	87	18.93	8.16	33.90	47.25	38.19	7.44	2.71	1.09	3.26	25.12
	98	17.75	8.48	33.00	48.57	39.32	7.67	3.02	1.17	2.60	25.62
	101	16.78	8.09	38.10	50.39	39.50	8.11	3.14	0.89	3.21	24.67
	103	16.02	8.34	38.60	48.65	39.61	8.58	2.34	0.92	2.76	26.57
	113	16.28	7.56	35.30	52.53	40.94	8.64	2.79	1.21	2.30	24.13
$\bar{x}$		19.96	8.67	32.57	44.38	35.56	6.68	2.77	1.03	3.24	26.52
Sd		3.37	1.11	5.94	6.63	5.13	1.70	0.29	0.09	0.53	2.08
CV (%)		16.90	12.77	18.23	14.92	14.45	25.43	10.57	9.62	16.31	7.84
III	102	36.54	11.05	12.70	22.87	15.48	2.27	5.03	0.64	4.41	30.16
	108	31.34	13.14	15.40	24.22	18.95	3.62	3.29	0.67	4.44	30.14
	110	29.63	11.94	18.60	29.17	20.71	3.51	4.36	0.88	4.17	29.45
	114	25.91	11.35	22.10	31.60	24.33	4.69	2.86	0.91	3.95	30.05
	123	20.95	9.67	27.80	40.06	29.21	5.71	3.82	1.03	3.11	30.03
	131	19.95	10.02	29.00	40.19	30.69	6.24	2.64	0.97	3.37	29.12
	135	19.95	9.21	30.80	42.50	32.28	6.46	3.10	1.33	3.21	28.23
	138	19.43	10.29	31.80	38.99	29.39	5.75	2.92	0.80	2.94	31.27
	140	19.34	9.17	34.40	41.93	31.47	6.44	2.41	0.75	3.05	28.93
	143	17.81	9.19	29.40	44.75	32.54	6.73	2.45	0.84	3.14	27.56
$\bar{x}$		24.09	10.50	25.20	35.63	26.50	5.14	3.29	0.88	3.58	29.49
Sd		6.41	1.34	7.48	7.98	6.19	1.54	0.87	0.20	0.60	1.08
CV (%)		25.24	12.12	28.17	21.24	22.15	28.35	24.97	21.73	15.83	3.46

** $\bar{x}$ – arithmetic mean; Sd – standard deviation; CV – coefficient of variation expressed in %*

In case of the NRC 2001 system, since the test was carried out on Lucerne as nutrient and not as a diet/meal, the values of 62%, 68%, 74% and 80% were taken for concentration of the total nutrients (TDNconc), while for energy consumption (DMI) the levels were taken that are two, three, and four times greater than their maintenance needs (2×, 3× and 4×).

With the increase in DMI levels, as well as TDNconc, the energy value of nutrients decreased. Based on the calculated parameters, the highest NEL values were observed for samples from the third harvest, at TDNconc of 62%, at the level of DMI 2×. This can be explained by the highest values of crude proteins and crude fats on the one hand, while at the same time lower values of NDF, ADF and ADL in the 3rd harvest, on the other hand. The lowest values of NEL were obtained in the 1st harvest, at TDNconc of 80% and at the level of DMI4×. There is a noticeable deviation of the 3rd harvest from the 1st and 2nd ($p<0.01$). When the same TDNconc and DMI are observed, the largest differences are between the 3rd and 2nd harvests, followed by differences between the 3rd and the 1st harvest, while the least differences were between 1st and 2nd. However, although the differences between the harvests (1st and 2nd, 1st and 3rd, as well as 2nd and 3rd) at the level of the same TDNconc and the same levels of consumption exist, the variance analysis found that they were not statistically significant ($p> 0.05$).

By comparing the NEL values from the same harvests, but with the same or different TDNconc, differences exist in certain cases. Thus, if the values of the same harvests are compared, and the same DMI, but different TDNconc, significant differences exist only at the level of 4× by comparing the highest and least TDNconc values (62% and 80%). If the values of different TDNconc, different DMI, but the same harvests are compared, variance analysis showed statistically significant differences in almost all cases when comparing DMI 2 × and 4 × in all harvests. No statistically significant differences were found in the analysis of variance when comparing the values of TDNconc 62% and 68% ($p> 0.05$).

In the Yugoslav energy system, the highest average NEL value was given to samples from the first harvest ($\bar{x}$ = 5.38 MJ/kg), while the lowest values were obtained for samples from the second harvest ($\bar{x}$ = 5.2 MJ/kg). The average energy value of the samples from the third harvest was very close to the average value of samples from the first (the absolute difference is 0.0122 MJ/kg). Using the analysis of variance, it was found that the differences in energy values between the harvests were not statistically significant, and based on these data it can be concluded that there were no statistically significant differences between the harvests in this energy system.

In the gas production system, the highest values of NEL were obtained in the third harvest ($\bar{x}$ = 5.83 MJ/kg), but by analysing the variance, it was found that there were no statistically significant differences in relation to the first or second harvest ($p>0.05$). Also, by comparing the values of NEL of the first and second harvests, statistically significant differences were not observed ($p> 0.05$). This means that the NEL value of lucerne in this system does not depend on the harvest in which it is mowed, similar to the Yugoslav energy system.

By comparing the NEL values of the same samples, evaluated on the basis of different systems, it is noted that the highest and lowest average values were obtained in the NRC 2001 system (Table 2). The highest average NEL values were obtained in the third harvest, with TDNconc 62% and DMI 2 × (hereinafter $NEL_{62\%2x}$). On the other hand, the lowest NEL values were observed in the 2nd harvest, with TDNconc 80% and DMI 4 × (hereinafter $NEL_{80\%4x}$).

By comparing the NEL value of samples from the first harvest, it was found that there were no significant differences between the systems. When comparing NEL values from the second harvest, significant differences were observed in the comparison of the NRC and the Yugoslav system, with TDNconc 62% and 68%, at all levels of the DMI.

The variance analysis found that there were statistically very significant differences between $NEL_{62\%2x}$ and values for the third harvest of the Yugoslav energy system ($p<0.01$). The same conclusions were reached in the analysis of the variance of $NEL_{62\%2x}$ and values for the third harvest in the gas production system ($p<0.01$). Statistically very significant differences were observed in the comparison of the 3rd harvest, the NRC and the Yugoslav system, while in the comparison of the NRC and the gas production system, very significant differences were observed only at TDNconc 62%.

Table 2. Average NEL values of samples by harvests, based on NRC 2001, Yugoslav energy system and gas production system expressed in MJ/kg DM

			1st harvest			2nd harvest			3rd harvest		
NEL MJ/kg	**TDNconc**	**DMI**	□	**Sd**	**CV (%)**	□	**Sd**	**CV (%)**	□	**Sd**	**CV (%)**
NRC 2001	**62%**	**2x**	6.28	1.01	16.09	6.18	0.70	11.25	6.85	0.86	12.62
		3x	6.16	0.99	16.17	6.07	0.69	11.31	6.72	0.85	12.68
		4x	6.04	0.98	16.26	5.95	0.68	11.37	6.59	0.84	12.74
	68%	**2x**	6.15	0.99	16.18	6.06	0.69	11.31	6.71	0.85	12.68
		3x	5.91	0.97	16.35	5.82	0.67	11.43	6.45	0.82	12.81
		4x	5.67	0.94	16.55	5.58	0.65	11.57	6.20	0.80	12.94
	74%	**2x**	6.05	0.98	16.25	5.96	0.68	11.36	6.6	0.84	12.73
		3x	5.70	0.94	16.52	5.61	0.65	11.55	6.23	0.81	12.92
		4x	5.35	0.90	16.82	5.27	0.62	11.76	5.86	0.77	13.14
	80%	**2x**	5.95	0.98	16.38	5.87	0.67	11.45	6.51	0.83	12.78
		3x	5.52	0.92	16.67	5.44	0.63	11.65	6.04	0.79	13.03
		4x	5.09	16.67	17.08	5.01	0.60	11.94	5.58	0.74	13.32
YU energy system	-	-	5.38	0.65	12.02	5.20	0.51	9.72	5.36	0.58	10.89
Gas production	-	-	5.59	0.68	12.14	5.62	0.45	8.06	5.83	0.54	9.181

** *$\bar{x}$ – arithmetic mean; Sd – standard deviation; CV – coefficient of variation expressed in %*

This can be explained by the fact that the NEL value in the NRC system decreases with the increase of TDNconc and DMI, and the value of NEL in the Yugoslav energy system decreases with the aging and maturation of the plant, or depending on the vegetation phase of the plant.

However, the analysis of variance of NEL values from the third harvest of the Yugoslav energy system and gas production system did not show statistically significant differences ($p>0.05$). The same conclusion was made when comparing the values of the first, i.e. the second harvest of these two systems. This indicates that there are no statistically significant differences between the Yugoslav energy system and the gas production system.

In order to check the estimated NEL value of lucerne, it is necessary to test the precision of energy systems based on animal productivity. *Weiss (2001)* tested the precision of the NRC 2001 by calculating the energy consumption of cows in lactation and energy needs and the use of this energy. In this test, the author relied on results from 25 papers and concluded that the 2001 NRC system was very

precise in calculating energy input, and on average it only showed 2% more than real values. On the other hand, *VandeHaar (2002),* by examining the formulation of the meal, noticed several problems in the NRC 2001 system. In his paper, it is stated that the value of the protein is overestimated, because it is estimated that the digestibility of protein nutrients is about 60%, while the real values are 30-40%. Lignin affects the digestibility of NDF, but the effect is variable depending on the type of meal. Feeds whose digestibility has been determined according to the NDF In vitro method (48 hours) are not directly compatible with the calculated NDF digestibility value (*VandeHaar, 2002*).
There is insufficient data in the expert literature on the validation of the method of nutrient energy value calculated on the basis of the Yugoslav energy system and the gas production system, and in the future period it is necessary to carry out more comparative tests, which, in addition to the evaluation of the energy value of the nutrients, will include the real production of animals. The best method of checking the energy value of food is the comparison of the determined values with the real energy balance.

Conclusion

Based on the collected samples, the samples collected in the third harvest were found to have the highest nutritional value.
The highest NEL values were assessed in the NRC 2001 system, in the 3rd harvest, at the consumption level 2 × and TDNconc 62%.
In case of the Yugoslav energy system, the highest average NEL values were obtained in the 1st harvest, while the lowest were in the second. The average NEL value of the 3rd harvest was very close to the average value for the 1st (difference 0.0122 MJ/kg). The analysis of the variance determined that there were no statistically significant differences between the energy values of different harvests. The values of the samples were more than those given in the norms.
In the gas production system, the highest values were obtained in the 3rd harvest. However, the variance analysis found that there was no significant statistical difference between the harvests ($p > 0.05$).
By comparing these three systems, it was concluded that both the highest and lowest average NEL values were obtained in the NRC system, with different TDNconc and DMI (6.85 MJ/kg and 5.01 MJ/kg, respectively).
There is not enough literature on the precision of the Yugoslav energy system and the gas production system. Surely it is necessary to be careful when making conclusions. More comparative studies, such as those that compare systems, and

those that compare the estimated energy values of nutrients and real animal production, are necessary.

Acknowledgment

This research was funded through Project III 46012 by the Ministry of Education, Science and Technological Development of the Government of the Republic of Serbia.

Poređenje različitih energetskih sistema za određivanje energetske vrednosti lucerke u ishrani mlečnih krava

Bogdan Cekić, Aleksa Božičković, Dragana Ružić Muslić, Nevena Maksimović, Violeta Caro-Petrović, Nenad Mićić, Vladimir Živković

Rezime

U radu su ispitivani uzorci lucerke iz tri otkosa, sa ciljem određivanja neto energetske vrednosti (NEL) na osnovu tri različita sistema. Obrađeni su NRC 2001 sistem, jugoslovenski energetski sistem i energetski sistem zasnovan na merenju In vitro produkcije gasa. Na osnovu hemijskog sastava uzoraka procenjena je energetska vrednost za sve uzorke. Najveću hranljivu vrednost su imali uzorci iz trećeg otkosa. Komparacijom ova tri sistema, došlo se do zaključka da su i najveće i najmanje prosečne NEL vrednosti dobijene u NRC 2001 sistemu, u zavisnosti od koncentracije pravo svarljivih hranljivih materija (TDNconc) i nivoa konzumacije energije (DMI) (6,85 MJ/kg, odnosno 5,01 MJ/kg, redom). U jugoslovenskom energetskom sistemu i u sistemu zasnovanom na merenju In vitro produkcije gasa ne postoje značajne razlike između otkosa, kao ni između samih sistema. Potrebno je pored komparativnih ispitivanja, koji upoređuju sisteme, sprovesti i one koji upoređuju procenjene energetske vrednosti hraniva i realnu proizvodnju životinja.

Ključne reči: lucerka, energetski sistemi, neto energija, mlečne krave

References:

AOAC (2000): Official Methods of Analysis, 17th ed., Association of Official Analytical Chemists, Arlington, VA, USA

BOŽIČKOVIĆ, A. (2013): Procena hranljive vrednosti lucerke u ishrani preživara na osnovu morfoloških parametara. Doktorska disertacija, Univerzitet u Beogradu, Poljoprivredni fakultet

ISO (2005) Animal feeding stuffs - Determination of nitrogen content and calculation of crude protein content - Part 2: Block digestion/steam distillation method. ISO 5983-2, 14 pp

ISO (2008): Animal feeding stuffs - Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents. ISO 13906, 17 pp.

MENKE, K.H., STEINGASS, H. (1988): Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid; Animal Research and Development, vol. 28, pg. 7–55.

NRC – National Research Council (2001): Nutrient Requirements of Dairy Cattle, 7th rev. ed., National Academy Press, Washington, D.C.

OBRAČEVIĆ, Č. (1990): Tablice hranljivih vrednosti stočnih hraniva i normativi u ishrani preživara, Naučna knjiga, Beograd

SNIFFEN, C.J., O'CONNOR, J.D., VAN SOEST, P.J., FOX, D.G., RUSSEL, J.B. (1992): A net carbohydrate and protein system for evaluation cattle diets. II. Carbohydrate and protein avaliability; Journal of Animal Science, vol. 70, no. 11, pg. 3562-3577

SPSS: IBM SPSS Statistics 20. 1 New Orchard Road, Armonk, IBM.

VANDE HAAR, M. J. (2002): Energy and protein in the 2001 Dairy NRC: Challenges for a ration formulation program, Tri-State Dairy Nutrition Conference, pg. 81-98

VAN SOEST, P. J. (1994): Nutritional Ecology of the Ruminant; 2nd ed. Cornell University Press, Ithaca, NY

WEISS, W. P. (2001): Dairy NRC 2001: Energy system; Proceeding of Four-State Applied Nutrition and Management Conference, pg. 37-42

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

BENTONITE IN NUTRITION OF DAIRY CATTLE

Nenad Mićić [1], Goran Grubić [2], Milan M.Petrović [1], Vlada Pantelić [1], Bogdan Cekić [1], Miloš Marinković [1], Marina Lazarević [1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: micicnenad@hotmail.com
Original scientific paper

Abstract: There are numerous methods for decontamination of animal feed, and a relatively inexpensive method is the application of inorganic adsorbents. Adsorbents from the group of alumosilicates are particularly important, especially bentonite. Inorganic adsorbents are able to detoxify foods contaminated with mycotoxins by their adsorption power, while minimizing the negative effects on the organism. The aim of this study was to examine and determine whether the use of natural bentonite has an effect on adsorption of aflatoxins from food. The influence of daily intake of bentonite at different doses (30 and 50 g / head) on the content of aflatoxin M_1 (AFM_1) in milk of examined cows was examined. By mycotoxicological analysis of the feed it was determined that maize grains were infested with aflatoxin B_1 (1.24 μg/kg) and that this was the cause of the AFM_1 metabolite in raw milk of cows. Based on the results obtained, it was concluded that the content of AFM_1 in milk of cows consuming food with a daily intake of 30 and 50 g bentonite was significantly reduced.

Key words: dairy cows, aflatoxins, bentonite

Introduction

A significant problem in the field of intensive livestock production is the occurrence of mycotoxycoses that are directly reflected as a problem of contamination of raw milk, reduced growth, condition and preservation of health of all categories of cattle.

In some years, natural conditions (high temperatures during the summer, heavy rainfall during autumn, etc.) can be very suitable for the development and activity of the muolds. In addition to the detection of mycotoxins in food, it is also important to specify their amount (*Žust et al., 1989).* About 30-40% of all moulds create mycotoxins dangerous for humans and animals. The most common are moulds from the genera *Aspergillus, Penicillium, Fusarium, Stachybotrys* and others. Apart from the general toxicity to the cells of an organism, some mycotoxins especially have pronounced effect on the liver (hepatotoxicant), the kidneys (nephrotoxins), nerves (neurotoxins), hematopoiesis (hematotoxins), heart (cardiotoxins), or digestive organs (gastrointestinal toxins). Some have a specific cytostatic, carcinogenic, mutagenic, teratogenic, emetic, immunosuppressive, estrogenetic or photosensitive effect (*Djordjevic and Dinić 2011*). The main producers of aflatoxin are *Aspergillus* species, in particular *A. flavus, A. parasiticus, A. orizae* and others. In addition, they are synthesized by some of the *Penicillium* and *Rhizopus* species. There are several aflatoxins in animal feed: B_1, B_2, G_1, G_2, and colostrum and milk secrete their metabolites M_1 and M_2. Aflatoxins inhibit the synthesis of DNA and RNA (*Diekman and Green, 1992*). It is believed that Aflatoxin B_1 is one of the strongest known hepatic carcinogens, which causes liver cancer in animals and humans, and also has mutagenic, teratogenic, and immunosuppressive action (*Jurić and Pupavac, 1994, Jurić et al., 2003*). Biochemical blood tests show a change in the activity of individual enzymes, which is a sign of damage to the liver (*Đorđević and Dinić, 2011*). *Škrinjar et al. (2011)* state that 91% of feeds for cows are contaminated with moulds of 20 genera and 72 types of moulds. Also in the tropical region with extremely hot conditions for contamination of the fodder with aflatoxin B_1, aflatoxin M_1 occurs in milk of cows after 10 hours subsequent to the consumption of food (*Sumantri et al., 2012*). Aflatoxin M_1 as a natural metabolite of aflatoxin B_1, which occurs in the liver of animals, is excreted in the milk, feces and urine (*Polovinski-Horvatović et al., 2009-a*). EU legislation is one of the most restrictive in regard to this mycotoxin and allows the concentration of 0.05 mg/litre, while in other countries of Europe, Asia, Africa and America ten times the amount of 0.5 g/l (*Polovinski-Horvatović et al., 2009-b*) is permitted.

Bentonite is an aluminosilicate clay made of colloidal and plastic materials, predominantly of montmorillonite minerals. The toxic effect of clay has not been recorded so far, but the elimination ability of toxins is observed (*Stojiljković, 2010*). Sodium bentonite as a binding agent improves the quality of the pellets, in the pelleting of animal feed, it has a positive effect on the utilization of ammonia nitrogen in the rumen of ruminants, positive effect on the ratio of the concentration of acetates and propionates in the rumen, exerts a buffering effect in the rumen and absorbs efficiently mycotoxins present in feeds. Sodium bentonite is included in the mixture for the appropriate categories of cattle when using diets with a high proportion of the concentrate, and diets with a high protein content particularly degradable in the rumen (*Stojanović et al., 2008*). The effects of using bentonite concentrates for calves, indicate realized higher average daily gain and higher average feed intake, better conversions and a higher pH of the rumen at the age of 80 and 120 days (*Stojanović et al., 2009, Adamović et al., 2011- Adamović et al., 2011-b, Adamović, 2005*). Bentonites, as well as zeolites, significantly influence the degree of acidity of the rumen and blood parameters (*Nešić et al., 2010)*. The positive effects of the use of bentonite are also shown for the detoxification of the organism and the prevention of diarrhea of animals of all species (*Trackova et al., 2004*). Bentonite reduces the level of radioactivity in animal feed and their products (*EFSA Journal 2010*), the milk has a higher content of calcium, phosphorus and glucose, while the value of urea in the blood is lower (*Radivojević et al., 2010*). Aluminosilicate clays (Min-a-Zel) in the sugar beet pulp silage, 60 days after ensiling, increase the lactic acid production of silage, reduce the content of acetic acid and pH (*Koljajić et al., 2003*), very significantly influence all the parameters of the maize silage quality, its chemical composition and biochemical changes (*Đorđević et al., 2006*).

HSCAS (hydrated sodium calcium aluminosilicate) in diet for dairy cows reduces the concentration of aflatoxin M_1 in milk, similarly also in the nutrition of lambs (*Harvey et al., 1991*), i.e. it immobilizes mycotoxins in the gastrointestinal tract of animals (*Phillips et al., 1990*). Bentonite is a good transporter of immunomodulators and vitamin complexes (vitamins A, C, E), and a reliable mould antagonist (*Neustroy and Tarabukin, 1995*).

Bentonite can adsorb the harmful gases (ammonia and carbon dioxide) and thus improve the microclimate of livestock facilities (*Avakumović et al., 1990*). Bentonite is material often used in environmental protection and water treatment (*Ranđelović et al.2011*).

Due to the possible risk of the occurrence of increased concentrations of aflatoxins M_1 in cow's milk, the aim of this study was to determine the effect of the application of bentonite in animal feed on the production performance, the health condition and the quality of the milk of the examined cows.

Material and methods

The trial studies were carried out at the farm of Holstein-Friesian cows in Bečej, where the animals were reared in the free housing system. Within 40 days, starting from mid-January 2014, the quality of milk obtained from cows at different stages of lactation was examined. According to the AT_4 method of milk control, average milk fat content and crude protein content were examined. The average milk fat content was determined by the Gerber method (*Rulebook on methods for sampling and methods of chemical and physical analyses of milk and milk products*

- *"Official Gazette of SFRY", No. 32/83*), and the content of raw proteins was determined by the method of total combustion.

The fodder base for feeding of dairy cows throughout the year on the farm is: whole maize plant silage and triticale silage, alfalfa hay, sugar beet noodles, sugar beet pulp, maize and wheat grains, soya cake, sunflower grain, bran, retread palm fats, sodium-carbonate ($NaCO_3$) and premixture for dairy cows. Meal is prepared in the form of TMR (*total mixed ration*), twice a day, with balanced cows' needs for daily production of 35-38 kg of milk.

The random samples of four kinds of feeds (maize silage, sunflower meal, sugar beet pulp and the maize kernel) intended for the feeding of the test group of dairy cows were analysed to determine the content of aflatoxin B_1 (AFB_1), at the beginning of the trial, prior to supplementation of the diet with bentonite. Aflatoxin M_1 content (AFM_1) was determined in the milk of test dairy cows from the samples of milk from animals that consumed feed without the addition of the bentonite and milk samples from animals that have received food containing the bentonite. AFB_1 and AFM_1 analyzes were performed according to the ELISA method.

Biochemical analysis of blood, which comprised the determination of the concentration of glucose, total protein, total β-hydroxy butyric acid (BHBA), the concentration of total bilirubin, total albumin, the concentration of the urea, and the concentration of calcium and phosphorus minerals was carried out on the eight randomly selected animals from free housing system. All the parameters were analysed from blood, except for glucose derived from blood serum. The comparison of the obtained values was performed according to "The Merck Veterinary manual" (www.merckmanuals.com) based on reference values. Blood samples for analysis of the metabolic profile were taken at the beginning and at the end of the experiment, by the method of puncture the tail vein (*lat. vena coccigea*) with manual compression in vacutainer tubes. Statistical analysis of the obtained data of the analysis of blood was carried out with the program package "Statistica Statsoft V. 6, 2003" (www.statsoft.com).

Results and discussion

Table 1 shows the results of the analysis of aflatoxin B_1 (AFB_1) in feed for the examined dairy cows sampled prior to the addition of bentonite. The average level of AFB_1 in all tested feed samples did not exceed the maximum allowed limit (5 μg/kg) according to the Rulebook on the quality of animal feedingstuffs of the Republic of Serbia (*Official Gazette of the Republic of Serbia, 4/2010, and 27/2014 113/2012*). Among the examined samples of dairy cow feeds, the highest content of AFB_1 was found in maize kernel samples (1.24 μg/kg) (Table 1).

Table 1. Average content (Mean level) of AFB_1 in examined samples of feed for dairy cows

Feed for dairy cows	AFB_1 (µg/kg)
Maize silage	< 0.03
Sunflower meal	< 0.03
Sugar beet pulp	< 0.03
Mize, kernel	1.24

In the milk samples tested, the average level of aflatoxin M_1 (AFM_1) was not above the maximum allowed limit (0.05 µg/kg) according to the Rulebook on the amendment of the Rulebook on maximum permitted quantities of residues of plant protection products in food and animal feed and on food and animal feed for which maximum residue limits for plant protection products are determined (*Official Gazette, 2014*) (Table 2).

Table 2. Average content (Mean level) of AFM_1 in examined samples of milk from trial dairy cows

Date of sampling	AFM_1 (µg/kg)	The amount of daily bentonite intake (g)
06.12.2013.	0.303	0
10.01.2014.	0.390	0
07.02.2014.	0.150	30
20.02.2014.	0.126	50

Bentonite has shown the ability and efficacy of aflatoxin adsorption, but not completely, for several reasons; bentonite, although it has a 70-90% share of montmorillonite, can not bind to all mycotoxins in its crystal lattice, it is not selective for aflatoxin but also for other mycotoxins, as well as all other free radicals from the animal organism. This is similar to the results of *Harvey et al. (1991)* and *Phillips et al., (1990).* Other authors have confirmed that bentonite adsorbs many vitamins, minerals and organic molecules with a free group (*Neustroy and Tarabukin, 1995, Tomašević - Čanović et al., 2000*).

During regular monthly AT_4 milk controls, milk production, average milk fat content and average content of raw milk proteins were monitored.

Table 3. Milk performance parameters during the trial

Periods within the trial	Number of milking cows	Average milk yield (kg)	Average milk fat content (kg)	Average protein content (kg)	Daily bentonite intake (g)
I - 10 days	81	40.80	4.55	3.61	0
II - 10 days	70	41.44	4.54	3.62	0
III - 10 days	82	48.16	4.39	3.48	30
VI - 10 days	91	47.12	4.26	3.59	50

The content of bentonite slightly affected the higher milk yield and protein content, and did not have a significant effect on fat content, which decreased with the stage of cow lactation.

Table 4. Metabolic profile of cows, initial and final

Trial phase	Number of animals	Glucose	BHBA	Bilirubin	Proteins	Albumins	Urea	Calcium	Phosphorus
	Ref. values	2,2-4,2 mmol/l	0,7-1,0	0,7-8,5 µmol/l	58-81 g/l	28-40 g/l	2,0-7,5 mmol/l	2,0-3,0 mmol/l	1,4-2,7 mmol/l
Beginning	**Average**	**1,443**	**0,683**	**3,900**	**77,493**	**36,843**	**5,750**	**3,335**	**1,799***
	St. dev.	0,397	0,251	2,300	4,341	3,607	1,349	0,255	0,235
	St. error	0,141	0,089	0,813	1,535	1,275	0,477	0,090	0,083
End	**Average**	**1,339**	**0,836**	**5,486**	**77,660**	**37,205**	**4,509**	**3,255**	**2,125***
	St. dev.	0,667	0,248	1,159	4,146	2,668	1,031	0,088	0,335
	St. error	0,236	0,088	0,410	1,466	0,943	0,365	0,031	0,118

* Differences are statistically significant (P=0.04)

The values of the metabolic profile parameters were within the limits of the reference values, similarly to *Nešić et al., (2010).* Glucose was at a somewhat lower level because all selected dairy cows were in the lactation. Total β-hydroxybutyric acid (BHBA) was within normal limits. Cows at the beginning of lactation have an indicative level of β-hydroxybutyric acid, sometimes even higher than 1.20 mmol/l in blood, which is characteristic of subclinical ketosis (*Đoković et al., 2013*), and for this reason this parameter has been monitored. If the concentrations of albumin, glucose, β-hydroxybutyric acid (BHBA) and calcium are significantly below normal values, the metabolic profile can be predicted and then confirmed by incidence of fatty livers in the cows after calving (*Šamanc et al., 2011*). The concentration of bilirubin in the blood of all sampled cows was within the limits of the reference values. Proteins, albumin and urea were also within optimal limits. Values for calcium were over 3.0 mmol/l in all examined cows, the reason being that the basic roughage feed was alfalfa hay. Thus, the needs of cows in calcium were overrated. Phosphorus values were in the range of average values.

After 40 day trial and consumed amounts of bentonite, the blood of the same cows was taken again for the analysis of the metabolic blood profile (Table 4).

The glucose values remained below the average values for almost all animals, and in case of cow 8 the value was within the limits of the average values. The total β-hydroxybutyric acid (BHBA) was slightly increased in animals 1 and 5, and decreased in animal 8. The values shifted slightly towards the upper limit during the duration of the experiment, but no cows entered the state of the metabolic imbalance or ketosis. This is also confirmed by the parameters of bilirubin and urea, which kept their values in line with the reference. For all proteins and albumin, the values were optimal, except for slight transitions of animals 4 and 8 towards the upper limit. The calcium intake was too high, and phosphorus changed its values. Analysis of the variance of the obtained data (Table 4) showed that there were no statistically significant differences between the observed parameters, except for the phosphorus share in the blood. At the end of the experiment, there was significantly more phosphorus in the blood of 2.125 mmol/l ($P = 0.04$) than at the beginning 1.799 mmol/l, although the values in both cases were within physiologically normal limits.

Conclusion

- The examined food for the dairy cows in which the bentonite was added was balanced according to the cow requirements and contained all the necessary ingredients for satisfying the nutritional needs of the cows.
- The mycotoxicological analysis of feeds used in the diet for dairy cows, showed that only maize kernels were infected with AFB_1 (1.24 μg / kg) and that this was the cause of AFM_1 in raw milk of the animals from the examined dairy farm.
- It has been confirmed that daily intake of bentonite in the amount of 30 and 50 g/animal significantly reduces the content of AFM_1 in milk. Both doses of bentonite administered during the experiment had a similar AFM_1 adsorption effect.
- The effect of bentonite added to the dairy cow's diet did not show significant influence on milk parameters and on the biochemical parameters of the blood, condition and health condition of the examined dairy cows. The obtained values of all examined blood constituents were within normal physiological limits.
- Based on the conducted tests, it can be concluded that the use of bentonite, as an inorganic mycotoxin adsorbent, is one of the important and justifiable preventive measures in reducing the content of aflatoxins in the food chain.

Bentonit u ishrani muznih krava

Nenad Mićić, Goran Grubić, Milan M.Petrović, Vlada Pantelić, Bogdan Cekić, Miloš Marinković, Marina Lazarević

Rezime

Postoje brojne metode kojima se može izvršiti dekontaminacija stočne hrane, i relativno jeftin metod jeste primena neorganskih adsorbenata. Poseban značaj pripada adsorbentima iz grupe alumosilikata, u okviru njih i bentonitu. Neorganski adsorbenti su u stanju da svojom adsorpcionom moći izvrše detoksikaciju hrane kontaminirane mikotoksinima, a da pri tom negativni efekti na organizam budu što manji.
Cilj ovoga rada je bio da se ispita i utvrdi da li upotreba prirodnog bentonita ima efekat na adsorpciju aflatoksina iz hrane. Ispitivan je uticaj dnevnog unosa bentonita u različitim dozama (30 i 50 g/grlu) na sadržaj aflatoksina M_1 (AFM_1) u mleku ispitivanih krava. Mikotoksikološkom analizom hraniva utvrđeno je da je zrno kukuruza infestirano aflatoksinom B_1 (1,24 µg/kg) i da je to uzrok pojave metabolita AFM_1 u sirovom mleku krava. Na osnovu dobijenih rezultata zaključeno je da je značajno smanjen sadržaj AFM_1 u mleku krava koje su konzumirale hranu sa dnevnim unosom bentonita od 30 i 50 g.

Ključne reči: muzne krave, aflatoksini, bentonit

Acknowledgment

The research was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia – project No 31053 The authors would like to thank Bentoproduct d.o.o., Šipovo, Bulevar Vojvode Stepe Stepanovića 181c, 78000 Banjaluka, and dairy farm Džigurski from Bečej.

References

ADAMOVIĆ M., BOČAROV-STANČIĆ ALEKSANDRA, RADIVOJEVIĆ M. (2011-a): Uticaj 'Bentopela' na kvalitet i efikasnost korišćenja peletiranih krmnih smeša. Journal on Processing and Energy in Agriculture, 15, 2, 74-78.

ADAMOVIĆ M., ŠAMANC H., STOJIĆ V., VUJANAC I., GRUBIĆ G., STOJANOVIĆ B., LEMIĆ J. (2005): Mineralne materije - regulatori elektrohemijske reakcije sadržaja buraga visokomlečnih krava. Poljoprivredne aktuelnosti, 3-4, 81-96.

ADAMOVIĆ M., BOČAROV-STANČIĆ A., PANTIĆ V., VUKIĆ-VRANJEŠ M., JOVANOVIĆ R., PANIĆ M. (2011-b): Effect of pelleting agent on microbiological and mycotoxicological safety of feed mixtures. XXVII Biotechnology in Animal Husbandry, 1209-1217.

AVAKUMOVIĆ Đ., JANKOVIĆ B., MARKOVIĆ S., VUKIĆEVIĆ O., SABO D. (1990): Iskustva u primeni zeolita u periodu odgoja prasadi od 6-25kg, s obzirom na proizvodne rezultate i zdravstveno stanje prasadi. Zbornik radova – X skup svinjogojaca Jugoslavije, Pančevo, 291-294.

DIEKMAN A. M., GREEN L. M. (1992): Mycotoxins and reproduction in domestic livestock. Journal of animal science, 70, 1615-1627.

ĐORĐEVIĆ N., GRUBIĆ G., ADAMOVIĆ M., STOJANOVIĆ B., SIMIĆ A. (2006): Uticaj dodavanja minazela plus na kvalitet silaže kukuruza oplemenjenih neorganskim i organskim azotom. XXII Biotechnology in Animal Husbandry, p. 549-559, Beograd- Zemun.

ĐORĐEVIĆ N., DINIĆ B. (2011): Proizvodnja smeša koncentrata za životinje. Institut za krmno bilje Kruševac, Kruševac.

ĐOKOVIĆ R., KURČUBIĆ V., ILIĆ Z., PETROVIĆ M.D., STOJKOVIĆ J., MILOŠEVIĆ B., CINCOVIĆ M. (2013): Metabolički status mlečnih krava simentalske rase za vreme tranzicionog perioda. Biotechnology in Animal Husbandry, vol. 29, 1, 29-36.

EUROPEAN FOOD SAFETY AUTHORITY (EFSA), (2010): Scientific Opinion on the safety and efficacy of bentonite as a technological feed additive for all species. EFSA Panel on Additives and Products or Substances used in Animal Feed EFSA Journal, Parma, Italy.

HARVEY B.R., PHILLIPS D.T., ELLIS J.A., KUBENA F.L., HUFF E.W., PETERSON H.D. (1991): Effect on aflatoxin M1 residues in milk by addition of Rydrated sodium calcium aluminosilicate to aflatoxin contaminated diets of dairy cows. Amer. J. Vet. Res. 52:1556.

JURIĆ V., PUPOVAC V. (1994): Uticaj mikotoksina na kvalitet stočne hrane. Savetovanje agronoma, veterinara i tehnologa Jugoslavije: „Kvalitet stočne hrane u svetlu novih propisa“. 7-9. decembar 1994. Zbornik referata, 121-127.

JURIĆ VERICA B., RADANOV-PELAGIĆ VESELINA T., JAJIĆ I. (2003): Aflatoksin M_1 u mleku i siru. Savremena poljoprivreda, vol. 52, 3-4, 343-346.

KOLJAJIĆ V., ĐORĐEVIĆ N., GRUBIĆ G., ADAMOVIĆ M. (2003): The influence of zeolite on the quality of fresh beet pulp silages. Journal of Agricultural Sciences vol. 48, No 1, 2003, p. 77-84. Belgrade-Zemun.

NEUSTROYEV M.P., TARABUKINA N.P. (1995): Perspectives of zeolite use in veterinary medicine. Internacional Simposium and Exhibition on Natural Zeolites, Sofia, 114-115.

NEŠIĆ S., GRUBIĆ G., ADAMOVIĆ M., ĐORĐEVIĆ N., STOJANOVIĆ B., BOŽIČKOVIĆ A. (2010): The use of zeolite as zearalenone adsorbent in the nutrition of calves. Cuban Journal of Agricultural Science, 44, 3, 221-225.

PHILLIPS D.T., KUBENA F.L., HARVEY B.R., HUFF E.W., CORRER E.D., ROTTINGHAUS E.G. (1990): Efficacy of a sodium calcium aluminosilicate to reduce the toxity of aflatoxin and T-2 toxin. Poultry Science 69: 1078-1086.

POLOVINSKI-HORVATOVIĆ MIROSLAVA S., JURIĆ VERICA B., GLAMOČIĆ D. (2009-a): Učestalost pojavljivanja aflatoksina M_1 u konzumnom mleku na teritoriji Vojvodine. Zbornik Matice srpske za prirodne nauke, 116, 75-80.

POLOVINSKI-HORVATOVIĆ M., JURIĆ V., GLAMOČIĆ D. (2009-b): Dvogodišnje istraživanje učestalosti aflatoksina M_1 u mleku na području Srbije. Biotechnology in Animal Husbandry, 25, 5-6-2, 713-718.

RADIVOJEVIĆ M., ADAMOVIĆ M., ŠAMANC H., RADOMIR B., PROTIĆ G. (2010): Efikasnost mineralnih materija u saniranju i preveniranju kiselih indigestija buraga krava. Zbornik naučnih radova Instituta PKB Agroekonomik, 16, 3-4, 61-70.

RANĐELOVIĆ M.,PURENOVIĆ M.,ZARUBICA ALEKSANDRA, MLADENOVIĆ I., PURENOVIĆ JELENA, MOMČILOVIĆ M. (2011): Fizičko-hemijska karakterizacija bentonita i njegova primena u uklanjanju Mn^{2+} iz vode. Hemijska industrija, 65, 4, 381-387.

SLUŽBENI GLASNIK RS 4/2010, 113/2012 i 27/2014/ OFFICIAL GAZETTE OF RS 4/2010, 113/2012 i 27/2014. Pravilnik o kvalitetu hrane za životinje/ Regulation on the quality of animal feed.

SLUŽBENI GLASNIK RS (2014): Pravilnik o izmeni Pravilnika o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja, br. 37/2014.

STOJANOVIĆ B., GRUBIĆ G., DJORDJEVIĆ N., ADAMOVIĆ M., RADIVOJEVIĆ M. (2008): Značaj peletiranja i korišćenja Na-bentonita u proizvodnji smeša za ishranu goveda. XXIV Biotechnology in Animal Husbandry, 435-444.

STOJANOVIĆ B., GRUBIĆ G., ADAMOVIĆ M., RADIVOJEVIĆ M., ŠAMANC H. (2009): Effect of bentonite in pelleted feed for calves. XIII Symposium feed technology, 29 September - 1 October 2009, p. 162-167, Novi Sad, Serbia.

SUMANTRI I., MURTI1 T.W., VAN DER POEL A.F.B., BOEHM J., AND AGUS A. (2012): Carry-over of aflatoxin B1-feed into aflatoxin M1-milk in dairy

cows treated with natural sources of aflatoxin and bentonite. The 5th International Seminar on Tropical Animal Production, 19th-22nd October, 2010, Yogyakarta, Indonesia, 282-285.
TOMAŠEVIĆ - ČANOVIĆ M.,DAKOVIĆ A., MARKOVIĆ V., RADOSAVLJEVIĆ - MIHAJLOVIĆ A.,VUKIĆEVIĆ J. (2000): Adsorpcioni efekti mineralnih adsorbenata, III deo - ispitivanje u prisustvu vitamina B6 i mikroelemenata. Acta veterinaria, 50, 23-29.
TRACKOVA M., MALTOVA L., DVORSKA L., PAVLIK I. (2004): Kaolin, bentonite, and zeolites as feed supplements for animals: health advantages and risks. Veterinary Research Institute, Brno, Czech Republic. Review 389, 389–399.
ŠAMANC H., KIROVSKI D., STOJIĆ V., STOJANOVIĆ D., VUJANAC I., PRODANOVIĆ R., BOJKOVIĆ-KOVAČEVIĆ S. (2011): Primena metaboličkog profila u predviđanju i dijagnozi masne jetre kod krava holštajn rase. Acta veterinaria, 61, 5-6, 543-553.
ŠKRINJAR M., JAKIĆ-DIMIĆ D., BLAGOJEV N., ŠOŠO V. (2011): Rezultati jednogodišnjeg istraživanja kontaminacije hrane za krave muzare i sirovog mleka plesnima i mikotoksinima. Biotechnology in Animal Husbandry, 27, 3, 985-992.
ŽUST J., VENGUŠT A., PESTEVŠEK U., KLEMENC N., KABAJ Z. (1989): Aktuelni problemi kontaminacije stočne hrane mikroorganizmima, posebno gljivicama i mikotoksinima. Krmiva, 31, 5-6: 93-108.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

TOTAL ANTIOXIDANT CAPACITY AND HEMATOLOGICAL CHANGES FOLLOWING EXPOSURE TO MODERATE ALTITUDE IN EWES POSSESSING LOW OR HIGH BASELINE HEMATOCRIT

Penka Moneva, Ivan Yanchev, Marina Dyavolova, Dimitar Gudev

Institute of Animal Science, 2232 Kostinbrod, Bulgaria
Corresponding author: pv_moneva@abv.bg
Original scientific paper

Abstract: The object of the present paper was to investigate the effect of baseline hematocrit level on total antioxidant capacity, hematological adjustments and adrenal activity in ewes following exposure to moderate altitude (1440 m above sea level) for 4 months and transport to low altitude. Ewes from Ile de France breed were allocated into two groups according to their baseline hematocrit level: group I – low baseline hematocrit and group II – high hematocrit level. Blood samples were taken before ascent to altitude, 14 d following exposure to altitude, immediately after transport to low altitude (500 m above sea level) and 7 d later. All samples were analyzed for hematocrit, reticulocyte count and cortisol. Total antioxidant capacity was measured before exposure to altitude, following transport from high to low altitude and 7d later. There was different pattern of hematological adjustments to altitude among the groups. Plasma cortisol levels were within the normal range at 14 d after exposure to altitude but the differences in the baseline levels of hematocrit among the groups persisted at that time. The ewes in group II unlike those in group I did not increase plasma cortisol level in response to transport. Total antioxidant capacity increased in both groups immediately after transport to low altitude and following 7d of stay at low altitude. These results suggest that raising ewes at altitude pasture increases total antioxidant capacity irrespective of the differences in their baseline hematocrit and pattern of hematological adjustments to altitude hypoxia.

Key words: total antioxidant capacity, cortisol, reticulocytes, stress, sheep

Introduction

Oxidative stress may be defined as a disruption of redox signaling and control which regulate the redox balance and the antioxidant response to oxidative insults (*Jones, 2006*). Total antioxidant capacity (TAC) is widly used as an indicator of oxidative stress in a number of desease conditions.

Antioxidant activity is of particular interest, since it is one of the mechanisms that contribute to the neutralization of excessive ROS production. Most of the investigations accentuate on individual vitamins and enzymatic antioxidants (*Schmidt et al., 2002; Pfeiffer et al., 1999; Subudhi et al., 2006*) and to a much less extent on total antioxidant activity (*Droge, 2002*). The literature concerning antioxidant activity in different forms of stress is contradictory (*Hermans et al, 2007; Khajehnasiri et al., 2013; Wadley et al., 2014*). Oxidative stress can play a role in the regulation of important processes through the modulation of signal pathways, influencing synthesis of antioxidant enzymes, repair processes, inflammation, apoptosis, and cell proliferation (*Duracková, 2010*).

There is evidence that the baseline values of hematocrit are closely related to the adrenal response and the metabolic pathway for energy supply (aerobic, anaerobic) (*Evans and Whitlock, 1964; Jones et al., 1967; Grace et al., 1992; Mason, 2000;, Stark and Schuster, 2012, Fiems, 2012; Shirasawa et al., 2003*). Altitude has been reported to trigger high mitochondrial reactive oxygen species production (*Cano et al., 2014*). Glucocorticoids are implicated in erythropoiesis and have potential to stimulate proliferation of erythroid cell in the presence of limiting amounts of erythropoietin (*Udupa et al., 1986*).

Hypoxia-induced oxidative stress at high altitude (3600 m above the sea level) exerts negative effect on sheep reproductive performance (*Parraguez et al., 2013; Parraguez et al., 2015*).

Three different patterns of human adaptation to high altitude have been described in literature (*Beall et al., 2002*).

We did not find any data concerning hematological adjustments and adaptation to moderate altitude in ewes.

Based on the existing literature we set ourselves the goal of investigating total antioxidant activity, plasma cortisol, reticulocyte count and hematocrit dynamics in shorn ewes during 4-month long exposure to moderate altitude, transport to low altitude and 7 d of stay at low altitude.

Materials and Methods

One hundred one Ile de France ewes (1-7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May following estrus synchronization. The animals were allocated into two groups following hematocrit measurement in all ewes. Group I comprised individuals with low level of hematocrit (low hematocrit group; n=10) and group II comprised individuals which had high level of hematocrit (high hematocrit group; n=10). Two additional measurement of baseline hematocrit at intervals of 10 days were performed in the sheep of both groups to verify hematocrit values of both groups, since hematocrit is known to be influenced by many factors and fluctuates from day to day. The average age of the ewes in groups I and II was 3.9± 0.795 and 3.1± 0.745 years respectively. The ewes were shorn on June 2nd and were immediately transported from the experimental base of the Institute of Animal Science, Kostinbrod (500 m above sea level) to the Petrohan Pass region (Balkan mountains), located at 1440 m above sea level. Minimum and maximum temperatures on that day were 13.9 and 25^{o}C for the region of Kostinbrod (low altitude) and 8.2- 13.6^{o}C for the region of Petrohan Pass (high altitude) respectively. The animals remained at high altitude for 4 months where they were on pasture for 10 h during the day. At night they stayed in a barn. The ewes had free access to a NaCl licking stone and water. In addition to pasture, they were offered concentrate once per day. Mean air temperature range in the region of Petrohan pass during the summer months of 2015 was 12 to 20^{o}C. At the end of the grazing season the ewes were transported back to low altitude. At that time the ewes in groups I and II were at 131±6.652 and 140± 4.015 d of gestation respectively as estimated by the day of parturition.

All samples were taken via jugular venipuncture within 3 min in the morning before feeding in order to minimize handling stress and avoid possible interference caused by cortisol diurnal variation. Blood samples were collected in EDTA tubes, centrifuged and stored at -20^{o}C until analyzed. Hematocrit was measured by the microhematocrit method using EDTA-anticoagulated blood. Reticulocytes were stained with New methylene blue and counted microscopically. We followed the procedure described by Briggs and Bain (*Dacie and Lewis, 2012*). Three drops of the dye were delivered into a plastic tube by means of a plastic Pasteur pipette. The same volume of EDTA-anticoagulated blood was added to the dye solution and mixed. The mixture was kept at 37^{o}C for 15-20 min. Blood films were made on glass slides and were allowed to dry before being examined without fixing. Plasma cortisol was measured using a commercial cortisol ELISA kit. Plasma total antioxidant capacity was measured using ELISA kit (IBL International GMBH). Total antioxidant capacity is quantified as millimolar Trolox equivalents. The results of one factor analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and Discussion

Total antioxidant capacity (TAC) increased in both groups of ewes following 4 months of stay at high mountain pasture and transport to low altitude as compared to basal level (Figure 1). It is known that increased autoxidation of membrane-bound hemoglobin during hypoxia enhances the production of red blood cells (RBC)-derived H_2O_2 on the RBC membrane. Under normoxic conditions ,reactive oxygen species (ROS) release from RBC is minimal and the bulk of the ROS are neutralized by the RBC antioxidant system in the red blood cell cytosol despite the relative inaccessibility of the membrane bound hemoglobin to the predominantly cytosilic RBC antioxidant system. (*Kiefmann et al., 2008*). However, the inaccessibility of the autoxidation of hemoglobin bound to the membrane becomes more pronounced under hypoxic conditions when hemoglobin is partially oxidated (*Mohanty et al., 2014*). Exposure to hypoxic stress has been shown to induce overproduction of reactive oxygen species (ROS) in multiple animal models (*Hoshikawa et al., 2001; Matsui et al., 2004*).

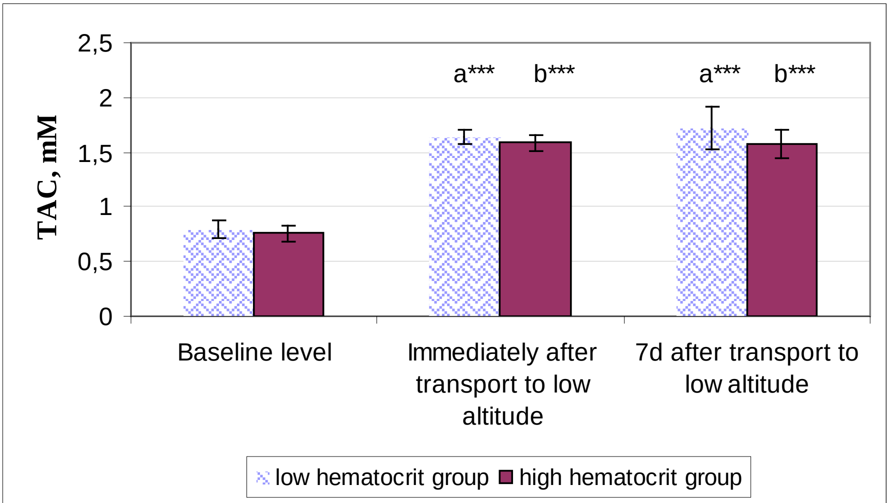


Figure 1. Total antioxidant capacity in sheep with high and low baseline hematocrit values before and following exposure to moderate altitude and transport to low altitude.
*** - p<0.001
a,b- significantly different versus baseline levels

Our experimental design did not envisaged measurement of sheep TAC during their stay at high altitude. However, there is a fair amount of database research literature that allows us to assume that exposure of the shorn ewes to moderate altitude in our experiment may have caused hypoxic stress, aggravated by the concomitant low ambient temperatute that was below the lower critical point at the start of exposure. According to *Cadena and Tattersal (2014)* hypoxic exposure is more pronounced in cold than in room temperature. Our assumption is in agreement with the reported higher level of TAC in male lowlanders and highlanders following ascent to high altitude (*Sinha et al., 2009; Arroyo, et al., 2014*). Besides, our data about hematocrit level and erythrocyte count during the pasture period at moderate altitude (Figure 3) are valuable source of indirect information for the process of adaptation and total antioxidant capacity in particular.

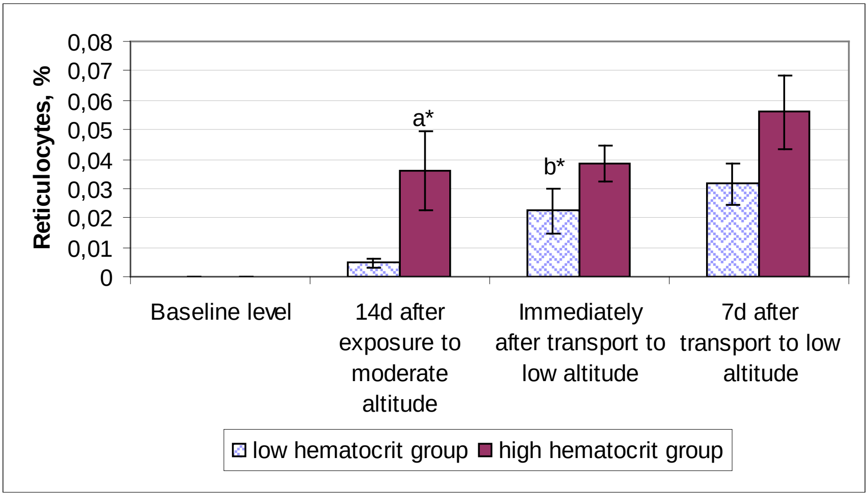


Figure 2. Reticulocyte count in sheep with high and low baseline hematocrit values before and following exposure to moderate altitude and transport to low altitude.
* - $p<0.05$
a -significantly different among the groups at 14 d after exposure to moderate altitude;
b- significantly different versus reticulocytes at 14 d after exposure to moderate altitude

Reticulocyte count increased in both groups of ewes at 14 d following exposure to high altitude (Figure 2). However, the rate of reticulocyte increase, used as a marker of erythropoiesis, was significantly higher in the second group of sheep indicating different adaptive dynamics between the two groups. Increased reticulocyte count in the ewes of goup II at 14 d following exposure to high altitude coincided with an increase in plasma cortisol at that time and suggests that cortisol may be implicated in the process of erythropoiesis. This view is in agreement with the reported cortisol potential to stimulate proliferation of erythroid cell (*Udupa et al., 1986; Hu and Lin, 2012*). These fndings are hardly incidental. In our previous paper (in press) we suggested that ewes with elevated basal hematocrit (II group), unlike those with low basal hematocrit (I group), possess hemoglobin variant with high oxygen affinity that releases less oxygen in the tissues. There is evidence showing that increased hemoglobin-oxygen affinity favors oxygen loading in the lung and survival in a hypoxic environment (*Eaton et al., 1974; Hebbel et al., 1978*). Based on these data we presume that the higher rate of erythropoiesis in the II group of ewes makes them less vulnerable to hypoxic stress as compared to first group of ewes due to the increased reticulocyte count that leads to diminished average age of the population of circulating red blood cells. The younger red cells are characterized by higher 2,3-diphospho glycerate, increased metabolic activity and deformability which improve tissue oxygen supply during hypoxia (*Mairbäurl, 2013*). Consequently, the better oxygen uptake in the lung in the second group of ewes, combined with improved oxygen unloading from hemoglobin in the tisssues, achieved by the increased 2,3-diphospho glycerate content in the reticulocytes, is assumed to contribute for better oxygen supply in the tissues. In contrast, oxygen supply to the tissues in the ewes of group I is supposed to be lower due to the lower affinity of hemoglobin to oxygen that leads to decreased oxygen binding by hemoglobin in the lung. The decreased oxygen uptake in these ewes is accompanied by slower rate of erythropoiesis which ultimately may diminish oxygen supply to the tissues. Our view is further supported by the reported higher sensitivity to hypoxia due to low tissue oxygen delivery in a mouse model of sickle trait (*Noguchi et al., 2001*). Besides, the increased rate of autoxidation under hypoxic conditions when hemoglobin is partially oxygenated, enhances the production of superoxide radical (*Balagopalakrishna et al., 1996*). Therefore, the ewes of group I which are considered to have low hemoglobin affinity for oxygen are expected to have higher rate of hemoglobin autoxidation and free radicals production than those of group II. These results give further support to the concept that basal hematocrit level is closely related to the affinity of hemoglobin for oxygen.

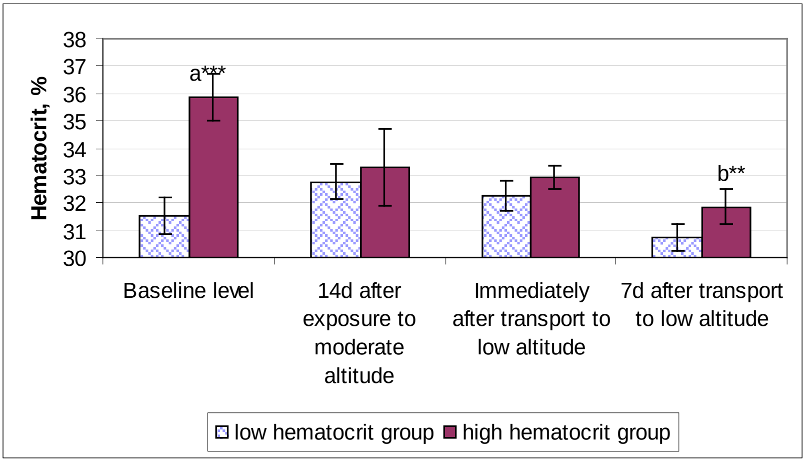


Figure 3. Hematocrit in sheep with high and low baseline hematocrit values, before and following exposure to moderate altitude and transport to low altitude.
** - p<0.01; *** - p<0.001
a-significantly different among the groups
b- significantly different versus baseline level

Basal hematocrit level provides valuable information on the hemathological adjustments under hypoxia. Hematocrit levels in the both group of ewes were similar at 14 d following exposure to high altitude and did not differ significantly from the respective basal values (Figure 3). However, it is worth noting that the observed difference in basal hematocrit levels between the groups was not present at 14 d following exposure to high altitude. This data show that basal hematocrit level is related to the dynamics of hematological adjustment to hypoxia. It is intriguig that reticulocyte count at that time increased sharply in the ewes of group II relative to that in group I against the background of similar hematocrit values between the groups (Figure 2). The observed discrepancy could hardly be due to an increase of plasma volume that was shown to reach a plateau after a certain period of time following the initial exposure to acute hypoxia (*Mason, 2000*). Increased erythropoiesis against the background of unchanged hematocrit level at 14 d following exposure to high altitude suggest that the lack of change in hematocrit level at that time was most probably due to intravascular hemolysis of senescent red blood cells rather than to the plasma volume expansion. Our view is supported by the reported increase in red blood cell destruction during alpine mountaineering (*Martin et al., 1992*). Increased erythropoiesis was shown to decrease red blood cell age (*Mairbäurl, 2013*). Thus, the increase of younger red blood cells could provide better oxygen supply in the tissues due to their higher deformability and lower hemoglobin –oxygen affinity. Besides, the observed declining trend in hematocrit level at 14 d after exposure to high altitude relative to basal hematocrit in the ewes of group II suggests that blood viscosity in these animals was diminished due to changes in plasma erythrocytes that are known as one of the major determinants of whole blood viscosity (*Mairbäurl, 2013*). Therefore, the decreased hematocrit values in these ewes is expected to facilitate blood flow in the microcirculation and improve oxygen supply to the tissues. This view is consistent with the observed difference in the model of adaptation between Himalayans and Andeans concerning hemoglobine response to high altitude as well as the time interval for adaptation (*Jansen and Basnyat, 2011; Beall et al., 1998*) Furthermore, the supposed difference in the hemoglobin affinity for oxygen between the groups signifies that each group has different rate of autoxidation which on its turn affects the rate of free radical production, because of the increased affinity of partially oxygenated hemoglobin for the red blood cell membrane that limits the efficiency of the antioxidant system (*Mohanty et al., 2014*).

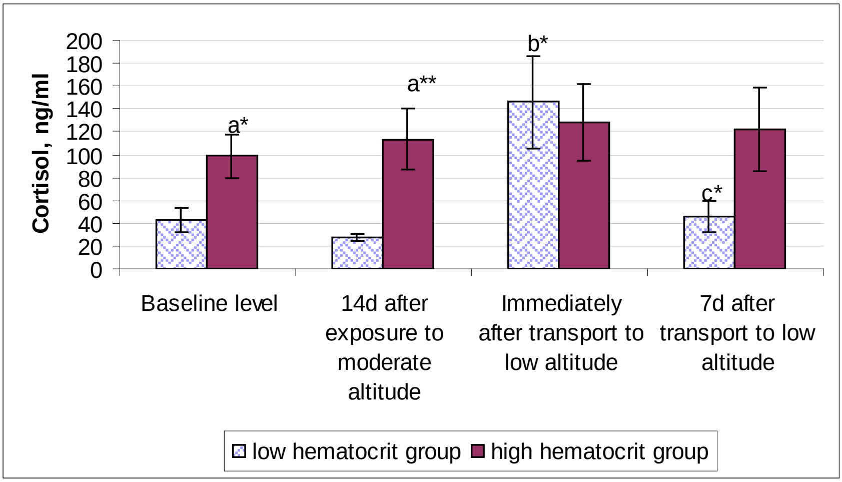


Figure 4. Cortisol levels in sheep with low and high baseline hematocrit values before and following exposure to moderate altitude and transport to low altitude.
* - p<0.05; ** - p<0.01
a - significantly different among the groups
b - significantly different versus baseline level
c - significantly different versus cortisol level at 14 d

Cortisol levels in the ewes of both groups at 14 d after exposure to high altitude were similar to their respective basal levels (Figure 4). Elevated cortisol is known to decrease plasma TAC (*Niki, 2010*). Therefore, it may be assumed that exposure of ewes to high altitude for 14 d was enough for their adaptation to moderate altitude hypoxia as judged by the normal values of cortisol at that time. This assumption suggests that the achieved adaptation in the ewes of both groups requires an increase in TAC production aimed at counteracting the elevated production of free radicals during hypoxia (*Hoshikawa et al., 2001; Matsui et al., 2004; Mohanty et al., 2014*). This view is further supported by the increased TAC immediately after the transport to low altitude and seven days later (Figure 1). In our earlier paper (in press) we postulated (based on our results and literature review) that the ewes of group II rely more on glycolytic pathway for energy supply whereas the ewes of group I rely more on oxidative phosphorylation. Therefore, increased plasma cortisol level in the ewes of group I immediately after the transport to low altitude can be attributed to the higher reliance on aerobic energy pathway in these ewes that leads to increased oxygen deficit. It is well known that the speed of ATP production by oxidative phosphorilation is less than that of glycolysis. Consequently, the ewes of group I will need to increase glucocorticoid - activated glycogenolysis and gluconeogenesis in order to meet increased demand for energy caused by the transport stress.

It is worth noting that the increase of TAC in the ewes of group I immediately after transport as compared to basal level was similar to that in the ewes of group II, despite the lack of adrenal response to transport stress in the latter (Figure 4). These results suggest that long-term exposure to moderate altitude increases TAC and the ability of ewes to neutralize transport-induced increase in free radicals production irrespective of the extent of the adrenal activation. Moreover, TAC remained higher at 7 d after the transport to low altitude (Figure 1). These findings are consistent with the reported local and systemic adaptive responses such as an increase in blood haemoglobulin concentrations and tissue oxygen delivery following chronic hypoxic preconditioning (*Cervos-Navarro and Diemer, 1991; Monge and Leon-Velarde, 1991*).

An alternative explanation over the lack of increase in cortisol at 14 d following exposure to altitude is that specific physiological adjustments to altitude hypoxia require lower metabolic rate as a protective mechanism for reducing oxygen demand and eliminating costly thermogenesis (*Tattersall et al., 2002*).

Conclusions

- Exposure to moderate altitude pasture for 4 months during the summer period increased total antioxidant capacity in sheep following transport to low altitude and 7 d of stay at low altitude irrespective of the baseline hematocrit level.

- Ewes with low and high baseline hematocrit had distinct pattern of hematological adjustment to moderate altitude hypoxia.
- Cortisol levels after 14 d long exposure to altitude was within the normal range despite the existing difference in plasma cortisol values between the ewes with low and high baseline hematocrit.

Ukupni antioksidativni kapacitet i hematološke promene nakon izlaganja umerenoj nadmorskoj visini kod ovaca sa niskim ili visokim osnovnim hematokritom

Penka Moneva, Ivan Yanchev, Marina Dyavolova, Dimitar Gudev

Rezime

Cilj ovog rada je bio da se ispita efekat nivoa osnovnog hematokrita na ukupan antioksidativni kapacitet, hematološka podešavanja i nadbubrežnu aktivnost kod ovaca nakon izlaganja umerenoj nadmorskoj visini (1440 m nadmorske visine) tokom 4 meseca i transporta na malu visinu. Ovce rase il de frans su raspoređene u dve grupe prema nivou osnovnog hematokrita: grupa I - nizak osnovni hematokrit i grupa II - visok nivo hematokrita. Uzorci krvi su uzeti pre transporta na veću nadmorsku visinu, 14 dana nakon izlaganja nadmorskoj visini, odmah nakon transporta na nisku nadmorsku visinu (500 m nadmorske visine) i 7 dana kasnije. Svi uzorci su analizirani na hematokrit, broj retikulocita i kortizol. Ukupan antioksidativni kapacitet je meren pre izlaganja nadmorskoj visini, nakon transporta sa visine na nisku visinu i 7dana kasnije. Postojao je različit obrazac hematoloških prilagođavanja na nadmorsku visinu među grupama. Nivoi plazma kortizola su bili u normalnom opsegu od 14 dana nakon izlaganja nadmorskoj visini, ali su razlike u nivoima osnovnog hematokrita među grupama u to vreme ostale postojane. Ovce u grupi II, za razliku od onih u grupi I, nisu uvećale nivo plazma kortizola kao odgovor na transport. Ukupan antioksidativni kapacitet povećan je u obe grupe odmah nakon transporta na malu visinu i nakon 7 dana boravka na maloj visini. Ovi rezultati ukazuju na to da podizanje ovaca na pašnjake na većoj nadmorskoj visini povećava ukupan antioksidativni kapacitet bez obzira na razlike u njihovom osnovnom hematokritu i uzorku hematoloških podešavanja na hipoksiju nadmorske visine.

Ključne reči: ukupan antioksidativni kapacitet, kortizol, retikulociti, stres, ovce

References

ARROYO C.C.N., DE ARCO R.K.N., PINTO J.V.F., ZENTENO C.S.F., BRAVO J.A., ALVARADO J.A., SORIAA R., PEÑARRIETA J.M. (2014): Plasma antioxidant capacity in highland subjects exposed at 5200 meters of altitude. Revista boliviana de química, 31, 1, 1-4.

BALAGOPALAKRISHNA C., MANOHARAN P.T., ABUGO O.O., RIFKIND J.M. (1996): Production of superoxide from hemoglobin-bound oxygen under hypoxic conditions. Biochemistry, 35(20), 6393-8.

BEALL C.M., DECKER M.J., BRITTENHAM G.M., KUSHNER I., GEBREMEDHIN A., STROHL K.P. (2002): An Ethiopian pattern of human adaptation to high-altitude hypoxia. Proc Natl Acad Sci U S A., 99(26), 17215–17218.

BEALL C.M., BRITTENHAM G.M., STROHL K.P., BLANGERO J., WILLIAMS- BLANGERO S., GOLDSTEIN M.C., DECKER M.J., VARGAS E., VILLENA M., SORIA R., ALARCON A.M., GONZALES C. (1998). Hemoglobin concentration of high-altitude Tibetans and Bolivian Aymara. Am J Physic Anthropol, 106, 385–400.

CADENA V., TATTERSALL G.J. (2014): Body temperature regulation during acclimation to cold and hypoxia in rats. J Therm Biol., 46, 56-64.

CANO I., SELIVANOV V., GOMEZ-CABRERO D., TEGNÉR J., ROCA J., WAGNER P.D., CASCANTE M. (2014): Oxygen Pathway Modeling Estimates High Reactive Oxygen Species Production above the Highest Permanent Human Habitation. PLoS ONE 9(11): e111068. doi:10.1371/journal.pone.0111068

CERVOS-NAVARRO J., DIEMER N. H. (1991): Selective vulnerability in brain hypoxia. Crit. Rev. Neurobiol., 6,149–182.

DACIE J.V., LEWIS S.M. (2012): Practical haematology, in chapter 3, p.33, ed., Churchil Livingstone, Elsevier.

DROGE W. (2002): Free Radicals in the Physiological Control of Cell Function. Physiol Rev, 82, 47–95.

DURACKOVÁ Z. (2010): Some current insights into oxidative stress. Physiol Res. 59(4), 459-469.

EATON, J.W., SKELTON T.D., BERGER E. (1974): Survival at extreme altitude: protective effect of increased hemoglobin-oxygen affinity. Science, 183(4126):743-744.

EVANS, J.V., WHITLOCK J.H. (1964): Genetic Relationship between Maximum Hematocrit Values and Hemoglobin Type in Sheep. Science, 145(3638): 1318.

FIEMS L.O. (2012): Double Muscling in Cattle: Genes, Husbandry, Carcasses and Meat. Animals, 2(3), 472-506.

GRACE R. J., GOVER P. A., TREACHER D. F., HEARD S. E., PEARSON T. C. (1992): Venesection in haemoglobin Yakima, a high oxygen affinity haemoglobin. Clin Lab Haematol., 14(3), 195-199.

HEBBEL R.P., EATON J.W., KRONENBERG R.S., ZANJANI E.D., MOORE L.G., BERGER E.M. (1978): Human llamas: adaptation to altitude in subjects with high hemoglobin oxygen affinity. J Clin Invest., 62(3), 593-600.

HERMANS N., COS P., DE MEYER G.R., MAES L., PIETERS L., VANDEN BERGHE. D., VLIETINCK A.J., DE BRUYNE T. (2007): Study of potential systemic oxidative stress animal models for the evaluation of antioxidant activity: status of lipid peroxidation and fat-soluble antioxidants. J Pharm Pharmacol.,59(1), 131-136.

HOSHIKAWA Y., ONO S., SUZUKI S., TANITA T., CHIDA M., SONG C., NODA M., TABATA T., VOELKEL N.F., FUJIMURA S. (2001): Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia. J. Appl. Physiol, 90, 1299–1306.

HU M., LIN W. (2012): Effects of exercise training on red blood cell production: implications for anemia. Acta Haematol., 127(3), 156-164. doi: 10.1159/000335620.

JANSEN G.F., BASNYAT B. (2011): Brain blood flow in Andean and Himalayan high-altitude populations: evidence of different traits for the same environmental constraint. J Cereb Blood Flow Metab, 31(2), 706-14, doi: 10.1038/jcbfm.2010.150

JONES D.P. (2006): Redefining oxidative stress. Antioxid Redox Signal, 8(9-10), 1865-1879.

JONES R. T., OSGOOD E. E., BRIMHALL B., KOLER R. D. (1967): Hemoglobin Yakina. I. Clinical and biochemical studies. J Clin Invest., 46(11), 1840-1847.

KHAJEHNASIRI F., BAGHER MORTAZAVI S., ALLAMEH A., AKHONDZADEH SH., HASHEMI H. (2013): Total Antioxidant Capacity and Malondialdehyde in Depressive Rotational Shift Workers. Hindawi Publishing Corporation, Journal of Environmental and Public Health Volume 2013, Article ID 150693, 5 pages http://dx.doi.org/10.1155/2013/150693

KIEFMANN R., RIFKIND J.M., NAGABABU E., BHATTACHARYA J. (2008): Red blood cells induce hypoxic lung inflammation. Blood, 15, 111(10), 5205-14. doi: 10.1182/blood-2007-09-113902.

MAIRBÄURL H. (2013): Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cells. Front Physiol., 4, 332- 345.
MARTIN D. T., WATTS P. B., NEWBURY V. S. (1992): The effect of alpine mountaineering on acute erythrocyte hemolysis. Int. J. Sports. Med. 13, 31–35, 10.1055/s-2007-1021230
MASON N. P. (2000): The physiology of high altitude: an introduction to the cardio-respiratory changes occurring on ascent to altitude. Current Anaesthesia & Critical Care, 11(1), 34–41.
MATSUI H., SHIMOSAWA T., ITAKURA K., GUANQUN X., ANDO K., FUJITA T. (2004): Adrenomedullin can protect against pulmonary vascular remodeling induced by hypoxia. Circulation, 109, 2246–2251.
MOHANTY J.G., NAGABABU E., RIFKIND J.M. (2014): Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol., 28, 5:84. doi: 10.3389/fphys.2014.00084. eCollection 2014.
MONGE C., LEON-VELARDE F. (1991): Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiol. Rev. 71, 1135–1172.
NIKI E. (2010): Assessment of Antioxidant Capacity in vitro and in vivo. Free Radical Biology & Medicine, 49, 503–515.
NOGUCHI C.T., GLADWIN M., DIWAN B., MERCIRIS P., SMITH R., YU X., BUZARD G., FITZHUGH A., KEEFER L.K., SCHECHTER A.N., MOHANDAS N. (2001): Pathophysiology of a sickle cell trait mouse model: human alpha(beta)(S) transgenes with one mouse beta-globin allele. Blood Cells Mol Dis.,27(6), 971-977.
PARRAGUEZ V. H., URQUIETA B., PÉREZ L., CASTELLARO G., DE LOS REYES M., TORRES-ROVIRA L., AGUADO-MARTÍNEZ A., ASTIZ S., GONZÁLEZ-BULNES A. (2013): Fertility in a high-altitude environment is compromised by luteal dysfunction: the relative roles of hypoxia and oxidative stress Reprod Biol Endocrinol., 11- 24.
PARRAGUEZ V.H., MAMANI S., COFRÉ E., CASTELLARO G., URQUIETA B., DE LOS REYES M., Aztiz S., Gonzales-Bulnes A. (2015): Disturbances in Maternal Steroidogenesis and Appearance of Intrauterine Growth Retardation at High-Altitude Environments Are Established from Early Pregnancy. Effects of Treatment with Antioxidant Vitamins. PLoS ONE 10(11): e0140902., doi:10.1371/journal.pone.0140902.
PFEIFFER J.M., ASKEW E.W., ROBERTS D.E.,WOOD S.M., BENSON J.E., JOHNSON, S.C., FREEDMAN, M.S. (1999): Effect of antioxidant supplementation on urine and blood markers of oxidative stress during extended moderate-altitude training. Wilderness Environ. Med., 10, 66–74.
SCHMIDT M.C., ASKEW E.W., ROBERTS D.E., PRIOR R.L., ENSIGN JR. W.Y., HESSLINK JR.R.E. (2002): Oxidative stress in humans training in a cold,

moderate altitude environment and their response to a phytochemical antioxidant supplement. Wilderness Environ. Med., 13, 94–105.

SHIRASAWA T., IZUMIZAKI M., SUZUKI Y., ISHIHARA A., SHIMIZU T., TAMAKI M., HUANG F., KOIZUMI K., IWASE M., SAKAI H., TSUCHIDA E., UESHIMA K., INOUE H., KOSEKI H., SENDA T., KURIYAMA T., AND HOMMA I. (2003): Oxygen Affinity of Hemoglobin Regulates O2 Consumption, Metabolism, and Physical Activity. The Journal of Biological Chemistry, 278 (7): 5035–5043.

SINHA S., SINGH S.N., RAY U.S. (2009): Total antioxidant status at high altitude in lowlanders and native highlanders: role of uric acid. High Alt Med Biol. Fall, 10 (3), 269-274. doi: 10.1089/ham.2008.1082.

STARK H., SCHUSTER S. (2012): Comparison of various approaches to calculating the optimal hematocrit in vertebrates. J Appl Physiol**.,** 113(3), 355-367.

SUBUDHI A.W., JACOBS K.A., HAGOBIA T.A., FATTOR J.A., MUZA S.R., FULCO C.S., CYMERMON A., FRIEDLANDER A.L. (2006): Changes in ventilatory threshold at high altitude: effect of antioxidants. Med. Sci. Sports Exerc., 38, 1425–1431.

TATTERSALL G.J., BLANK J.L., WOOD S.C. (2002): Ventilatory and metabolic responses to hypoxia in the smallest simian primate, the pygmy marmoset. J Appl Physiol, 92(1), 202-210.

UDUPA K.B., CRABTREE H.M., LIPSCHITZ D.A. (1986): In vitro culture of proerythroblasts: Characterization of proliferative response to erythropoietin and steroids. Br. J. Haematol. 62: 705–714.

WADLEY A.J., VELDHUIJZEN VAN ZANTEN J.J, PAINE N.J., DRAYSON M.T., ALDRED S. (2014): Underlying inflammation has no impact on the oxidative stress response to acute mental stress. Brain Behav Immun.,40,182-190, doi: 10.1016/j.bbi.2014.03.009.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EFFECT OF ALTITUDE HYPOXIA ON LEUCOCYTE COUNT IN LOW AND HIGH HEMATOCRIT SHEEP

Penka Moneva, Ivan Yanchev, Marina Dyavolova, Dimitar Gudev

Institute of Animal Science, BG-2232 Kostinbrod, Bulgaria
Corresponding author: pv_moneva@abv.bg
Original scientific paper

Abstract: The object of the present paper was to investigate the effect of altitude hypoxia on leukocyte count in ewes having different baseline hematocrit levels. Ewes from Ile de France breed were allocated into two groups according to their baseline hematocrit level: group I – low baseline hematocrit and group II – high hematocrit level. Blood samples were taken before shearing (baseline level), immediately after shearing, 3 h after shearing, 14 d following exposure to moderate altitude (1440 m above sea level), immediately after transport to low altitude (500 m above sea level) following 4 months long exposure to altitude and 7 d later. All samples were analyzed for hematocrit and total leucocyte count. There was different pattern of hematological adjustments to altitude and transportation to low altitude among the groups. Inverse relationship between hematocrit and white blood cell count was also found. Physiological adjustment to altitude hypoxia resulted in similar leukocyte values among the ewes having low and high baseline hematocrit values. Transport from high to low altitude caused more pronounced decline of leukocyte number in the ewes having high basal leukocyte number than those having low basal leukocyte count. These results suggest that physiological adjustment of leukocyte number in response to stress depends not only on the nature of stress stimuli and stress duration but also on the baseline hematocrit level.

Keywords: hematocrit, leucocyte count, moderate altitude, stress, sheep

Introduction

White blood cells count (WBC) is used as an indirect indicator of stress in animals and human. However, investigations on leukocytes response to stress have yielded contradictory results. Stress has been shown to induce a significant decrease in blood leukocyte numbers in a range of different species *(Dhabhar et* *al., 1994; Dhabhar et al., 1995; Musa et al., 2016).* In apparent contrast, studies have also shown that stress can increase rather than decrease blood leukocyte numbers *(Naliboff et al., 1991; Schedlowski et al., 1993; Brosschot et al., 1994; Mills et al., 1995; Bosch et al., 2003).*

There is some evidence suggesting that basal values of hematocrit are closely related to hemoglobin type and therefore may be linked to the adrenal response and the metabolic pathway for energy supply (aerobic, anaerobic) *(Evans and Whitlock, 1964; Jones et al., 1967; Grace et al., 1992; Mason, 2000; Shirasawa et al., 2003; Stark and Schuster, 2012).*In the recent years immunologists have accentuated on the possibility of using bioenergetic profiles of leukocytes with the realization that leukocytes metabolism is closely tied to immunity *(Kramer et al., 2014).* Recent findings support the concept that circulating leukocytes can serve as early sensors of mitochondrial function under conditions of metabolic stress *(Chacko et al., 2014).* Stress-induced immune changes have been widely studied during the recent years. Global immunosuppression model has been further transformed into biphasic model in which acute stress increases, and chronic stress suppresses, the immune response *(Dhabhar and McEwen, 1997; Dhabhar, 2009).* Exposure to hypoxia may prove to be a valuable experimental model for studying the relation between hematocrit and leukocyte count in ewes under hypoxia.

The object of the present paper was to investigate the effect of altitude hypoxia as well as the influence of transport following exposure to altitude on leukocyte count in ewes having different baseline hematocrit levels.

Materials and Methods

One hundred one Ile de France ewes (1-7 years old) were used in the present experiment. All ewes of the flock were artificially inseminated in May following estrus synchronization. The animals were allocated into two groups following hematocrit measurement in all ewes. Group I comprised individuals with low level of hematocrit (low hematocrit group; n=10) and group II comprised individuals which had high level of hematocrit (high hematocrit group; n=10). Two additional measurement of baseline hematocrit at intervals of 10 days were performed in the sheep of both groups to verify hematocrit values of both groups, since hematocrit is known to be influenced by many factors and fluctuates from day to day. The average age of the ewes in groups I and II was 3.9± 0.795 and 3.1± 0.745 years respectively. The ewes were shorn on June 2nd and were immediately transported from the experimental base of the Institute of Animal Science, Kostinbrod (500 m above sea level) to the Petrohan Pass region (Balkan mountains), located at 1440 m above sea level. Minimum and maximum temperatures on that day were 13.9 and

25^{o}C for the region of Kostinbrod (low altitude) and 8.2- 13.6^{o}C for the region of Petrohan Pass (high altitude) respectively. The animals remained at high altitude for 4 months where they were on pasture for 10 h during the day. At night they stayed in a barn. The ewes had free access to a NaCl licking stone and water. In addition to pasture, they were offered concentrate once per day. Mean air temperature range in the region of Petrohan pass during the summer months of 2015 was 12 to 20^{o}C. At the end of the grazing season the ewes were transported back to low altitude. At that time the ewes in groups I and II were at 131±6.652 and 140± 4.015 d of gestation respectively as estimated by the day of parturition.

Blood samples were collected before shearing (baseline level), immediately after shearing, 3 h after shearing, at 14 d following exposure to moderate altitude, immediately after transport to low altitude and following 7d of stay at low altitude.

All samples were taken via jugular venipuncture. Hematocrit was measured by the micro hematocrit method. Total leukocyte count was determined by manual hemocytometer chamber count. The results of one factor analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and Discussion

Hematocrit levels increased significantly after shearing in both groups of ewes and returned to near the baseline level 3 h later($P<0.001$). The observed increase of hematocrit was hardly due to a change in erythrocyte number because it takes at least 15 hours after stimulation for erythropoietin to start erythropoiesis *(McArdle et al., 2010)* and around 2 days to reach peak response *(Mason, 2000).*The rapid increase of hematocrit immediately after shearing was most probably due to the shift of water out of the vascular system. This interpretation is consistent with the view that plasma volume is prone to acute changes, whereas changes in total red blood cell mass (or volume) are slow due to slow rates of erythropoiesis *(Sawka et al., 2000).* Increased plasma volume and extracellular fluid were reported in shorn sheep *(Blunt et al., 1975).* It is intriguing that the existing difference in the baseline levels of hematocrit among the groups persisted after the shearing and 3 h later (Figure 1).

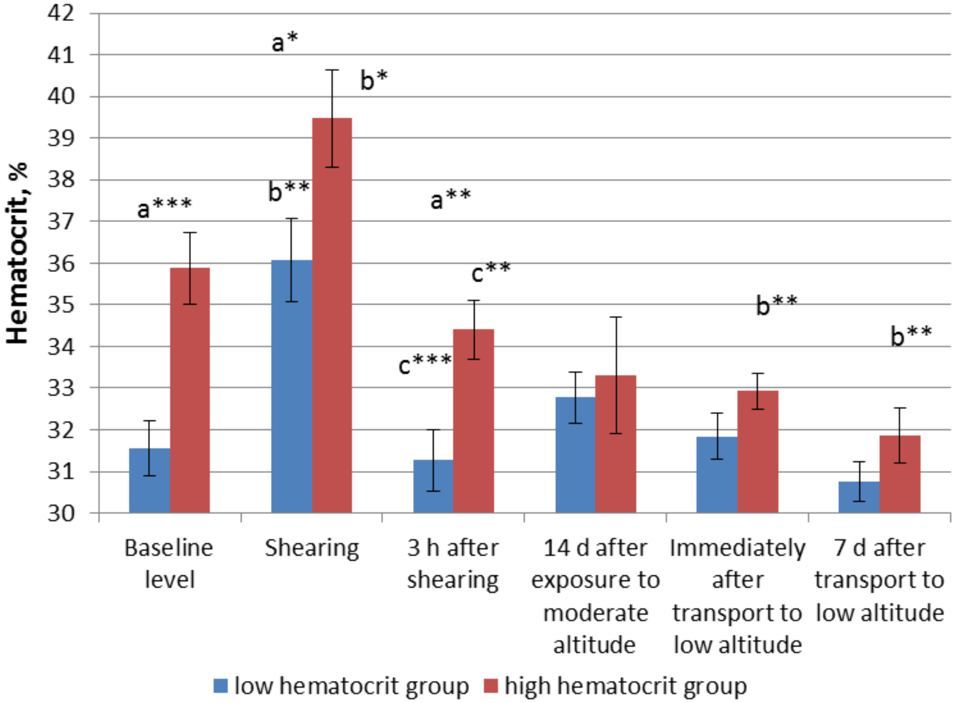


Figure 1. Hematocrit values, following shearing, exposure to moderate altitude and transport to low altitude in sheep with high and low baseline hematocrit values.
* P<0.05, ** P<0.01, ***P<0.001

a- significantly different among the groups
b- significantly different versus baseline level
c- significantly different versus shearing

Hematocrit values between the groups did not differ significantly at 14d following exposure to moderate altitude when compared with the corresponding baseline levels. However, hematocrit level in the ewes of group II unlike that in the ewes of group I, declined more sharply, although not significantly. This data may indicate different physiological adjustment of hematocrit to the existing hypoxia between the groups. This view is further supported by the registered decline of hematocrit value in the ewes of group II immediately after transport to low altitude and 7 d later relative to baseline level (P<0.05). Hematocrit values in the ewes of group I were similar to baseline levels at these points (Figure 1). We assume that the observed decline of hematocrit level in the ewes of group II was an adaptive response aimed at decreasing blood viscosity. Oxygen transport was found to be

inversely related to viscosity *(Lim, 1999)*. In our early study having similar experimental design *(Moneva et al., 2016)* we suggested that the ewes in group II unlike those in group I have higher affinity of hemoglobin for oxygen. A detailed comparison of oxygen transport in llama, with a high hemoglobin oxygen affinity, and sheep, with a low hemoglobin oxygen affinity, indicated that the low O2 affinity in sheep was clearly more beneficial at moderate altitude (1600-2800 m), while at higher altitude (4500 – 6400 m) the high oxygen affinity of llama was more advantageous *(Horstman et al., 1980)*. Lower hematocrit level keeps blood viscosity lower and improves blood flow rate which improves oxygen transport and transfer of oxygen to the muscles *(Sherwood et al., 2014; Waltz et al., 2015)*.
There was statistically significant difference in the basal values of leukocytes between the two experimental groups (Figure 2).

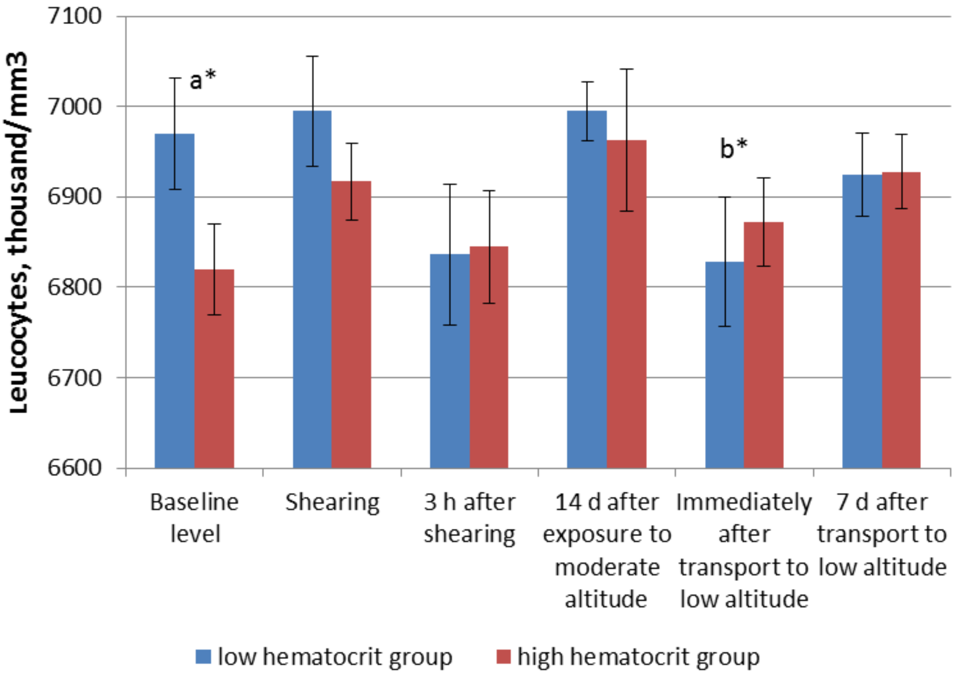


Figure 2. Total leucocyte count, following shearing, exposure to moderate altitude and transport to low altitude in sheep with high and low baseline hematocrit values.
***** P<0.05
a - significantly different among the groups
b - significantly different versus 14 d after exposure to moderate altitude

This data indicate that there is relation between hematocrit and white blood cell count. There was no significant difference in WBC count between the groups immediately after shearing and 3 h later. However, leukocyte count in the ewes of group I tended to decline at 3h after shearing relative to baseline level while in group II it was similar to the baseline level. *Dhabhar (1995)* hypothesized that leukocyte number increases immediately after exposure to stress due to spontaneous release of catecholamines and sharp increase of sympathetic activity, and then it declines 15-20 min. later due to the increased level of glucocorticoids which counteracts the effect of catecholamines on leukocyte count. However, the observed dynamics in WBC count in our case was most probably due to the shift of water out of the vascular system. This view is consistent with the reported reduction in the volume of water in the blood at shearing and its recovery 3 h later *(Piccione et al., 2006; 2010; Hashem, 2014).* Similar decline in WBC count was observed immediately after transport to low altitude in both groups. However, WBC count decline was more pronounced in the ewes of group I ($P<0.05$), whereas it was insignificant ($P>0.05$) in the ewes of group II.

After 14-day stay of sheep at 1440 meters altitude the number of leukocytes in the first group was comparable to the basal value, while sheep of Group II had a slight upward trend. The established difference in baseline leukocyte number between the groups did not exist anymore following 14 d exposure to altitude due to the insignificant increase of leukocyte number in the ewes of group II. Increased leukocyte number has been reported to have adverse effect on overall microvascular hemodynamics and vascular resistance because of their larger volume, rigidness and enhanced interaction with endothelium as compared to red cells *(Kaul and Hebbel, 2000).*These results suggest that the physiological adjustment of leukocyte number in response to stress depends not only on the nature of stress stimuli and stress duration but also on the baseline hematocrit level.

Conclusions

1. There was inverse relation between basal hematocrit values and WBC count.
2. Physiological adjustment to altitude hypoxia resulted in similar leukocyte values among the ewes having low and high baseline hematocrit values.
3. Transport from high to low altitude caused more pronounced decline of leukocyte number in the ewes having low basal leukocyte number than those having high basal leukocyte number.

Uticaj visinske hipoksije na broj leukocita kod ovaca niskog i visokog hematokrita

Penka Moneva, Ivan Yanchev, Marina Dyavolova, Dimitar Gudev

Rezime

Cilj ovog rada je bio da se ispita uticaj visinske hipoksije na broj leukocita kod ovaca sa različitim osnovnim nivoima hematokrita. Ovce rase il de frans su bile raspoređene u dve grupe prema osnovnom nivou hematokrita: grupa I - nizak osnovni hematokrit i grupa II – visok osnovni hematokrit. Uzorci krvi su uzeti pre strižе (osnovni nivo), odmah nakon strižе, 3 h nakon strižе, 14 d nakon izlaganja umerenoj nadmorskoj visini (1440 m nadmorske visine), odmah nakon transporta na malu visinu (500 m nadmorske visine) nakon 4 meseca dugotrajne izloženosti nadmorskoj visini i 7 dana kasnije. Svi uzorci su analizirani na hematokrit i ukupni broj leukocita. Postojao je različit obrazac hematoloških prilagođavanja nadmorskoj visini i transportu na nisku nadmorsku visinu među grupama. Inverzna veza između hematokrita i broja belih krvnih zrnaca takođe je utvrđena. Fiziološko prilagođavanje visinskoj hipoksiji dovelo je do sličnih vrednosti leukocita kod ovaca sa nižim i visokim osnovnim vrednostima hematokrita. Transport sa velike na nisku nadmorsku visinu prouzrokovao je izrazitiji pad broja leukocita kod ovaca sa visokim bazalnim leukocitnim brojem od onih koji imaju nizak broj leukocita. Ovi rezultati ukazuju na to da fiziološko prilagođavanje broja leukocita u odgovoru na stres zavisi ne samo od prirode stresnih stimulusa i trajanja stresa, već i od nivoa baznog hematokrita.

Ključne reči: hematokrit, broj leukocita, umerena visina, stres, ovce

References

BLUNT M.H., COX R.I. , CURTAIN C.C., DARGIE J.D., FERGUSON K.A., HOLMES T.H.J., HUISMAN P.H., LEAT W.M.S., LINDSAY D.B., MACFARLANE W.M., MOODIE E.W., TUCKER E.M. (1975): The Blood of Sheep: Composition and Function, Ed. M.H. Blunt, Springer, pp 224.

BOSCH J.A., BERNTSON G.G., CACIOPPO J.T., DHABHAR F.S., MARUCHA P.T. (2003): Acute stress evokes selective mobilization of T cells that differ in chemokine receptor expression: a potential pathway linking immunologic reactivity to cardiovascular disease. Brain Behav Immun., 17, 4, 251-259.

BROSSCHOT J.F., BENSCHOP R.J., GODAERT G.L., OLFF M., DE SMET M., HEIJNEN C.J., BALLIEUX R.E. (1994): Influence of life stress on immunological reactivity to mild psychological stress. Psychosom. Med.,56, 216-224.

CHACKO B.K., KRAMER P.A., RAVI S., BENAVIDES G.A., MITCHELL T., DRANKA B.P., FERRICK D., SINGAL A.K., BALLINGER S.W., BAILEY S.M., HARDY R.W., ZHANG J., ZHI D., DARLEY-USMAR V.M. (2014): The Bioenergetic Health Index: a new concept in mitochondrial translational research. Clin Sci (Lond)., 127,6, 367-373.

DHABHAR F.S., MILLER A.H., MCEWEN B.S., SPENCER R.L. (1995): Effects of stress on immune cell distribution—dynamics and hormonal mechanisms. J. Immunol.,154, 5511-5527.

DHABHAR F.S., MILLER A.H., STEIN M., MCEWEN B.S., SPENCER R.L. (1994): Diurnal and stress-induced changes in distribution of peripheral blood leukocyte subpopulations. Brain Behav. Immun.,8, 66-79.

DHABHAR F.S., MCEWEN B.S. (1997): Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun., 11, 4, 286-306.

DHABHAR F.S. (2009): Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation, 16, 5, 300-317.

EVANS J.V., WHITLOCK J.H. (1964): Genetic Relationship between Maximum Hematocrit Values and Hemoglobin Type in Sheep. Science, 145, 3638, 1318.

GRACE R.J., GOVER P.A., TREACHER D.F., HEARD S.E., PEARSON T. C. (1992): Venesection in haemoglobin Yakima, a high oxygen affinity haemoglobin. Clin Lab Haematol., 14, 3, 195-199.

HASHEM A.L.S. (2014): Effect of summer shearing on thermoregulatory, hematological and cortisol responses in balady and damascus goats in desert of Sinai, Egypt. World Applied Sciences Journal 30, 5, 521-533.

HORSTMAN D., WEISKOPF R., JACKSONET R.E. (1980): Work capacity during 3-wk sojoun at 4,300 m: Effect of relative polysysthemia. J.Appl. Physiol.: Envir. Exercise Physiol., 49, 311-318.

JONES R.T., OSGOOD E.E., BRIMHALL B., KOLER R. D. (1967): Hemoglobin Yakina. I. Clinical and biochemical studies. J Clin Invest., 46, 11, 1840-1847.

KAUL D.K., HEBBEL R.P. (2000): Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. J Clin Invest., 106, 3, 411-420.

KRAMER P.A., RAVI S., CHACKO B., JOHNSON M.S., DARLEY-USMAR V.M. (2014): A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol., 10, 2, 206-10.

LIM B. (1999): Modeling ultrasound imaging of red blood cell aggregation in shear flow. PhD Thesis, Institute of Biomedical Engineering, Department of Electrical and Computer Engineering, University of Toronto.
MASON N.P. (2000): The physiology of high altitude: an introduction to the cardio-respiratory changes occurring on ascent to altitude. Current Anaesthesia & Critical Care, 11, 1, 34-41.
MCARDLE W.D., KATCH F.I., KATCH V.L. (2010): Exercise at medium and high altitude,chapter 24:592-608, In: Exercise Physiology: Nutrition, Energy, and Human Performance, Lippincott Williams & Wilkins.
MILLS P.J., BERRY C.C., DIMSDALE J.E., ZIEGLER M.G., NELESEN R.A., KENNEDY B.P. (1995): Lymphocyte subset redistribution in response to acute experimental stress: effects of gender, ethnicity, hypertension, and the sympathetic nervous system. Brain Behav Immun., 9, 61-69.
MONEVA, P., YANCHEV I., DYAVOLOVA M., GUDEV D. (2016): Hematocrit as a potential marker of acclimatization capacity and stress sensitivity in sheep exposed to transport and high altitude. Bulg. J. Agric. Sci., 22, 999-1005.
MUSA I., MABROUK M.A., TANKO Y. (2016): The effect of exercise and training status on leucocytes count. International Journal of Current Research in Life Sciences, 5, 1, 525-528,
NALIBOFF B.D., BENTON D., SOLOMON G.F., MORLEY J.E., FAHEY J.L., BLOOM E.T.,MAKINODAN T., GILMORE, S.L. (1991): Immunological changes in young andold adults during brief laboratory stress. Psychosom. Med.,53, 121-132.
PICCIONE G., CASELLA S., ALBERGHINA D., ZUMBO A., PENNISI P. (2010): Impact of shearing on body weightand serum total proteins in ewes. Spanish Journal of Agricultural Research, 8, 2, 342-346.
PICCIONE G., GRASSO F., FAZIO F., GIUDICE E., PENNINI P. (2006). Evaluation of some physiological and haematological parameters in the ewes: influence of shearing and sheltering. Folia Vet., 50, 13-16.
SAWKA M.N.,CONVERTINO V.A., EICHNER E.R., SCHNIEDER S.M.,YOUNGA.J. (2000): Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. Med.Sci. Sports Exerc.,32,332-348.
SCHEDLOWSKI M., JACOBS R., STRATMAN G., RICHTER S.,HADICKE A.,TEWES U.,WAGNER T.O.F., SCHMIDT R.E. (1993): Changes of natural killer cells during acutepsychological stress. J. Clin. Immunol. 13, 119-126.
SHERWOODJ. M., KALIVIOTIS E., DUSTING J., BALABANI S. (2014): Hematocrit, viscosity and velocity distributions of aggregating and non-aggregating blood in a bifurcating microchannel. Biomechanics and Modeling in Mechanobiology,13, 2, 259-273.

SHIRASAWA T., IZUMIZAKI M., SUZUKI Y. , ISHIHARA A., SHIMIZU T., TAMAKI M., HUANG F., KOIZUMI K., IWASE M., SAKAI H., TSUCHIDA E., UESHIMA K., INOUE H., KOSEKI H., SENDA T., KURIYAMA T., HOMMA I.(2003): Oxygen Affinity of Hemoglobin Regulates O2Consumption, Metabolism, and Physical Activity. The Journal of Biological Chemistry, 278,7, 5035-5043.
STARK H.,SCHUSTER S.(2012): Comparison of various approaches to calculating the optimal hematocrit in vertebrates. J Appl Physiol**.,**113,3,355-367.
WALTZ X., HARDY-DESSOURCES M.D., LEMONNE N., MOUGENEL D., LALANNE-MISTRIH M.L., LAMARRE Y., TARER V., TRESSIRES B., ETIENNE-JULAN M., HUE O., CONNES P. (2015): Is there a relationship between the hematocrit-to-viscosity ratio and microvascular oxygenation in brain and muscle?Clin Hemorheol Microcirc., 59, 1, 37-43.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EFFECT OF NUTRITION ON PHENOTYPE CHARACTERISTICS OF CROSS-BRED LAMBS IN FATTENING

Almira Softić, Velija Katica, Vedad Šakić, Aida Kavazović, Maja Varatanović, Dinaida Tahirović

Veterinary Faculty University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina
Corresponding author: almira.softic@vfs.unsa.ba
Original scientific paper

Abstract: The objective of studie was to examine effect of nutrition on phenotype characteristics of cross-bred lambs fattening. The study was conducted on 20 cross-bred lambs from different pramenka types, divided in two groups. The age of the lambs at the start of experiment was 55 - 60 days, and the fattening lasted for five weeks. The lambs of one group were fed with mixture of cereals and sunflower meal without mineral-vitamin premix, but for the feeding of second group of lambs were used feed mixture for older lambs. During the fattening period the lambs from both group were fed hay ad libitum. Body weight and linear body measures (height at withers, hight at sacrum, length of the trunk, width of the chest, depth of the chest, circumference of the chest, circumference of the leg) were measured on the begining and the end of every week of the study. The results were statisticly analyzed, statistically significant differences and correlation coeficient were determined. The gain of individual body measures was determined within the both groups according to age of lambs during fattening. The results indicated that the group lambs fed mixture of cereals and sunflower meal achieved better but not statisticaly significant ($p>0,05$) body weight, and all linear body measures comparing to lambs fed with feed mixture.

Key words: lamb, croos-bred, feed mixture, fattening

Introduction

Sheep breeding is a very important branch of animal husbandry, as well as whole agricultural production. The importance of sheep breeding is particularly evident in countries and regions with large pastures and meadows available. The economic importance of breeding is based on the biological characteristics of sheep, which allow that even relatively sparse vegetation of pastures can be turned into highly valuable products: meat, milk, wool. Significant place in sheep breeding takes testing and characterisation of different breeds which has great future interest for this branch of animal husbandry *(Ugarte, 2007; Kurt and Horst, 2008).* In Bosnia and Herzegovina, the breed compositions are different depending on the production direction, and the only indigenous breed is Pramenka breed that has a larger number of strains of different phenotypic and breeding characteristics. Breeding traits of sheep depend on numerous genetic and non-genetic factors *(Petrovic et al., 2009).* Fattening of early weaned lambs lasts short and provides good meat quality using protein nutriments. Protein nutriments, besides providing animals with high quality proteins have a positive influence on breeding indicators such as consumption, degree of food utilization, daily gain, yield and meat quality *(Memiši 2002).* The aim of the research is to examine the impact of nutrition by using mixtures with different protein content on phenotypic characteristics of crossbred lambs for fattening.

Materials and Methods

The study of phenotypic characteristics was conducted on 20 crossbred lambs of Dubska and Sjenica Pramenka divided in two groups. Age of lambs at the beginning the fattening period was 55 to 60 days, and fattening lasted 35 days. Lambs of one group were fed a mixture of grain and sunflower pellets without the addition of mineral and vitamin premix containing 12.26% protein (Group I), while the nutrition of the other group of lambs was conducted using complete feeds for feeding older lambs with a protein content of 14, 69% (group II). Lambs from both groups during fattening, received hay *ad libitum*.

Body weight and linear body measurements (withers height, height of cross, body length, chest width, chest depth, chest circumference, leg circumference) were measured at the beginning and at the end of each week the of the fattening period. Body measurements were taken by using Lydtin rod and the measuring tape, while the body weight was determined by animal scale. Indexes of physical development, as well as correlation coefficient of lambs were done. The obtained results were statistically analysed using Microsoft Excel 2010, module Data Analysis. To indicate the level of significance, different superscripts and star rating system were used ($p<0.05^*$; $p<0.01^{**}$, $p<0.001^{***}$).

Results and Discussion

The obtained results and the phenotypic characterization of body weight of crossbred lambs are shown in Table 1. The results show that the lambs fed mixture of sunflower meal and grains without mineral-vitamin premix with 12.26% of protein (Group I) achieved better phenotypic characteristics and weight compared to a group of lambs, fed complete feeds with 14.69% of protein (Group II) but there was no statistical significance ($p>0.05$). The increase in individual body measurements was determined within both groups with increasing age of lambs during fattening period. Linear body measurements of live animals can be used to estimate body conformation *(Salako, 2006),* growth rate, weight, feed utilization and characteristics of meat quality, and for monitoring the relations of production indicators between production characteristics, visual and physical measures *(Lawrence and Fowler, 1998; Fourie et al, 2002).* The results obtained in the course of our studies have been compared with results of other authors who have researched impact of nutrition and age on production traits and phenotypic characteristics of different breeds of lambs. Comparing the results *Maric et al. (2013)* for his research conducted on of lambs breed Dubrovacka ruda, used the compound feed with 16% protein, the results of our research show that crossbred lambs Pramenka from both groups achieved greater weight and volume of the breast fed by mixtures with a lower protein content, while results for other body measurements were similar.

The average body weight lambs achieved in our research is in line with the results that have been conducted on lambs breed Tsigai *(Antunovic et al. 2010; Antunovic et al. 2012)*, as well as Lipska and Svrljig breed *(Petrovic et al. 2012).* Additionally, it is important to emphasize that *Petrovic et al (2012)* during his researches used a mixture of concentrate with 18% protein which is significantly more than in mixtures that are used during our study (12.26% and 14.69%) which can be explained by the production characteristics of different breeds (better utilization of food, the higher growth rate).

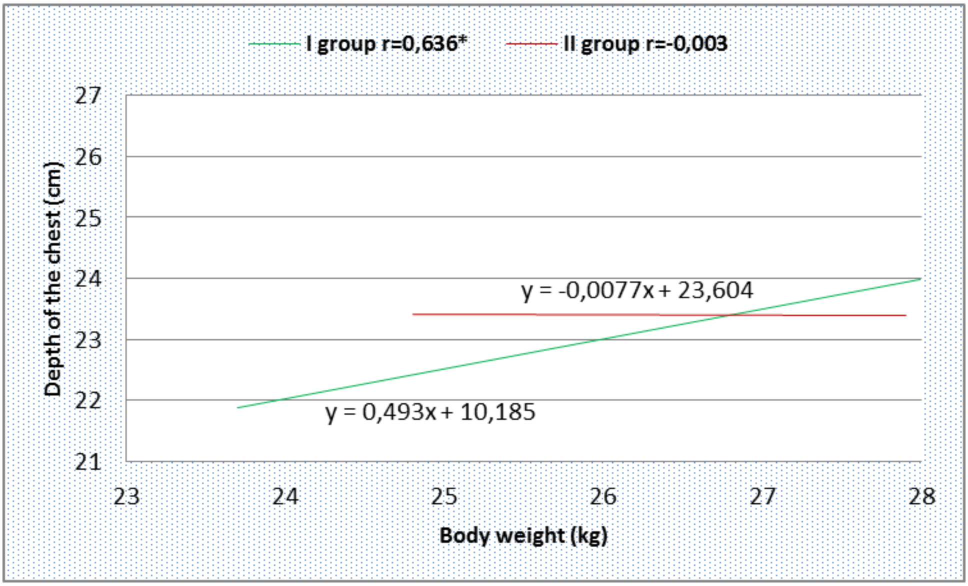
In a study of phenotypic characteristics and indexes of physical development of lambs of Travnicka sheep in the average age of four and a half and six months *Bradešić (2014)* has found a good and harmonious development of lambs with age increases, as well as better results in male lambs in relation to female. The results of our studies are mainly in line with the results of the aforementioned authors, except for less weight and body length which can be explained by the difference in the average age of lambs.

Table 1. Phenotypic characteristics of hybrids lambs for fattening

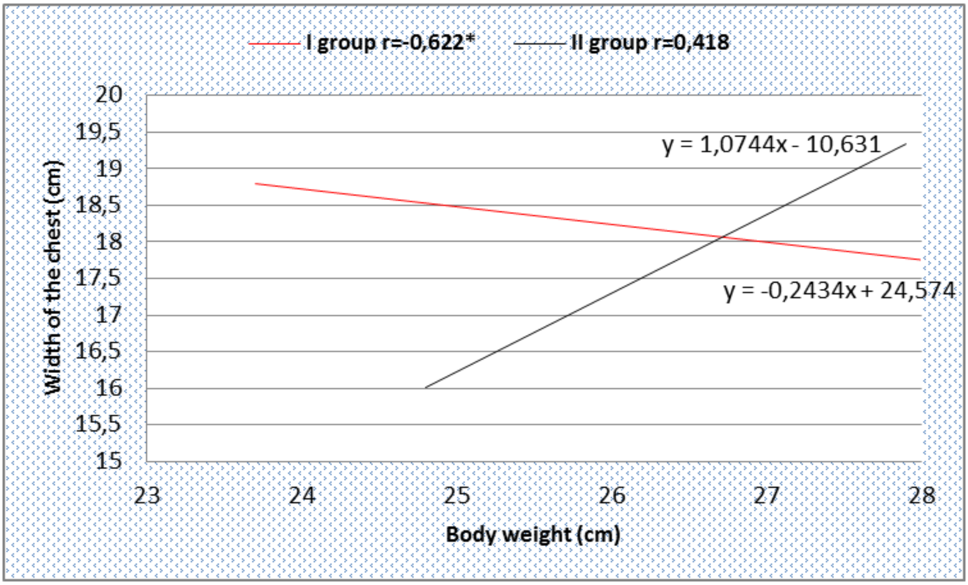
Week	**Start**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group I	18,90±2,03	54,99±2,38	55,83±2,30	55,10±1,96	15,15±1,70	21,10±1,41	66,90±0,87	7,61±0,32
Group II	18,40±1,05	53,88±1,99	54,11±1,89	54,50±1,32	14,95±1,87	20,30±1,66	65,10±0,28	7,59±0,26
week	**First week**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group 1	20,50±20,8	56,22±1,95	56,31±2,35	55,99±5,77	15,30±1,15	21,60±1,64	67,76±5,16	7,67±0,38
Group 2	19,80±1,24	55,04±1,74	54,99±2,03	55,44±6,02	15,15±1,37	21,10±1,66	66,98±4,38	7,63±0,34
week	**Second week**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group 1	22,20±2,11	57,33±1,40	57,76±1,76	57,02±2,98	16,00±1,49	22,00±3,01	68,08±4,49	7,72±0,44
Group 2	21,50±1,22	56,34±1,80	56,82±1,86	56,15±4,05	15,85±0,70	21,90±1,52	67,24±4,48	7,69±0,59
week	**Third week**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group 1	24,10±2,20	57,81±1,99	58,23±2,30	57,97±3,98	16,70±1,41	22,80±2,44	68,80±4,38	7,79±0,39
Group 2	23,40±1,17	57,38±1,23	57,46±2,94	57,02±2,53	16,59±1,36	22,60±3,13	68,36±3,35	7,76±0,80
week	**Fourth week**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group 1	25,80±2,27	59,58±1,70	58,97±1,98	59,25±5,23	17,30±1,05	23,19±2,27	70,90±6,85	7,86±0,39
Group 2	25,0±1,12	57,94±2,19	58,07±1,73	58,29±2,38	17,13±2,36	23,05±2,40	69,95±3,20	7,77±0,79
week	**Fifth week**							
parameters	**BW (kg)**	**HW (cm)**	**HC (cm)**	**LT (cm)**	**WCH (cm)**	**DCH (cm)**	**CCH (cm)**	**CL (cm)**
Group 1	27,20±2,12	59,82±2,01	59,83±0,90	60,21±2,40	17,95±0,83	23,60±1,64	72,22±4,09	7,97±0,54
Group 2	26,40±1,01	58,31±0,87	58,70±2,01	59,10±2,04	17,70±2,75	DCH (cm)	70,75±4,10	7,88±0,40

BW - Body weight; HW - Height of withers; HC - Height of cross; LT - Length of the trunk; WCH - Width of the chest; DCH - Depth of the chest; CCH- Circumference of the chest; CL - Circumference of the leg

At the end of the fattening period, the correlation coefficient determined in relation to the weight and breast depth (r = 0.636 *) showed a significant statistical evaluation (P <0.05) in the group of lambs fed with a mixture of grains and sunflower pellets without the addition of vitamin-mineral premix (Graph 1), and also with a negative coefficient of correlation (r = -0.622 *) between body weight and the breast width (Graph 2).



Graph 1. Correlation of body weight compared to the depth of the chest at the end of fattening

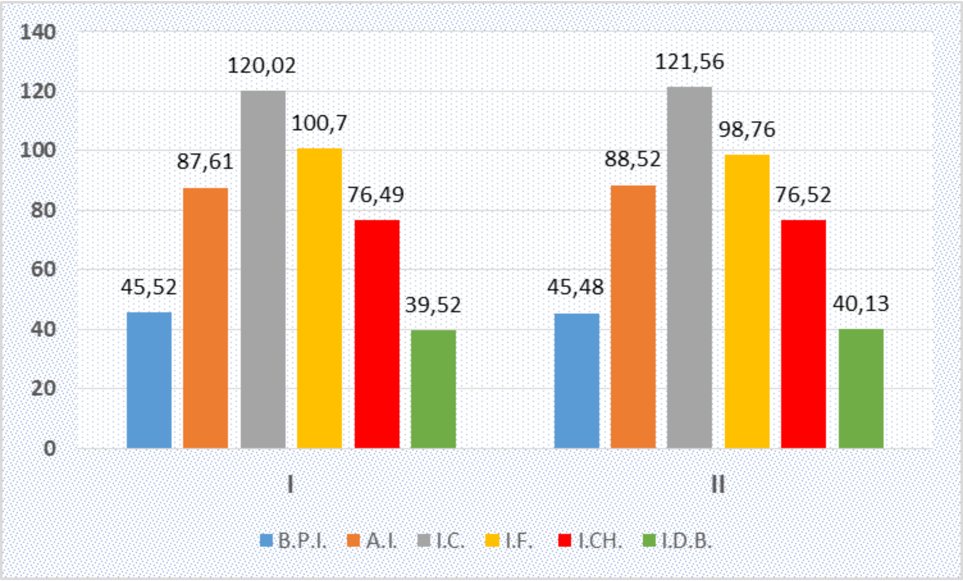


Graph 2. Correlation of body weight compared to the width of the chest at the end of fattening

By examining genetic and phenotypic characteristic aspects of physical measures of lambs from Merinolandschaf breed sheep, *Petrovic et al. (2012)* found a variation of genetic correlation between body measurements in the positive range from 0,873 (Height to withers-Girth of Chest) to 0.999 (Body length-Girth of Chest), while the value of phenotypic correlations was lower in comparison with genetic ones.

According to *Petrovic et al. (2012)* phenotypic correlations are positive and highly significant between the characteristics of growth, as opposed to genetic correlations which were significant, but not in between all the study periods of ageing of lambs Lipska and Svrljig breed. The results obtained in the course of our studies it is difficult to compare with literature allegations of *Petrovic et al. (2012)* who nominated various experimental designs.

The chest development indicates a large volume of the chest cavity that allows good ventilation, easier breathing and good circulation. Chest index as a measurement indicates the curvature and position of the ribs onto vertically set sidelong ribs with small kurtosis which is the characteristics of a low selected natural populations and the characteristics of fattening breeds. At the end of the fattening period, the obtained results of chest index in both groups were 76.49 and 76.52 (Figure 3). The breasts are the most important part of the body and on their size heavily depends productive capacity of an animal, their health status and resistance *(Krajinović at al. 2004).* Index value of breasts depth of 40,13 and 39,52 achieved in both groups of lambs during our research are in line with statements of *Marić at al. (2013).* A something better anamorphosis index at the end of fattening period was achieved in lambs from second group (88,52) without statistical significance ($p>0,05$) in comparison to lambs from the first group (87,61). Comparing our results, they are higher in relation to research by *Antunovic et al. (2010)* who have found in their experiment on Tsigai lamb breed in fattening period, the average age of 70 days fed a mixture of grains and soybean meal with the addition of alfalfa hay at will, and *Maric et al. (2013)* has found lower values (74.77) in the fattening period of 90 days on lambs Dubrovacka ruda, fed with fodder mixture with 16% protein (Graph 3). Body proportions index was similar in both groups of lambs, and amounted to 45.52 and 45.48, respectively (Graph 3), and were higher compared to the research by *Antunovic et al. (2010); Maric et al (2013).* The index format of 100,70 in lambs from the first group points out to an extremely compact build of an animal body, and together with the index of compactness of the body indicates a strong constitution of crossbred lambs (Graph 3).



Graph 3. The indicies body conformation of cossbred lambs

B.P.I. – Body proportions indeks; **A.I. –** Anamorphosis index; **I.C. –** index compaction**; I.F.** – index format; **I.CH**.- Index chest; **I.D.B.** – index depth breasts

Conclusion

Based on obtained results it can be concluded that by using a mixture of grain and sunflower meal, without the addition of mineral and vitamin premixes with a protein content of 12.26%, better but not statistically significantly better (p <0.05) phenotypic characteristics can be achieved of crossbred lamb Pramenka in fattening period, which makes production of lamb meat more economical.

Uticaj ishrane na fenotipske karakteristike jagnjadi meleza u tovu

Almira Softić, Velija Katica, Vedad Šakić, Aida Kavazović, Maja Varatanović, Dinaida Tahirović

Rezime

Cilj istraživanja je bio ispitati uticaj ishrane na fenotipske karakteristike jagnjadi meleza u tovu. Istraživanje je sprovedeno na 20 jagnjadi meleza različitih sojeva pramenke podeljenih u dve grupe. Starost jagnjadi na početku tova bila je 55-60 dana, a tov je trajao 35 dana. Jagnjad jedne grupe hranjena je smešom žitarica i suncokretove sačme bez dodatka mineralno-vitaminskog premiksa, dok se ishrana jagnjadi druge grupe provodila korišćenjem kompletne krmne smeše za ishranu starije jagnjadi. Jagnjad iz obe grupe je tokom tova dobijala seno po volji.
Telesna masa i linearne telesne mere (visina grebena, visina sapi, dužina trupa, širina grudi, dubina grudi, obim grudi, obim cevanice) mereni su na početku i na kraju svake sedmice istraživanja. Izvršena je statistička obrada dobijenih rezultata, određena statistička opravdanost razlika, te koeficijent korelacije. Porast pojedinih telesnih mera utvrđen je unutar obe grupe s porastom dobi jagnjadi za tovni period. Rezultati istraživanja pokazuju da su jagnjad grupe hranjene smešom žitarica i suncokretove sačme, ostvarila veću ali ne i statistički značajno veću ($p>0,05$) prosečnu telesnu masu i linearne telesne mere (visina grebena, visina sapi, dužina trupa, širina grudi, dubina grudi, obim grudi, obim cevanice) u odnosu na jagnjad koja je hranjena kompletnom krmnom smešom.

Ključne reči: jagnjad, melezi, smeša, tov

References

ANTUNOVIĆ Z., NOVOSELEC J., ŠPERANDA M., DOMAĆINOVIĆ M., DJIDARA M. (2010): Praćenje hranidbenog statusa janjadi iz ekološkog uzgoja. Krmiva 52 , Zagreb, 1; 27-34.

ANTUNOVIĆ Z., SENČIĆ Đ., ŠPERANDA M., NOVOSELEC J. (2012): Fenotipske i metaboličke značajke janjadi cigaja pasmine. 47 th. Croatian & 7 th. International Symposium on Agriculture. February 13 – 17, 2012. Opatija, Croatia.

BRADEŠIĆ D. (2014): Fenotipske odlike janjadi travničke pramenke na području zapadne Slavonije. Završni rad. Sveučilište Josipa Jurja Strossmayera u Osijeku. Poljoprivredni fakultet u Osijeku. Osijek.

FOURIE P. J, NESER F. W. C., OLIVIER J. J., DER WESTHUIZEN C. V. (2002): Relationship between production performance, visual appraisal and body measurements of young Dorper rams. S. Afr. J. Anim. Sci. 32,256-262.

KRAJINOVIĆ M., ŠAHINOVIĆ R., VEGARA M., VILIĆ H. (2004): Osnove opšteg stočarstva. Univerzitetski udžbenik. Izd. „Grafičar“ Bihać. Bihać.

KURT G., HORST R. B.(2008): Development and characterization of lambs' coats of East Prussian Skudden and Rough-coated Pomeranian Landrace sheep. Arch. Tierz., Dummerstorf. 51, 467- 478.

MARIĆ I., ANTUNOVIĆ Z., ŠPERANDA M., STEINER Z., NOVOSELEC J. (2013): Utjecaj hranidbe i dobi na proizvodna svojstva i fenotipske odlike janjadi pasmine dubrovačka ruda. 48th Croatian & 8th International Symposium on Agriculture. 17th to 22nd February 2013 Dubrovnik, Croatia.

MEMIŠI N., BAUMAN F., GRUBIĆ G., KOLJAJIĆ V., PAVLOV B. (2002): Uticaj različitih izvora nerazgradivog proteina u obroku na proizvodna svojstva rano odlučene jagnjadi u tovu. Biotechnology in Animal Husbandry, 18 (5-6), 213-219.

PETROVIĆ M.P., PETROVIĆ V. CARO, RUŽIĆ MUSLIĆ D., ILIĆ Z., SPASIĆ Z., STOJKOVIĆ J., MILENKOVIĆ M. (2012): GENETIC AND PHENOTYPIC ASPECTS OF THE BODY MEASURED TRAITS IN MERINOLANDSCHAF BREED OF SHEEP. Biotechnology in Animal Husbandry 28 (4), 733-741.

PETROVIĆ P. M., RUŽIĆ-MUSLIĆ, D., MAKSIMOVIĆ, N., MEMIŠI, N.(2009): Effect of environmental and paragenetic factors on birth mass variability of Mis sheep populations. Biotechnology in Animal Husbandry. 25,213-219.

PETROVIĆ V. CARO, PETROVIĆ M. P., PETROVIĆ M. M,. ILIĆ Z., MAKSIMOVIĆ N., RUŽIĆ MUSLIĆ D., STOLIĆ N. (2012): Estimation of phenotypic and genetic trends of the growth traits in lipska and svrljig sheep.Biotechnology in Animal Husbandry 28 (4), p 743-749.

SALAKO, A.E. (2006): Principal component factor analysis of the morphostructure of immature Uda sheep. Int. J. Morphol.,24(4):571-4, 2006.

UGARTE E. (2007): The Breeding program of Latxa Breed. Biotechnology in Animal Husbandry, 5-6, 97-111.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE INFLUENCE OF THE LEVEL OF NON-DEGRADABLE PROTEIN AND THE CROSSING SYSTEM ON THE TORTUOSITY, STRENGTH AND EXTENSIBILITY OF LAMB WOOL FIBER

Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro-Petrović, Nevena Maksimović, Violeta Mandić, Bogdan Cekić

Institute for Animal Husbandry, Belgrade-Zemun, 11080, Serbia
Corresponding author: Dragana Ružić-Muslic, e-mail: muslic.ruzic@gmail.com
Original scientific paper

Abstract: The paper presents the influence of a different level of non-degradable protein and a system of crossing on the tortuosity, strength and stretchability/ extensibility of the lamb wool fibre. The researches were carried out according to the scheme of the repeated factorial experiment 3 x 2 (three levels of non-degradable protein and two systems of crossing of lambs). The trial included 30 lambs - crosses of F1 generation: Pirot Pramenka (50%) x Württemberg (50%), and 30 crosses of F1 generation: Pirot Pramenka (12.5%) x Württemberg (37.5%) x Ille de France (50%), which were weaned at 60 days of age, an average body weight of 18.0 kg. The animals were fed with compound feeding stuffs and alfalfa hay in groups and ad libitum. The mixtures differed in proportion of non-degradable protein I (43%): II (51%): III (58%). The trial lasted 75 days. The final weight of the lambs was about 35.0 kg. To test the tortuosity, strength, and extensibility of the wool fibres, samples from all the animals in the experiment were taken from three places: the left side of the shoulder, the back and the rump. The average fibre tortuosity in lambs on treatments I: II: III was 2.80, 2.86 and 2.90 whorls/cm of fibre length, respectively. Two breed crosses had 2.82, and the triple breed crosses 2.89 whorls/cm of fibre length. The average absolute extensibility of the fibres in lambs on treatments I: II: III was 8.04, 7.51, and 9.18g, respectively, and in two breed crosses (PxW) and triple breed crosses (PxWxIDF): 8.31 and 8.18g, respectively. The average fibre extensibility in lambs on treatments I: II: III was 26.74, 29.41 and 33.18%, respectively, and for two breed crosses (PxW) and triple breed crosses (PxWxIDF): 26.07 and 33.49%, respectively. The level of non-degradable protein in food mixtures, as well as the system of crossing of the lambs, significantly influenced the tortuosity, strength and stretchability/ extensibility of the fibres.

Key words: lambs, non-degradable protein, crosses, tortuosity, strength, stretchability/ extensibility

Introduction

During the intrauterine development of the lambs, the formation wool follicles begins to the extent determined by the genetic potential of the animal. Studies have shown that the development of wool fibres starts with 50 and ends with the 110 days of embryonic development (*Jovanović, 1996, 2001*). Their growth is a continuous process, which, in addition to the hereditary basis, is greatly influenced by maternal nutrition during the last stage of pregnancy, while the level of lambs' nutrition in the postnatal period determines the number of follicles that will mature and become functional. Inadequate nutrition of lambs results in, among other things, the slowing of follicular maturation, which in the end results in a reduction in the yield of wool *(Masters et al., 1998).* In addition to the yield of wool, its quality is of particular importance, contained in a number of properties that determine its commercial value (*Wood, 2003; Jones et al., 2004; Purvis and Franklin, 2005; Bidinost et al., 2008).*

Considering that the wool fibres consist of a protein (keratin), it is understandable that the dietary proteins are the primary factors determining the growth and the quality of wool, which is determined by the physical-mechanical properties: diameter (fineness), height, length, tortuosity, strength and stretchability/ extensibility *(Ružić-Muslić, 2006).* The effectiveness of the conversion of dietary proteins into wool proteins amounts to 15-20%, which is, among other things, a consequence of microbiological degradation of the protein into the rumen. The degradation of dietary proteins in the rumen makes it impossible to supply the organism of sheep with larger amounts of amino acids containing sulphur and which are responsible for the quality of wool. Proteins which avoid the bacterial hydrolysis in the rumen (non-degradable protein) increase the wool production by increasing the supply of the organism with amino acids, in particular cystine which is a limiting factor *(Ružić-Muslić, 2006).*

The aim of the research was to examine the effect of the level of non-degradable protein (43: 51: 58%) of the feed mixture) and crossing system (two breed and three breed crosses) on the tortuosity, strength and stretchability/ extensibility of the wool fibre in lambs.

Material and Methods

The trial was designed according to the scheme of the repeated factorial experiment 3x2 (three levels of non-degradable protein: 43: 51: 58%) and two systems of crossing (two and three breed crosses). The research involved 30 lambs – crosses of F1 generation: Pirot Pramenka (50%) x Württemberg (50%) and 30 crosses of F1 generation: Pirot Pramenka (12.5%) x Württemberg (37.5%) x Ille de France (50%), which were weaned with 60 days of age, with an average body weight of 18.0 kg. They were fed with fodder mixtures and alfalfa hay in groups and ad libitum. Table 1 shows the nutritional value of the mixtures.

Table 1. Nutritional value of mixtures

Nutritional indices	Concentrate mixtures		
	I	II	III
*Dry matter, g kg^{-1}	870	860.5	860.8
*OFU	1.2	1.2	1.2
*NEM,MJ	7.51	7.98	7.91
**UFV	0.99	1.05	1.04
*Total protein,g kg^{-1}	142	137	141
RUP	**43**	**51**	**58**
**PDIN g $animal^{-1}$ day^{-1}	102	103	107
**PDIE g $animal^{-1}$ day^{-1}	102	112	118
*Ashes, g kg^{-1}	25	23	27
*Ca,g kg^{-1}	8.4	8.2	10.6
*P,g kg^{-1}	4.6	3.7	5.0

RUP- rumen non-degradable protein; PDIN - protein digested in small intestine depending on the fermenting nitrogen; PDIE - protein digested in small intestine depending on the fermenting organic matter **INRA (1988) *Obračevic (1990)

Feed mixtures differed in the share of non-degradable protein: 43: 51: 58%. The average animal weight at the end of the experiment, which lasted 75 days, was about 35.0 kg. In order to examine the quality of the wool from all the animals, samples from three places were taken: the left side of the shoulder, the back and the rump of the animals. For each sample, 10 fibres were measured from the places indicated, i.e. 30 per animal, that is, a total of 1800 fibres were measured. Samples were cut with scissors, along the skin, 2-3 cm in thickness and stacked in a form with data on the identity of the animal and the location on the body from which the sample was taken. As an indicator of the quality of the wool, the following properties were analysed: tortuosity, absolute strength, stretchability/ extensibility of the wool fibre. The torsion was determined by the number of whorls/cm of fibre length. The indicated measurements were made according to JUS.F.$B_1 0_{11}$ and JUS.F.$B_1 0_{12}$. Measurement of the strength and extensibility of the wool fibre was carried out according to JUS F.$S_{2.}$213 on the dynamometer for determining the breaking force and elongation of fibres, yarns and fabrics of the type "Instron" at the Textile Institute in Belgrade. The strength of the fibre (breaking force) is expressed in cN/tex (centinewton by tex), where: cN is the force and the tex-length mass. Tex is the main representative of the mass-numbering system of fibre fineness and shows how much mass in grams has a fibre length of 1000 meters. In order to express the strength of the fibres in grams, it is necessary to translate the cN into grams, which is achieved by dividing the obtained value with the factor 0.981. The statistical processing of the obtained data is executed on a computer using Stat.Soft, Inc. (2003). STATISTICA (data analysis software system), version 6, using standard mathematical - statistical methods that imply the analysis of variance (according to the plan of factorial experiment 3x2, where the levels of non - degradable protein are one, and the lamb crossing system, the second observed factor) and the evaluation of the significance of the differences obtained, for this purpose with a suitable test (Tukey honest significant difference test).

Results and discussion

The tortuosity is a feature of the wool fibres to form a bend. It is expressed by the number of whorls per 1 cm of fibre length. The results of the average values for the tortuosity of wool fibres are shown in Table 2. The results show that the average number of whorls, according to the treatments I: II: III, was 2.80, 2.86 and 2.90 per cm of fibre length, respectively. The observed differences were minimal and were not statistically confirmed (P<0.05). The tortuosity of fibres in crosses was 2.82 (two breed crosses) and 2.89 (triple breed crosses) on average. The analysis of this feature from the aspect of the crossing system shows that the differences were statistically very significant (P = 0.002). It is known that the greatest tortuosity of fibres is on the shoulders and the weakest on the back *(Mitić, 1987).*

Table 2. The tortuosity of wool fibres (number of whorls per 1 cm of fibre length)

Non-protein	Crosses	Location	Indicators Average	CV
		Shoulder	2.96±0.27	9.18
		Back	2.63±0.24	9.14
	2	Rump	2.87±0.20**	7.17
		Average	2.82	
I (43%)		Shoulder	2.97±0.25	8.55
		Back	2.74±0.38	13.78
	3	Rump	2.67±0.12**	4.69
		Average	2.79	
Average I			**2.80**	
		Shoulder	3.11±0.42	13.51
		Back	2.77±0.27	9.63
	2	Rump	2.90±0.18**	6.29
		Average	2.93	
II (51%)		Shoulder	2.87±0.27	9.30
	3	Back	2.85±0.33	11.61
		Rump	2.68±0.18**	6.77
		Average	2.80	
Average II			**2.86**	
		Shoulder	3.05±0.22	7.12
	2	Back	2.82±0.20	7.25
		Rump	2.91±0.21**	7.32
		Average	2.93	
III (58%)		Shoulder	3.04±0.30	9.83
		Back	2.73±0.20	7.34
	3	Rump	2.84±0.24**	8.33
		Average	2.87	
Average III			**2.90**	
Average	2		2.82	
	3		2.89	

2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF) ; *(P<0.05); ** (P<0.01); ***(P<0.001)

Table 3 shows the results of the average absolute load (strength) of the fibre. The strength or the load of the fibres is its trait to resist the breaking force that seeks to break it. It is expressed in grams. The highest absolute fibre strength (9.18 g) was determined in lambs for treatment III and the lowest (7.51 g) for treatment II, while the load capacity of the fibres of animals in the treatment I was 8.04. The difference in fibre strength on the back of the animal, between triple crosses, on treatments III and I of 4.64g was statistically significant (P = 0.02). Also, the three breed crosses on treatments III and II differed in respect to the stated traits by 4.92g, which was statistically confirmed (P=0.01). The average fibre strength (PxW) and (PxWxIDF) was 8.31 and 8.18 g, respectively. A statistically significant difference (P = 0.01) of 4.57g was determined between the strength of the fibres on the back between the two breed crosses on treatment I and the triple crosses on treatment III. The established absolute load capacity of the fibres ranges from 1.6 to 60 g as indicated by *Mitić (1987).* The results of our research support the finding that the strength of the wool fibre depends on the nutrition of the animals *(Mitić, 1987; Petrović, 2000).*

Table 3. Average absolute strength of wool fibre, (g)

Non-degradable protein	Crosses	Location	Average	CV
		Shoulder	8.54±4.50	5.76
	2	Back	7.78±4.48*	7.58
		Rump	8.16±4.49	5.04
I (43%)		Average	8.16	
		Shoulder	7.5	2.24
	3	Back	7.71±0.56	7.25
		Rump	8.55±2.38	4.89
		Average	7.93	
Average I			**8.04**	
		Shoulder	8.53±1.07	3.17
	2	Back	9.24±0.57	6.18
		Rump	5.93±0.19	3.26
II (51%)		Average	7.90	
		Shoulder	6.17±0.60	6.53
	3	Back	7.43±0.29*	3.85
		Rump	7.80±1.74	5.69
		Average	7.13	
Average II			**7.51**	
		Shoulder	9.15±0.44	4.85
	2	Back	8.45±1.53	5.05
		Rump	9.00±1.05	4.75
III (58%)		Average	3.11	
		Shoulder	7.28±0.55	7.60
	3	Back	12.35±0.44	3.61
		Rump	8.83±1.04	3.22
		Average	9.49	
Average III			**9.18**	
Average	2		8.31	
	3		8.18	

2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF) ; *(P<0.05);

The stretchability/extensibility of the wool fibre implies an increase in the length of the fibre until breaking/tearing, expressed in cm (absolute stretching). However, if this increase is expressed in % relative to the length of the fibre prior to stretching, relative stretching is obtained. The results of the measurement of the relative extensibility of the lamb's wool fibres are shown in Table 4.

Table 4. Average stretchability/ extensibility of the wool fibre, %

Non-degradable	Crosses		Indicators	
		Location	Average	
		Shoulder	24.19±4.18	5.04
	2	Back	22.51±6.40***	7.71
		Rump	24.69±7.85	7.27
I (43%)		Average	23.80	
		Shoulder	29.17±3.52	6.88
	3	Back	28.32±2.57*	6.06
		Rump	31.55±2.82	4.38
		Average	29.68	
Average I			**26.74**	
		Shoulder	25.21±2.88	2.42
	2	Back	26.56±3.22**	6.70
		Rump	25.18±2.27	4.00
II (51%)		Average	25.65	
		Shoulder	25.11±2.24	8.91
	3	Back	41.09±1.83***	4.44
		Rump	33.35±1.73	5.20
		Average	33.18	
Average II			**29.41**	
		Shoulder	34.46±1.44	4.17
	2	Back	25.02±3.31*	5.22
		Rump	26.79±5.13	3.74
III (58%)		Average	28.76	
		Shoulder	35.36±3.25	4.18
	3	Back	41.07±3.60**	4.76
		Rump	36.36±2.00	5.53
		Average	37.60	
Average III				
Average	2			
	3			

2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF) ; *(P<0.05); ** (P<0.01); ***(P<0.001)

The multiple stretchability/extensibility of fibres in lambs on treatments I: II: III was 26.74, 29.41 and 33.18%, respectively. The differences in this feature between samples taken from the back were statistically highly significant (P = 0.000027). The three breed crosses on treatments II and I significantly differed (P = 0.02) by 12.77% in terms of fibre stretching on the back. Analysing the average fibre stretching/expansion, depending on the crossing system, we can conclude that the increase of the length of fibres in triple crosses was greater by 7.42% in relation to two breed crosses. The difference in the stretchability/ extensibility of fibres on the back of 16.05% between double and triple crosses at the source of protein III were statistically confirmed (P = 0.04). Also, the examined populations of crosses differed in the mentioned trait at the source of protein II by 14.53%, which is statistically very significant (P = 0.008). At the same time, the established difference of 18.58% in the stretching/expansion of the fibres between the two breed crosses on the treatment I and the three breed crosses on treatment II was statistically significant (P = 0.000185), as well as the difference of 18.56% between the two breed crosses on the type of diet I and triple crosses on the type of diet III (P = 0.006). The stretchability/ extensibility of fibres of two breed crosses on treatment III and three breed crosses on treatment II differed by 16.07%, which was statistically significant (P = 0.002). Our results are within the limits stated by *Mitić (1987)* for this feature, which emphasize that the stretchability/ extensibility of the wool fibres ranges from 2 - 79% of their length, depending on the complex of exogenous and endogenous factors.

Judging from the results of research of physical and mechanical properties of wool fibres (tortuosity, strength, stretchability/ extensibility) of trial lambs, we can conclude that the test rations which were different in the ratio of non-degradable protein in total (43.51: 58%) contributed to the achievement of a difference in the values for said traits. In addition, the wool of the lambs fed with a mixture containing 58% NP, in terms of the mentioned properties, as quality parameters, was characterized as the wool of the highest quality. Slightly lower values for the tortuosity, the strength and the stretchability/ extensibility of fibres were determined in lambs which consumed the mixture with 51% of NP, and the lowest in lambs which had been on the treatment with 43% NP. This is consistent with the fact that the proteins which avoid the breakdown in rumen increase the growth and improve the quality of wool, by increasing the supply of amino acids to the organism (*Whitney et al., 2011).* The degradation of the dietary protein in the lamb rumen helps prevent the supply of an organism with higher amounts of amino acids. Proteins which avoid bacterial hydrolysis in the rumen (NP) increase the wool production, by increasing the supply of the organism with amino acids, in particular cystine, which represents a limiting factor for the production of wool. According to *Jovanović (2001),* the infusion of cystine to abomasum or blood may double the growth of wool, while infusion of methionine increases the growth of wool by providing sulphur for the synthesis of cystine.

Slen (1969), in the study of the impact of protein sources in the diet on the growth of high quality wool a fibres in yearlings of Romney breed, has found that animals consuming diet with the higher proportion of non-degradable protein have by 172% greater height of the fibres, superior tortuosity and by 206% stronger and heavier fibres. By studying the effect of different concentrations of dehydrated lucerne (0, 5, 10, 15 and 20%), as a source of non-degradable protein in the diets for lambs of 17.0 to 36.0 kg of body weight, on the production of wool, *Urbaniak (1994)* has found that the largest accumulation of the protein in wool fibres (4.11 g/day) is obtained in lambs fed with a concentrate containing 10% dehydrated alfalfa.

Viewed from the aspect of the influence of the system of crossing on the quality parameters of wool, it is obvious that three-breed crosses exceeded the two breed crosses, as a result of the heterosis effect. Similar results are stated by *Ćeranić (1970)* in the study of the impact of two-breed and three-breed crossing system on some properties of wool fibres. The following genotypes were used in the experiment: F2 (Domestic Merino x Prekos); F2 (Domestic Merino x Stavropol breed); (F1 Domestic Merino x Prekos) x Caucasian breed; (F1 Domestic Merino x Stavropol breed) x Caucasian breed. The absolute strength of the fibres respective to treatment is 7.46, 7.50, 7.47 and 7.80, while the stretching (expressed in %) is 32.51, 31.31, 32.36 and 36.78, respectively. The author emphasized the advantage of the triple breed combination in all the tested wool properties.

Conclusion

Based on the conducted research, the following conclusions can be made:

- Average fibre tortuosity in lambs on treatments with 43: 51: 58 % NP was 2.80, 2.86 and 2.90 whorls/cm of fibre length, respectively. The two breed crosses had 2.82, and the triple crosses 2.89 whorls/cm of fibre length, respectively.
- The average absolute load (strength) of lamb fibres in analogous treatments was 8.04, 7.51 and 9.18 g, and for two breed crosses (PXW) and three breed crosses (PxWxIDF): 8.31 and 8.18g.
- The average fibre stretchability/ extensibility in lambs on treatments 43: 51: 58% was 26.74, 29.41 and 33.18%, respectively, and for two breed crosses (PXW) and three breed crosses (PxWxIDF): 26.07 and 33.49%, respectively.
- The proportion of non-degradable protein in food mixtures, as well as the crossing system of the lambs, significantly influenced the tortuosity, strength and stretchability/ extensibility of the wool fibres.

Uticaj nivoa nerazgradivog proteina i sistema ukrštanja na vijugavost, jačinu i rastegljivost vunskog vlakna jagnjadi

Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro-Petrović, Nevena Maksimović, Violeta Mandić, Bogdan Cekić

Rezime

U radu je prikazan uticaj različitog nivoa nerazgradivog proteina i sistema ukrštanja na vijugavost, jačinu i rastegljivost vunenog vlakna jagnjadi. Istraživanja su obavljena po šemi ponovljenog faktorijalnog eksperimenta 3x2 (tri nivoa nerazgradivog proteina i dva sistema ukrštanja jagnjadi). U ogled je bilo uključeno 30 jagnjadi-meleza F_1 generacije: Pirotska pramenka (50%) x WÜRTEMBERG (50%) i 30 trorasnih meleza F1 generacije: Pirotska pramenka (12.5%) x WÜRTEMBERG (37.5%) x il de frans (50%), koja su zalučena sa 60 dana uzrasta, prosečne telesne mase 18.0 kg. Grla su hranjena krmnim smešama i lucerkinim senom grupno i po volji. Smeše su se razlikovale u pogledu udela nerazgradivog proteina 43:51:58%. Ogled je trajao 75 dana. Završna telesna masa jagnjadi je iznosila oko 35.0 kg. Za ispitivanje vijugavosti, jačine i rastegljivosti vunskih vlakana uzeti su uzorci od svih grla u eksperimentu, sa tri mesta: leve strane plećke, poslednjeg rebra i sapi. Prosečna vijugavost vlakana kod jagnjadi na tretmanima I: II: III je iznosila: 2.80 : 2.86 : 2.90 vijuga/cm dužine vlakna. Dvorasni melezi su imali 2.82, a trorasni 2.89 vijuga/cm dužine vlakna. Prosečna apsolutna nosivost (jačina) vlakana kod jagnjadi na tretmanima I : II: III je iznosila: 8.04 :7.51: 9.18 g , a kod dvorasnih (PXW) i trorasnih (PxWxIDF) meleza: 8.31: 8.18g. Prosečna rastegljivost vlakana kod jagnjadi na tretmanima I : II : III je iznosila : 26.74: 29.41: 33.18%, a kod dvorasnih (PxW) i trorasnih (PxWxIDF) : 26.07 : 33.49%. Nivo nerazgradivog proteina u smešama za ishranu, kao i sistem ukrštanja jagnjadi, su značajno uticali na vijugavost, jačinu i rastegljivost vlakana.

Ključne reči: jagnjad, nerazgradiv protein, melezi,vijugavost, jačina, rastegljivost

Acknowledgment

Research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR 31053.

References

BIDINOST F., ROLAND D.L.,DODERO A.M., CANO E.M.,TADDEO H.R.,MUELLER J. P., POLI M. A. (2008): Wool quantitative trait loci in Merino sheep.Small Ruminant Research, 74, 113-118.

ĆERANIĆ V. (1970): Izuĉavanje uticaja dvorasnog i trorasnog ukrštanja domaćeg merina na popravljanje nekih osobina vlakna. Arhiv za poljoprivredne nauke, 23, 82-86.

CHALUPA W. (1975): Rumen bypass and protection of proteins and amino acids. Journal Dairy Science, 58, 1198-1218.

JONES C., MENEZES F., VELLA F. (2004): Auction price anomalies: Evidence from wool auctions in Australia. Econ. Rec., 80, 271-288.

JOVANOVIĆ R. (1996): Ishrana ovaca, eds., Stylos, Novi Sad, pp. 420.

JOVANOVIĆ R., DUJIĆ D., GLAMOĈIĆ D. (2001): Ishrana domaćih životinja,eds., Stylos, Novi Sad, pp. 713.

MASTERS D.G., MATA G., LIU S.M., PETERSON A.D. (1998): The influence of live weight, live weight change and diet on wool growth and staple strength in young sheep. Australian Journal of Agricultural Science, 49, 269-277

MITIĆ M. (1984): Ovĉarstvo, eds., Zavod za udžbenike i nastavna sredstva,Beograd.

PETROVIĆ P.M. (2000):Genetika i oplemenjivanje ovaca. Naučna knjiga, Beograd, 365pp.

PURVIS I.W., FRANKLIN I.R. (2005): Major genes and QTL influencing wool production and quality: A review. Genetics Selection Evolution, 37, 97- 107.

RUŽIĆ-MUSLIĆ D. (2006): Uticaj razliĉitih izvora proteina u obroku na proizvodne rezultate jagnjadi u tovu. Doktorska disertacija, Poljoprivredni fakultet, Beograd-Zemun, pp. 160.

SLEN S. B. (1969): Nutrition in relation to wool production and body growth in sheep. In Nutrition of animals of agricultural importance, SIR DAVID CUTHBERSTON, eds., Pergamon press, London, 1969, 827-848.

URBANIAK M. (1994): Effect dehydrated lucerne on lamb performance and protein and energy deposition in the body. Journal of Animal and Feed Sciences, 3, 191-199.

WHITNEY T.R., LUPTON C.J., SMITH S.B.(2011): Redberry juniper as a roughage source in lamb feedlot rations: Wool and carcass characteristics, meat fatty acid profiles, and sensory panel traits. Meat Science,Volume 89, Issue 2, October 2011, Pages 160–165

ECONOMIC VALUES OF THE BASIC PRODUCTIVE AND FUNCTIONAL TRAITS OF SHEEP FROM BULGARIAN DAIRY SYNTHETIC POPULATION

Stanimira Slavova, Georgi Kalaydzhiev, Jivko Krastanov, Staika Laleva, Yovka Popova, Petia Slavova

Agricultural Institute – Stara Zagora, Bulgaria
Corresponding autor: mirka_sl@abv.bg
Original paper

Abstract: The subject of the study was to estimate economic values of the main productive and functional traits of the sheep flock from Bulgarian dairy synthetic population, raised in the Agricultural Institute in Stara Zagora, Bulgaria. The collected data was processed by the Program EWSH2 for sheep, version 1.0.2., using a bio-economic model. The study covered a period of five years (2010-2014), i.e. five reproductive cycles, at semi-intensive productive system with one lambing per year. The following indicators were investigated: rate of profitability, marginal economic values (MEV) of the traits of great economic importance, and their relative economic weights (REV) in the sum of the absolute values of the standardized economic values. The rate of profitability including governmental subsidies is estimated (-25.63%). Milk yield stands out with the highest relative economic weight of 19.01, followed by the litter size per ewe (16.99) and live weight (13.72). The production lifetime of ewes has a relative value of 8.70 and conception rate – 6.21, which is significantly lower than the results obtained from similar studies in other European countries in the years. The values of the average survival rate till 24 hour after birth and from birth until weaning are almost equal and relatively low.
Key words: Bulgarian dairy synthetic population, production traits, functional traits, marginal economic value, relative economic value, rate of profitability,

Introduction

The basic object of selection in sheep is improving breeding traits, which to provide sustainable development of the farms in the future. The general definition of breeding objectives is “development of vital animals ensuring profit under future commercial conditions", implying the inclusion of several traits in an aggregate genotype *(Fewson, D., 1993).* However, genetic changes in sheep sometimes may not be as profitable as expected. According to *James, J. W., (1982)*, an efficient selection for the wrong objective may be worse than no selection at all. On that reason it is of great importance to recognize the traits which have significant impact on the final economic effect in particular economic circumstances by calculating their economic values using different methods.

Ponzoni, R. W. and S. Newman (1989), stress that the derivation of the economic value of each trait influencing income and inputs is a crucial step in the development of breeding objective. Usually, subject of investigation are the economic values or weights of the most important traits as milk yield, mature live weight, litter size, daily gain of lambs, conception rate of ewes, productive lifetime etc. While the terms economic value and economic weight are often used synonymously, they may also be defined as the absolute and the relative benefit of improving a trait, respectively *(Amer et al., 2001)*. In order to establish a total merit index, the relative levels of economic values of the considered traits should be known *(Fuerst-Waltl, B. and R. Baumung, 2009).* These traits include dairy (milk yield, fat and protein content, somatic cells count), growth (birth and mature weights and growth rates from birth to weaning and in the rearing period of replacements) and functional traits (conception rate of ewes and ewe lambs, lamb survival rates at lambing and till weaning, productive lifetime, litter size). Therefore, the subject of our study was to estimate which of the investigated production and functional traits had a significant impact on the economic effect at given economic conditions.

The economic weight of a particular trait in the selection index is calculated on the basis of a complex assessment of the importance of each of the studied traits *(Krupova et al., 2009)*. In a number of European countries breeding programs in dairy sheep breeding are focused on milk production *(Oravcova et al., 2005; Smulders et al., 2007)* and litter size *(Margetin et al., 2006)*. But in recent years, functional traits have become increasingly important for the efficiency of breeding schemes, due to increased production costs that affect milk prices, consumer demand for safe and quality food and animal welfare *(Barillet, 2007)*, which implies their inclusion in the selection indexes.

A number of authors investigate economic values for production traits using different approaches in the years. *Legarra et al. (2007)* calculated economic values for the two Spanish sheep breeds Laksa and Manchega, and *Fuerst-Waltl and Baumung (2009)* - for some Austrian dairy sheep breeds respectively. *Morais and Madalena (2006), Lobo et al. (2011), Kosgey et al. (2004), Tolone et al. (2011)* in their researches identify the traits with the most significant impact on the revenue and profit under specific market conditions, for which the breeding objective would be profitable in the future.

Wolfová et al. (2009) develop a bio-economic model to estimate the effects of change in production and functional traits on profit in dairy or dual-purpose milked sheep under alternative management systems. Later, in the Czech Republic, the gene-flow methodology for the calculation of economic weights for direct and maternal traits and trait components in cattle was adapted for sheep, so that the economic weights for 12 production and functional traits for Suffolk sire breeds used in different breeding systems were estimated *(Wolfová et al., 2011).*

In the Slovak republic *Krupová et al. (2009)* calculate economic values of 14 production and functional traits for two dairy sheep breeds (Improved Valachian and Tsigai). In their subsequent study *(2013)* they make a sensitivity analysis of dairy sheep farming by investigating the impact of changes in economic conditions on the absolute and relative economic importance of the traits for the same two breeds, which are the most widespread in Slovakia.

Wolf, J. et al. (2011) develop a program for calculating economic weights in livestock and partially for sheep called Program EWSH2 for Sheep, Version 1.0.2 that we use to process the data we collected within our study.

In our country no other previous studies devoted to economic assessment of production traits of sheep and their impact on the function of profit have been made.

The subject of the present study was to evaluate profitability and calculate marginal and relative economic values of the most important traits in the sheep flock of Bulgarian dairy synthetic population, raised in the Agricultural institute in Stara Zagora, Bulgaria.

Material and Methods

The object of the study was the sheep flock from Bulgarian dairy synthetic population, bred in the Experimental farm of the Agricultural Institute in Stara Zagora, Bulgaria. It is necessary to specify that this sheep population is widespread - about 70% of the dairy sheep in the country. It is a so-called "composite breed", a result of long-standing internal selection after crossbreeding of different breeds, as well as such specialized for milk. The basis breed in the flock, raised in the Institute in Stara Zagora, is Starozagorska local breed, in which genetic plasma from the Pleven black-head breed and East Frisian breed has been infused. Subsequently, a part of the ewes have been crossed with rams of the Chios breed.

The study was conducted for 5 reproductive cycles, i.e. from 2010 to 2014. Production scheme included lambing during the winter in the premises and grazing in the summer. The flock was reproduced by its own replacements. In order to increase the genetic variation for milk productivity, in the recent years, a genetic component from the Lacaune breed was included. Therefore, rams from pure-bred imported flocks were used during the insemination. There was one breeding season per year, starting around 15th August and ending around 15th October. Ewes were being mated by artificial insemination. After the campaign, lasting for 45 days, rams were placed unlimited among ewes. Female replacements were mated after the age of 18 months. Conception rate of ewes and breeding ewe lambs was about 80% and 85%, respectively.

Lambs were weaned around the 60th day after birth, and subsequently sold or left per replacement. Ewes were milked twice a day and drained until the end of September. The milk yield, was 0,8 kg / day per ewe at a standard milking period of 120 days. Rams were used for 4 years in schemes of pure breeding or crossbreeding. The ratio of sheep to rams was 50:1. The average production lifetime of ewes was at about 5,5 years. The basic information related to their reproductive characteristics is shown on Table 1.

Table 1. Key indicators for ewes in terms of reproductive cycles

Reproductive cycle, n	Death rate of ewes, %	Ewes, culled due to health problems, %	Ewes, culled due to low milk production, %	Conception rate of ewes, %	% of single and twin births	
1	5	1	7	85	60	30
2	5	1	5	80	63	30
3	10	1	10	75	46	52
4	16	1	10	80	53	44
5	7	1	7	-	47	50

Note: the percentage of triplets is 100 minus the sum of the singles and twins

The proportion of ewes crossed with rams from Lacaune breed for a reproductive cycle was 35%, and ewe lambs - 40%. On Table 2 is presented the birth and weaning weight of purebred and crossbred male and female lambs. Purebred lambs are lambs, whose mothers from Bulgarian dairy synthetic population have been mated with rams from the same breed, and crossbred are lambs, whose mothers from Bulgarian dairy synthetic population were mated with Lacaune rams. The growth rate of lambs shows differences in both sex and birth type.

Table 2. Birth and weaning weight in purebred and crossbred lambs

Birth weight	Singles	Twins	Triplets
Purebred male lambs	4.5	3.9	2.9
Purebred female lambs	4.3	3.7	2.7
Crossbred male lambs	4.1	3.6	3.0
Crossbred female lambs	3.8	3.3	2.8
Weaning weight			
Purebred male lambs	20.5	17.9	15.6
Purebred female lambs	19.1	17.2	13.4
Crossbred male lambs	18.7	16.8	16.0
Crossbred female lambs	17.9	16.1	15.5

The economic efficiency in the studied flock was estimated by the synthetic indicator – rate of profitability, which was calculated as a profit to cost ratio. The profit was representing the difference between total revenues and total costs with addition of the governmental subsidies per ewe and year.

All revenue was discounted to the initial period by the discount rate of 0.08%. It was made up of sold milk, lambs for slaughter, breeding lambs, culled ewes and rams, and of wool. The price of milk was calculated on the basis of a physical unit (liter) and not as a function of milk fat, protein content and somatic cells as in other European countries. The milk was sold to dairies, so there was no benefit from processing it into cheese. Animals of all categories were sold per kg of live weight. The price per kg was the same for both purebred and crossbred animals. Wool was sold per kg, regardless of its quality.

The most significant costs were those for feed, labor, veterinary services and medicines, milking, shearing, electricity, fuels, materials etc. There were winter and summer feeding seasons, as during the summer period sheep were mainly fed by grazing, straw and hay. In the winter silage was also added. Lactating ewes and those in advanced pregnancy, as well as breeding male and female animals during the flushing period were supplemented with concentrated mixtures. Feed costs were calculated on the basis of the animals' energy and protein requirements, and the amount of dry matter in the rations for the relevant category. The Institute has its own feed production, as well as a fodder enterprise, where concentrates are prepared, so that their prices are at cost. Table 3 shows the prices per kg of forage in the ration according to the category, given that the values are averaged over the two feeding seasons. State subsidies per ewe for a reproduction cycle (or per year) were 34 BGN on average. They do not depend on productivity and do not affect the economic value of the traits.

Table 3. Price per unit of the main revenue and costs (BGN, equal to 0,5 EURO)

Revenue	BGN	Costs	BGN
Base price for milk, MU/kg	1.20	Price for fresh feed for feeding rations for ewes, MU/kg	0.1034
Price per kg wool of ewes	1.00	Price for fresh feed for feeding rations for rams, MU/kg	0.0832
Price per kg live weight of breeding lambs (age < 1 year), MU/kg live weight	6.50	Price for fresh feed for feeding rations for lambs till weaning, MU/kg	0.0361
Price per kg live weight of not mated ewe lambs sold between the first and the second breeding season, MU/kg live weight	4.00	Price of fresh matter for all seasonal variants of feeding ration for female breeding lambs (flushing), MU/kg	0.0992
Price of kg live weight of ram lambs before the i-th breeding season, MU/kg live weight	5.00	Price of fresh matter for all seasonal variants of feeding ration for female and male breeding lambs, MU/kg	0.0994
Price per kg live weight of ewes older than 12 months, MU/kg live weight	2.00	Price of fresh matter for all seasonal variants of feeding ration for female breeding lambs (high pregnancy),MU/kg	0.2866
Price per kg live weight of lambs slaughtered after weaning, MU/kg live weight	5.00	Costs for drugs per kg live weight and per drenching against worm (endo-parasites), MU/kg live weight	0.015
Governmental subsidy, MU/ewe per year	33.00	Costs for drugs per treatment against ecto-parasites and per animal, MU/animal	0.20
		Average costs for veterinary service and drugs (except for parasite treatment) per ewe per reproductive cycle, MU/ewe and reproductive cycle	4.30
		Price for water, MU/l	0.0001
		Number of man-hours per ewe and year (including lambs till weaning)	15
		Cost per man-hour (including insurance), MU/hour	2.9
		Cost per shearing per ewe, MU/animal	0.50
		Cost for milking per kg, MU/kg	0.10
		Other miscellaneous costs independent on animal performance per stable place per day for ewes (including lambs till weaning), MU/stable place per day	0.0056

The rate of profitability, which is the ratio between profit and production costs, is presented as an average value in % for the five reproduction cycles. The marginal economic value (MEV) of each trait is calculated as a private derivative of the profit function in relation to the relevant trait *(Wolfová et al., 2009).* It is an absolute value that indicates the change in the amount of profit by increasing by one unit the value of the trait. MEV is the value of the given trait in monetary units (BGN, which is equal to 0,5 EURO) per ewe per year. However, the function of profit is a complex function and the coefficient difference in the profit values at the beginning and end of the investment period must be taken into account. In order to compare the economic importance of the different traits, their marginal values are standardized by multiplying them by the genetic standard deviation of the respective traits (SD):): $\boldsymbol{evs_i = ev_i x \sigma_{gi}}$,, where $\mathbf{evs_i}$ is the standardized economic value of the trait i, and $\boldsymbol{\sigma_{gi}}$ is the genetic standard deviation for the trait i. The relative economic values (REV) of the traits present an expression of their percentages in the sum of the absolute values of the standardized economic values of all studied traits (Krupová et al., 2013): $\boldsymbol{evr_i=100x|evs_i|/\Sigma|evs_i|}$.

Genetic standard deviation (SD) values for the studied traits were obtained after processing of the collected data for the flock in the recent years. The marginal and relative values were established for all traits, which the program made evaluation for, but the final results summarized only those of the greater importance as milk yield, live weight, conception rate, litter size, productive lifetime, average daily gain and survival rate of lambs.

Profitability rate, marginal and economic values were calculated after processing the output data with EWSH2, version 1.0.2. *(Wolf, J., M. Wolfová, Z. Krupová and E. Krupa, 2011).*

Results and Discussion

Profitability rate amounted to (-25.63%) on average for the studied period, taking into account the payments from the government. The reason for the negative result was that costs are predominant in terms of revenue, so profit and hence profitability was of negative value.

Similar results are reported by *Michalickova et al., (2014)* in their study of the economic efficiency, using a database of the Animal Breeding Research Center in Nitra, Slovakia. The average profit-to-cost ratio in the investigated sheep farms for the period from 2006 to 2012 is (-48%).

Table 4 lists the marginal, standardized and relative economic values of the traits of great economic importance. Since in dairy sheep breeds the main focus is on milk productivity *(Carta and Ugarte, 2003),* milk yield was the first trait to be examined. Its economic value (EV) is 0.65 BGN/l (at a basic milk price of 1.20 BGN / l per 120 days milking period). For comparison, in Slovakia for the period 2004-2008, EV of the milk yield is 0.89 €/kg for the Improved Valachian breed and 0.86 €/kg for Tsigai at a milk price of 0.78 €/kg per 150 days milking period *(Krupová et al., 2009). Legarra et al. (2007)* report a marginal value of 0.69-1.44 €/l for some Spanish dairy breeds. In Bulgaria sheep milk is sold per kg (or liter) while in Slovak Republic, for example, the average milk price is calculated as a function of milk fat, protein content and somatic cell count.

The REV of milk yield in our study is 19.01 and has the highest percentage of the sum of the values of all the investigated traits. For comparison, in Slovakia it is 36.20 *(Krupová et al., 2013)* and 26.05 *(Krupová et al., 2009)* for the Improved Valachian breed. In this regard, the pricing system for the sale of sheep milk in Bulgaria is probably a restricting factor for acquiring additional benefits.

In our previous research *(Kalaydzhiev, G., 2014)*, we found a positive correlation between coagulation ability of sheep milk and milk components - fat and protein content, lactose, SNF etc. From this point of view, it is advisable to deepen the research in terms of improving the coagulation capacity of milk and optimizing its qualitative composition, which will have a positive economic effect when processing it into a final product.

It has been established that the litter size is an important reproductive trait not only in our study but also in those of *Fuerst-Waltl and Baumung, (2009)*, and *Legarra et al. (2007)*, where the marginal value of the trait is in the range of about 30 to 50 €/lamb. According to our calculations, MEV of the average litter size is 78 BGN per lamb. The relative economic value of the trait is 16.99, which means it is the second most important one in our investigation. For comparison, *Krupová et al. (2013)* report for REV of the trait is 9.10 in Improved Valachian at their own research.

The live weight of the ewes has a negative MEV (-1.62), which means that each unit of weight gain (in kg) leads to a reduction in profit. The relative value of the trait is 13.71 and its percentage makes it the third of economic importance. According to *Conington et al., (2004)* and *Morais and Madalena, (2006),* the economic effect of weight gain in sheep is usually negative.

Productive lifetime of ewes is also an important trait, although the reported relative value in our case is lower – 8.70, compared to the results of other authors. This can be explained by the greater relative share of other traits such as milk yield, letter size and live weight. Therefore, it would be more appropriate to compare SEV of the trait which is 12.20 for our flock, while for the Improved Valachian breed and Tsigai is respectively 5.39 and 5.50 *(Krupova et al., 2009).* The higher standardized economic value of the trait in our flock brings a higher profit per unit, hence the longer productive lifetime of the ewes is considered profitable.

Daily gain of lambs from birth to weaning usually has a greater economic significance in the sheep breeds for meat than in dairy sheep, and the share of the revenue from sold lambs in the total revenue in the meat sector is higher than in the dairy. *Conington et al. (2004)* estimate that the share of revenue from lambs sold out of total revenue is between 32% and 45%, depending on the production system. In our case, it is 35-45%, and the relative value of the trait is 6.927.

The conception rate of ewes has relative economic weight of 6.20, which is considerably lower than reported from *Krupova et al., (2009)* - 15.93 for the Improved Valachian breed and 14.46 for Tsigai. In the Slovak breeds the trait varies from 94% to 96%, while in our study the average conception rate of ewes is 80%.

Relative values of survival rate for lambs up to 24 hours after birth and from birth to weaning are relatively low – 5.60 and 5.27, respectively. These traits are not of a great economic importance because of the high survival rate of the lambs and low mortality.

Conclusion

On the basis of the results obtained, the following conclusions were drawn:

1. 1.Milk yield is the trait with the highest relative economic value – 19.011.
2. 2.The average litter size per lambed ewe has a relative weight – 16.99, so it is advisable to hold a selection in terms of increasing fertility, as well.
3. 3.The negative marginal value of the mature weight of ewes (-1.62) and its high REV – 13.71 have rather negative effect on the profit, which means that the live weight gain leads to reduced profitability and needs to be optimized.
4. 4.The lamb survival rate to 24th hour and from birth to weaning is relatively high - 96% and 98.5% respectively, so the relative economic values of the traits are relatively low – 5.60 and 5.27, which has a positive effect on the economic result in the flock.

Ekonomske vrednosti osnovnih produktivnih i funkcionalnih osobina ovaca bugarske mlečne sintetičke populacije

Stanimira Slavova, Georgi Kalaydzhiev, Jivko Krastanov, Staika Laleva, Yovka Popova, Petia Slavova

Rezime

Predmet studije bio je procena ekonomskih vrednosti glavnih proizvodnih i funkcionalnih osobina zapata ovaca bugarske mlečne sintetičke populacije, koje su odgajene na farmi Poljoprivrednog instituta u Staroj Zagori u Bugarskoj. Prikupljeni podaci su obrađeni u programu EVSH2 za ovce, verzija 1.0.2, koristeći bio-ekonomski model. Studija je pokrivala period od pet godina (2010-2014), tj. pet reproduktivnih ciklusa, u poluintenzivnom proizvodnom sistemu sa jednim jaganjenjem godišnje. Ispitivani su sljedeći pokazatelji: stopa profitabilnosti, marginalne ekonomske vrednosti (MEV) osobine od velikog ekonomskog značaja i njihovi relativni ekonomski ponderi (REV) u zbiru apsolutnih vrednosti standardizovanih ekonomskih vrijednosti. Procenjena je stopa profitabilnosti uključujući državne subvencije (-25,63%). Prinos mleka izdvaja se sa najvećom relativnom ekonomskom težinom od 19.01, praćen veličinom legla po ovci (16.99) i živom masom (13.72). Životni vek proizvodnje ovaca ima relativnu vrednost od 8,70 a stopa koncepcije - 6,21, što je znatno niže od rezultata dobijenih iz sličnih studija u drugim evropskim zemljama u godinama. Vrednosti prosečne stope preživljavanja do 24 sata nakon rođenja i od rođenja do odbijanja su skoro jednake i relativno niske.

Ključne reči: bugarska mleka sintetička populacija, proizvodne osobine, funkcionalne osobine, marginalna ekonomska vrednost, relativna ekonomska vrednost, stopa profitabilnosti

References

AMER, P.R, SIMM, G., KEANE, M.G., DISKIN, M.G.,WICKHAM, B.W. (2001): Breeding objectives for beef cattle in Ireland. Livestock Production Science 67:223-239.

BARILLET, F. (2007): Genetic improvement for dairy production in sheep and goats. Small Ruminant Research 70:60-75

CARTA, A., UGARTE, E. (2003): Breeding goals and new perspectives in dairy sheep programs. 54 th Annual Meeting EAAP, Roma, Italy, page 89

CONINGTON, J., S. C. BISHOP, A. WATERHOUSE AND G. SIMM (2004): A bioeconomic approach to derive economic values for pasturebased sheep genetic improvement programs. Journal of Animal Science, 82:1290-1304

FEWSON, D. (1993): Definition of the breeding objective. Design of livestock breeding programmes. Animal Genetics and Breeding Unit, University of New England ed., Armidale, NSW, Australia.

FUERST-WALTL, B., R. BAUMUNG (2009): Economic values for performance and functional traits in dairy sheep. Italian Journal of Animal Science, 8, 341–357.
JAMES, J. W. (1982): Construction, uses, and problem of multitrait selection indices. Proc. 2nd World Congress of Genetics of Applied Livestock Production 5:130-139.
KALAYDZHIEV, G. (2014): Genetic and environmental variability of coagulation ability of milk from different sheep breeds. Dissertation. Agricultural academy, Agricultural institute – Stara Zagora, Bulgaria
KOSGEY, I.S., VAN ARENDONK, J.A.M., BAKER, R.L. (2004): Economic values for traits in breeding objectives for sheep in the tropics: impact of tangible and intangible benefits. Livestock Production Science 88, 143–160.
KRUPOVA, Z., WOLFOWA, M., WOLF, J., ORAVCOVA, M., MAGRETIN, M., PESKOVICOVA, D., KRUPA, E. and DANO, J. (2009): Economic values for dairy sheep breeds in Slovakia. Asian-Australian Journal of Animal Science 22:1693–1702.
KRUPOVA, Z., KRUPA, E., WOLFOVA, M. (2013): Impact of economic parameters on economic values in dairy sheep. Czech Journal of Animal Science, 58, (1): 21–30
LEGARRA, A., RAMON, M., UGARTE, E., PEREZ-GUZMAN, M.D. (2007): Economic weights of fertility, prolificacy, milk yield and longevity in dairy sheep. Animal 2007, 1:193-203.
LOBO, R.N.B., PEREIRA, I.D.C., FACO, O. (2011): Economic values for production traits of Morada Nova meat sheep in a pasture based production system in semi-arid Brazil. Small Ruminant Research, 96, 93–100.
MICHALICKOVA, M., KRUPOVA, Z., KRUPA, E. (2014): Determinants of economic efficiency in dairy cattle and sheep. Slovak Journal of Animal Science, 47, 2014 (1): 39-50
MORAIS, O. R., MADALENA, F. E. (2006): Economic values for production traits in Santa Ines sheep. 8th World Congress on Genetics Applied to Livestock Production. CD-ROM. Paper 04-32).
ORAVCOVA, M., GROENEVELD, E., KOVAC, M., PESKOVICOVA, D., MAGRETIN, M. (2005): Estimation of genetic and environmental parameters of milk production traits in Slovak purebred sheep using test-day model. Small Ruminant Research 56:113-120.
PONZONI, R.W., NEWMAN, S. (1989): Developing breeding objectives for Australian beef cattle production. Animal Production, Volume 49 / Issue 01/ pp. 35-47
SMULDERS, J. P., SERRANO, M., PEREZ-GUZMAN, M. D., JIMENEZ, M. A., URIBE, H. and JURADO, J.J. (2007): Stochastic simulation of Manchega sheep breed selection scheme. Impact of artificial insemination, progeny testing system

and nucleus size on genetic progress and inbreeding. Livestock Scence. 2007;106:218–231.

TOLONE, M., RIGGIO, V., MAIZON, D.O., PORTOLANO, B. (2011): Economic values for production and functional traits in Valle del Belice dairy sheep using profit functions. Small Ruminant Research, 97, 41–47.

WOLF, J., WOLFOVA, M., KRUPOVA, Z., KRUPA, E. (2011): Package ECOWEIGHT (Programs for Calculating Economic Weights in Livestock), Version 5.1.1.Part 3A: Program EWSH2 for Sheep, Version 1.0.2

WOLFOVA, M., WOLF, J., KRUPOVA, Z., KICA, J. (2009): Estimation of economic values for traits of dairy sheep: I. Model development. Journal of Dairy Science 92:2183–2194.

WOLFOVA, M., WOLF, J., MILERSKI, M. (2011): Calculating economic weights for sheep sire breeds used in different breeding systems. Journal of Animal Science 89:1698–1711 doi:10.2527/jas.2010-3237

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

PRODUCTIVE TRAITS OF MORAVKA BREED - HAS ANYTHING CHANGED IN LAST SIXTY YEARS?

Radomir Savić[1], Milica Petrović[1], Marija Gogić[2], Čedomir Radović[2], Dragan Radojković[1], Nikola Stanišić[2], Marjeta Čandek-Potokar[3]

[1] University of Belgrade-Faculty of Agriculture, 11080 Belgrade-Zemun, Republic of Serbia
[2] Institute for Animal Husbandry, 11080 Belgrade- Zemun, Republic of Serbia
[3] Agricultural Institute of Slovenia, 1000 Ljubljana, Slovenia
Corresponding author: savic@agrif.bg.ac.rs
Review paper

Abstract: The main objective of this paper is to present characteristics of autochthonous pig breed Moravka, reared in the previous century, as well as results of investigations of production performance in the last ten years. A collection of available literature data on reproductive, growth, carcass and meat quality traits of Moravka breed was carried out. According to research studies done in the 50-ies of the last century, Moravka females reached sexual maturity at about 5-6 month, whereas breeding maturity was at age of 10-12 months. Average litter size (different parities) was 6.8-8.2 born alive piglets (1.2 kg birth weight). Lactation lasted around 60 days. At weaning piglets weighed 8.7-10.4 kg (i.e. 120-150 g daily gain). Recent studies show that in average the age at first farrowing was 373±10 days, litter size 8.6±1.7 born piglets, and duration of lactation 46.8±3.4 days. Regarding growing/fattening results, only one of old studies reported daily gain in growing phase which was in average 186-197 g (depending of sex), whereas depending on type of feeding and season daily gain for fattening phase was reported between 490 and 660 g (for pigs on feed mixture and intake between 2.5 to 3.2 kg). Reported feed conversion ratio was from 4.4 to 5.1 kg/kg feed mixtures. A more recent research reported piglets to have 31.9 kg at 192 days denoting a growth rate of about 160 g/day in growing phase, whereas pigs fattened from 32 to 94 kg with maize grew 385 g/day (reported feed conversion ratio was 3.74). Backfat thickness (average of measurements along split line) was app. 7 cm (from about 4 to 9 cm) in earlier and app. 4 cm (from about 3 to 6 cm) in recent studies, but it must be noted that slaughter weight was considerably lower in recent studies. The newest research on *Longissimus dorsi muscle* showed intramuscular fat content of 7.0±0.6%, 21.5±0.2% of proteins with the specific quality traits (pH^{45}=6.53±0.13, pH^{24}=5.65±0.05; CIE L*=48.9±1.4, a*=12.0±1.0, b*=5.9±0.4).

Key words: autochthonous breed, reproductive traits, growth, carcass and meat quality

Introduction

Moravka was created at the beginning of the XX century, in the area around the Velika Morava River Basin. In order to improve the production characteristics of Šumadinka, the allochthonous breeds Berkshire and Yorkshire have been imported, for which there are no relevant data, as well as English Large black pig. *Belić (1951)* states that for Moravka breed, it can be said to have been accidentally created, unplanned. It is reared in the municipalities of Despotovac, Ub, Ljig, Mionica, Mladenovac, Topola, Prokuplje and Kuršumlija.

The size of the Moravka population has decreased in recent years (Table 1). There is no official breeder register. The number of animals under productivity control is insignificant, which is a limiting factor for improving the existing situation. In recent years, efforts have been made to improve the situation, which in large part has been contributed to through the financial support of the competent ministry.

Table 1. Population size

Year	1993	2004	2008	2009	2012	2013	2014	2014*	2015*
Population	1000-1500	50-1000	50-1000	100-500	150-500	100-500	100-500		
Nº sows		30	45	90	140	14 (13#)	18 (18#)	12	12
Nº boars		5	5	10	10	2	4	4	4
Nº farms						3	5		

Source of data-DAD-FAO (www.dad.fao.org) access 29/06/2016
#Registered animals in Herd book.
*Source of data of Institute for Animal Husbandry *(Main Breeding Organisation)*, Annual Report (2014, 2015)-animals under the control of productivity.

Petrović et al. (2007a) indicate that the Moravka has a body covered with thick or rare black hair, which is smooth and straight. On the lower parts of the body the hair is scarce, and on the other rough and longer. The skin is relatively thin and black pigmented. The head is long, narrow, with a slightly clenched profile. Cheeks are not so clearly covered with muscle tissue. The ears are big, crushed, battered. The neck is of medium length and often narrow. The body is rather long, often narrow, so that the animals appear flat. Animals reared in the intensive system have well-defined exterior widths. The backline is slightly protruding or straight. The loin is quite long, but always down. There are 4-6 pairs of teats on the abdomen. The extremities are medium long, thin, gentle and slightly covered with muscle tissue.

Moravka has a good adaptive ability, it is distinguished by vitality and resistance to illness. It tolerates well extensive conditions of holding, so it can be successfully grown outdoors with the addition of smaller quantities of grain feeds. Today, autochthonous pig breeds are reared in open system or farm conditions and fed the traditional way or using complete mixtures *(Petrović et al., 2012).*

Consumer interest in products from Moravka pig breed is on the increase due to the specific quality of meat and meat products.

Knowing the production performance of Moravka can significantly contribute to the advancement of this breed. Therefore, the aim of this paper is to present the most important properties of fertility, growth, quality of carcasses and meat, as well as changes that have occurred in the last sixty years of the existence of this breed.

Reproductive performance

The Moravka gilts reach full maturity at the age of 5-6 months, however, the breeding maturity is reached at the age of 10-12 months (*Petrović et al., 2007a).*

Regardless of the fact that this is an autochthonous breed, the number of litters per sow annually is 1-1.5, indicating a low annual productivity of sows (Table 2). It is probable that the potential of this breed is not fully exploited and that the improvement of the rearing system the annual productivity can be improved.

Table 2. Reproductive traits of Moravka

Reference / Reproductive traits	*Živković and Kostić (1952b)*	*Lalević et al. (1953)*	*Petrović et al. (2007a)*	*Faculty of Agriculture (2009)*	*Institute for Animal Husbandry (2014)*
Number of sows recorded	98	24	-	16	12
Sow age/parity[1]	> 2 years	1.5[*]/2.5[**]/4[***] years of age		2.3±1.2 parities	373±10 (age at first farrowing)
Litters/sow and year				1.06	1-1.5
Piglets/litter	8.6±2.3 (4-16)				8.6±1.7
Piglets alive/litter	7.8±2.1 (4-13)		7.2±2.0 (5-14)	6.8±1.6 (3-9)	8.2±2.1
Piglet live birth weight (kg)	1.2				
Piglets weaned/litter	6.8±2.2 (2-12)				8.1±2.0
Piglet weaning weight (kg)	10.04				
Duration of lactation (days)	60	60			46.8±3.4
Average daily milk production (kg) measuring of litter weight before and after suck		2.2[*] (1.9-2.4) 2.3[**] (2.00-2.5) 2.3[***] (2.00-2.5)			

[1*] 1st farrowing, [**] 2nd farrowing, [***] 3th farrowing; Average value in the table (Mean±SD)

The number of piglets born in the litter varies by age (*Lalević, 1954*), as well as by the seasons (*Lalević, 1953*). The average litter size is 8.6 piglets, and the number of live-born piglets ranges from 6.8 to 8.2 (Table 2). When it comes to the size of the litter, there are no significant differences between previous and recent researches, which shows that Moravka has retained its reproductive potential. If the variation intervals are analyzed, the number of live-born piglets in the litter ranges from 3 to 14, indicating that this breed possesses fertility potential and that systemic selection can improve this trait.

Sows of autochthonous breeds have a well-defined maternal instinct, so the piglet losses in the suckling period are lower compared to allochthonous genotypes. The number of weaned piglets ranges from 6.8 to 8.1, and by comparison with the number of live piglets at birth, losses are 1.2-12.8%.

The average milk production of sows of different ages during lactation was 133.74 kg (*Lalević, 1953*). The lowest production of milk is found in the primiparous sows, while the highest is in multiparous sows. The same author points out that the yield of milk varies between sows of the same age during whole lactation, but also during the lactation decades, where the milk yield increases from the first to the fourth lactation decade (sows aged 4 years) and then decreases.

Research of *Petrović et al. (2007a)* reports that at weaning, at the age of 60 days, the average body weight of piglets is from 8.7 to 10.4 kg with differences relating to different methods of rearing during suckling period (i.e., 120-150 g daily gain). Recent data show that the age of primiparous sows is 373 days, while the suckling period is shorter compared to the earlier period. Shortening of the suckling period is probably a consequence of providing better conditions for rearing of weaned piglets.

The demonstrated variability of litter size traits, indicates the potential of this breed, but also the need for continuous productivity control and selection. In addition to improving the properties, it is necessary to define the most optimal system of rearing in order to make the breeding of this pig breed more sustainable over a longer period of time.

Growth traits

Moravka is an autochthonous breed of combined production capabilities. Table 3 shows the production traits of Moravka, which indicate differences depending on the intensity of the rearing, nutrition, gender, season and fattening system.

In the experiment, *Živković and Kostić (1952a)*, examined 25 animals with a balanced gender ratio and found the following results: in the first group with pre-slaughter body weight (BW) of 115 kg, average daily increase was 0.490 kg, while in the other group, at BW of 137 kg, ADG was 0.660 kg. The addition of protein feeds (soybean) into the diet had stimulating effect on the daily increase of pigs, which reflected on higher average daily intake and better utilization of food.

By comparing winter and summer fattening, *Mitrović and Kostić (1954)* have found a better growth during summer fattening, which may indicate that females of this breed better tolerate high summer temperatures, compared to the winter period when a large part of energy is spent on maintaining body temperature. During the lactation period, *Živković and Kostić (1952b)* have found an absolute gain of piglets of 8.8 kg (10-1.2), which indicates that the average daily gain in this period is about 150 g/day. During the growth phase, the same researchers have determined an average daily gain of 186-197 g, with higher gain observed in male piglets.

Research of *Petrović et al. (2007a)* reported that piglets to have 31.9 kg at 192 days denoting a growth rate of about 160 g/day in growing phase, whereas pigs fattened from 32 to 94 kg with maize grew 385 g/day (reported feed conversion ratio was 3.74).

By comparing earlier results with the recent research of *Radović et al. (2017)*, it is evident that the growth of fatteners of this breed has decreased (369 g/day). This decline in the growth rate is probably the result of fewer numbers of the population today, in-breeding and lack of selection procedures with aim to improve the growth traits.

Presented production performances show the potential of this breed, the need to increase the population, improve the system of rearing and to implement selection.

Table 3. Variability of growth traits

Reference / Growth traits	*Živković and Kostić (1952a)*		*Mitrović and Kostić (1954)*		*Živković and Kostić (1952b)*	*Radović et al. (2017)*
	Group feeding only with corn	Group feeding with 85% corn+15% soya	Winter fattening; concentrated mixture (85% corn+15% sunflower expeller)	Summer fattening; concentrated mixture (85% corn+15% sunflower expeller)	-	Semi-intensive system
Body weight (BW) at slaughter, kg	115 (83.5-151)	137 (110-174)	134 (118-154)	137 (110-174)	149.8 (121-214);	134.8
BW in the trial, kg	55-115	58-137	61-134	56-137	1.2-10.0 (lactation-60 days); ♂10.9-34.6 ♀10.4-32.7	30-150
Average daily gain (ADG), kg	0.490	0.660	0.600	0.660	♂ 0.197 ♀ 0.186	0.369
Average feed intake (ADFI), kg	2.45	2.99	3.16	-	-	-
Feed conversation ratio (FCR), kg/kg	4.95	4.44	5.13	-	-	-

Carcass and meat quality traits

The carcass side quality traits vary depending on the diet and the rearing system (Table 4). Depending on the period when the research was conducted, the differences in the body weight of pigs at slaughter are also evident. The slaughter weight was different and ranged from about 101 to 150 kg in a number of authors. In the earlier period, pigs were fattened to large body masses, which affected the higher content of fat tissue in the carcass. Today consumers' demands are focused on the leanness of the carcass sides, which caused pigs to be fattened to lower weights, similar to those in the intensive breeding system (about 100-110 kg). The slaughter yield ranged from 76 to 83%. The thickness of the fat tissue varied in the presented researches depending on the pre-slaughter body weight of pigs and the location on the carcass on which it was measured.

Table 4. Carcass performance of Moravka

Reference / Carcass traits		*Živković and Kostić (1952a)*	*Živković and Kostić (1952b)*	*Mitrović and Kostić (1954)*	*Petrović et al. (2007b)*	*Petrović et al. (2010)*
Feeding/Production system		Feeding with 85% corn+15% soya)	Concentrated feeding	Concentrated mixture (85% corn+15% sunflower expeller	Semi-intensive	Semi-intensive
Slaughter weight (kg)		145.33	149.80	131.57		101.22
Carcass weight (kg)		109.79	117.10	101.14		84.15
Carcass yield (% live weight)		75.54	78.17	76.88		83.00
Carcass length (os pubis-atlas, os pubis-1st rib; cm)					106.4±0.9 86.6±0.9	95.5 77.8
Backfat thickness (cm)	neck	6.08	6.20	6.08	6.32	
	ridge	9.00	9.37	9.07		5.87
	loins	8.41	8.15	7.73		3.51
	rump	7.50	8.32	6.89		4.16-4.31
	belly	5.08	3.73	4.42		2.86-4.08

The lowest weight of the carcass side (84.15 kg) is recorded in the research by *Petrović et al. (2010)*, but with the highest carcass side yield 83%. The length of the carcass side was 95.5 cm (*os pubis-atlas*) and 77.8 cm (*os pubis-1 rib*) in the research by *Petrović et al. (2010)* to 106.4 cm (*os pubis-atlas*) and 86.6 cm (*os pubis-1 rib*) in the research of *Petrović et al. (2007b).*

According to the research of *Petrović et al. (2010),* the weight of the round was 8.19 kg (3.55 kg of muscle tissue), the weight of the shoulder was 4.35 kg (1.90 muscle tissue) and the weight of the back-loin part 6.95 kg (2.09 kg muscle tissue). The weight of fast tissue and lard was 60.53 kg (*Mitrović and Kostić, 1954*) with a share of fat tissue of 59.85%; 69.79 kg (*Živković and Kostić, 1952a*) with the share of fat tissue of 63.57 kg and 76.50 kg (*Živković and Kostić, 1952b*) with the share of fat tissue of 65.33 kg. Muscle bone mass varied from 38.45 kg (*Živković and Kostić, 1952b*) to 40.61 kg (*Mitrović and Kostić, 1954*). The proportion of bones with muscle in the carcass side ranged from 32.84% (*Živković and Kostić, 1952b*) to 40.15% (*Mitrović and Kostić, 1954*). In the research *Živković and Kostić (1952a),* the bone mass with muscles is 40 kg, while their share in the carcass side is 36.43%.

The newest research (*Radović et al., 2017*) on *Longissimus dorsi* muscle shows intramuscular fat content of 7.0±0.6%, 21.5±0.2% of proteins with the specific quality traits (pH^{45}=6.53±0.13, pH^{24}=5.65±0.05; CIE L*=48.9±1.4, a*=12.0±1.0, b*=5.9±0.4). Research of *Petrović et al. (2014)* shows the specific fatty acid composition of the long back muscle (ΣSFA=41.6±0.6%, ΣMUFA=53.8±0.6%, ΣPUFA=4.5±0.3%).

Given the high heritability of slaughter traits, the improvement of this breed in terms of carcass quality is possible. The lower performances of the carcass side quality of this breed compared to allochthonous genotypes largely is compensated by the meat quality traits.

Conclusion

Knowing the production performance of Moravka can significantly contribute to the advancement of this breed. The observed variability of production characteristics indicates that the potential of this breed exists. The lower phenotypic value of some properties, in comparison with the previous period, is probably due to the decline in the number of animals in the population of this breed of pigs, which indicates the need to increase the population, to continuously control productivity, to improve the system of keeping and implement the systematic selection.

Proizvodne osobine moravke - da li se nešto promenilo u poslednjih šezdeset godina?

Radomir Savić, Milica Petrović, Marija Gogić, Čedomir Radović, Dragan Radojković, Nikola Stanišić, Marjeta Čandek-Potokar

Rezime

Glavni cilj ovog rada je prikaz proizvodnih osobina autohtone rase svinja moravka, gajene u prethodnom veku, kao i rezultata istraživanja u poslednjih deset godina. Sprovedeno je prikupljanje dostupnih literaturnih podataka osobina plodnosti, porasta, kvaliteta trupa i mesa. U skladu sa istraživanjima sprovedenim 50-tih godina prošlog veka, plotkinje moravke postižu polnu zrelost sa oko 5-6 meseci, a priplodnu zrelost u uzrastu 10-12 meseci. Prosečna veličina legla (različiti pariteti) je 6,8-8,2 živorođene prasadi (1,2 kg masa na rođenju). Laktacija je trajala oko 60 dana. Telesna masa prasadi na zalučenju bila je 8,7-10,4 kg (120-150 g dnevni prirast). Skorašnja istraživanja pokazuju da je uzrast prvopraskinja 373±10 dana, veličina legla 8,6±1,7 rođene prasadi i trajanje laktacije 46,8±3,4 dana. U pogledu rezultata porasta/tova, samo jedna starija studija pokazuje dnevni prirast u fazi porasta od prosečno 186-197 g (zavisno od pola), dok je u zavisnosti od tipa ishrane i sezone, dnevni prirast u fazi tova bio između 490 i 660 g (za svinje hranjene krmnim smešama i dnevnom unosu hrane od 2,5 do 3,2 kg). Utvrđena konverzija hrane bila je od 4,4 do 5,1 kg/kg krmne smeše. Novija istraživanja su pokazala da prasad imaju 31,9 kg pri 192 dana uzrasta, što znači da su ostvarila stopu porasta od 160g/danu u fazi porasta, dok su svinje tovljene od 32 do 94 kg sa kukuruzom prirastale 385 g/danu (konverzija hrane bila je 3,74). Debljina slanine (prosečne mere) je bila oko 7 cm (od oko 4 do 9 cm) u ranijim i oko 4 cm (od oko 3 do 6 cm) u novijim istraživanjima, s tim da je telesna masa pri klanju znatno niža u novijim studijama. Novija istraživanja mišića *Longissimus dorsi muscle* pokazala su sadržaj intramuskularne masti od 7,0±0,6%, sadržaj proteina od 21,5±0,2% sa specifičnim kvalitativnim osobinama (pH^{45}=6,53±0,13; pH^{24}=5,65±0,05; CIE L*=48,9±1,4; a*=12,0±1,0; b*=5,9±0,4).

Ključne reči: autohtona rasa, reproduktivne osobine, porast, kvalitet polutki i mesa

Acknowledgment

"This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634476 (Project acronym: TREASURE). The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains."

References

BELIĆ J. (1951): Specijalna zootehnika (ovčarstvo i svinjarstvo), Naučna knjiga, Beograd.

DAD-FAO (www.dad.fao.org) access 29/06/2016

INSTITUTE OF ANIMAL HUSBANDRY (2014): Annual Report, 2014.

INSTITUTE OF ANIMAL HUSBANDRY (2015): Annual Report, 2015.

LALEVIĆ D. (1953): Prilog poznavanju mlečnosti svinje moravke. Arhiva za poljoprivredne nauke, VI, 13-14, 123-136.

LALEVIĆ D. (1954): Uticaj načina i vremena pripusta na plodnost krmača. Zbornik radova Poljoprivrednog fakulteta Beogradskog Univerziteta, II, 1, 1-7.

MITROVIĆ D., KOSTIĆ J. (1954): Ispitivanje utroška hrane u zimskom tovu svinja. Arhiv za poljoprivredne nauke, godina VII, sveska 16, strane 46-58. Institut za stočarstvo NR Srbije-Zemun Polje.

PETROVIĆ M., MIJATOVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č., MARINKOV G., STOJANOVIĆ LJ. (2007a): Genetic resources in pig breeding-Moravka. Biotechnology in Animal Husbandry, 23, 1-2, 1-11.

PETROVIĆ M., MIJATOVIĆ M., RADOVIĆ Č., RADOJKOVIĆ D., JOSIPOVIĆ S. (2007b): Genetic resources in pig breeding – carcass quality traits of breeds Moravka and Mangalitsa. Biotechnology in Animal Husbandry, 23:421-428.

PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., MIJATOVIĆ M., RADOJKOVIĆ D., ALEKSIĆ S., STANIŠIĆ N., POPOVAC M. (2010): Quality traits of carcass sides and meat of Moravka and Mangalitsa pig breeds. Biotechnology in Animal Husbandry, 26:21-27.

PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R. (2012): Composition of carcass sides and quality of meat from swallow-belly mangalitsa reared in two systems. Biotechnology in Animal Husbandry, 28, 2, 303-311.

PETROVIĆ M., WÄHNER M., RADOVIĆ Č., RADOJKOVIĆ D., PARUNOVIĆ N., SAVIĆ R., BRKIĆ N. (2014): Fatty acid profile of m. longissimus dorsi of Mangalitsa and Moravka pig breeds. Archiv Tierzucht 57, 17, 1-12.
RADOVIĆ Č., PETROVIĆ M., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R., STANIŠIĆ N., GOGIĆ M. (2017): Carcass and pork quality traits of indigenous pure breeds (Mangalitsa, Moravka) and their crossbreads. Indian Journal of Animal Research, 51, 2, 371-376.
UNIVERSITY OF BELGRADE, FACULTY OF AGRICULTURE (2009): Annual Report, 2009.
ŽIVKOVIĆ R., KOSTIĆ I. (1952a): Iskorišćavanje hrane i tovnost crne i šarene svinje (moravke i resavke). Arhiva za poljoprivredne nauke, V, 8, 1-21.
ŽIVKOVIĆ R., KOSTIĆ I. (1952b): Prilog poznavanju crne i šarene svinje (moravke i resavke). Arhiva za poljoprivredne nauke, V, 10, 23-46.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

MEAT QUALITY OF KRŠKOPOLJE PIGS AS AFFECTED BY *RYR1* GENOTYPE

Urška Tomažin[1], Nina Batorek Lukač[1], Martin Škrlep[1], Maja Prevolnik Povše[1,2], Jernej Ogorevc[3], Peter Dovč[3], Marjeta Čandek-Potokar[1,2]

[1]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia
[2]University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia
[3]University of Ljubljana, Biotechnical faculty, Department of Animal Science, Groblje 3, SI-1230 Domžale
Corresponding author: Marjeta Čandek-Potokar, meta.candek-potokar@kis.si
Original scientific paper

Abstract: The effect of *RYR1* genotype on carcass and meat quality traits was investigated in the only Slovenian local pig breed Krškopolje. Thirty-six castrates originating from 12 litters were genotyped for c. C1843T (p. Arg615Cys) at *RYR1* locus (recessive allele further denoted as "n" and wild as "N"). Pigs with mutated recessive allele (N/n) had lower growth rate and leaner carcasses (exhibited greater lean meat content, muscle thickness and loin eye area, thinner backfat and smaller fat area over LD muscle). A pronounced effect of *RYR1* on meat quality was observed. The rate of pH fall in *longissimus dorsi* (LD) muscle of N/n pigs was faster (lower pH at 45 min after slaughter) but there was no effect of *RYR1* on ultimate pH. Lower water holding capacity (higher drip loss and higher thawing and cooking loss) of meat from N/n was noted. Meat of N/n pigs exhibited also higher shear force resistance (indicative of lower tenderness).

Key words: Krškopolje pig; RYR1 gene; growth; carcass; meat quality

Introduction

Krškopolje pig is the only Slovenian local breed. In the recent years, the census of this black pig breed with white belt has been steadily increasing. Among the breeders, these pigs are reputed for having good meat quality, especially suitable for processing into high quality dry-cured products. However, only a few studies objectively evaluating meat quality of Krškopolje pigs exist. In pigs, *RYR1* gene encoding the ryanodine receptor in the calcium channel of the sarcoplasmic reticulum is one of the major genes affecting meat quality. The recessive mutation c. C1843T (p. Arg615Cys) at *RYR1* locus, further denoted as "n" allele, is responsible for susceptibility to stress and appears to be incompletely recessive regarding meat quality. It has marked positive effects on muscle development and carcass lean content *(Monin, 2004).* The "n" allele at *RYR1* is associated with the loss of control of free calcium and is associated with a very fast post-mortem pH fall (below 6.0 within 30 minutes to 1 hour) which together with high body temperature causes muscle protein denaturation resulting in decreased water holding capacity and discoloration of meat *(Monin, 2004).* Relatively high frequencies of "n" allele at *RYR1* were reported for Krškopolje pigs; i.e. on a sample of 17 pigs; *Čandek-Potokar et al. (2003)* reported 70% of heterozygous (N/n) and 18% of homozygous (n/n) carriers, whereas earlier study on 10 pigs *(Kastelic, 2001),* showed 40% of N/n and 10% of n/n pigs, denoting allele "n" frequency of 0.53 and 0.30, respectively. In an earlier study, allele "n" frequency of 0.432 was reported for Krškopolje pigs by *Dovč et al. (1996).* All studies highlighted the problem of high incidence of the *RYR1* mutation to be taken into account in the breeding programme. The current situation with regard to *RYR1* gene and the effect on meat quality has thus been one of the objectives of the TREASURE project.

Materials and Methods

The subject of the present study are Krškopolje castrates (n=36) originating from 12 different litters reared at the same farm and fed complete feed mixtures adapted to the stage of growth (for more details see *Batorek et al., 2016*). At the average age (±SD) of 228±6 days and weight of 121±14 kg the pigs were slaughtered in a commercial abattoir according to the routine procedure. The samples of ears were collected for DNA extraction. Carcasses were weighed and measurements of fat and muscle thickness taken (by official classification body) for estimation of lean meat content according to the method approved for Slovenia (OJ EU L56/28, 2008). Back fat thickness at the level of last rib was additionally measured. Measurement of pH was taken in *longissimus dorsi* muscle (LD) at the level of last rib 45 minutes (pH45) post mortem using MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland). A day after slaughter the pH (pH24) was measured in LD muscle and a sample of LD with overlying subcutaneous fat taken for further assessments of marbling (1-7 scale), area of LD and corresponding fat, objective colour (CIE L*, a*, and b* colour parameters measured with Minolta Chroma Meter CR-300, Minolta Co. Ltd, Osaka, Japan). For chemical analysis, the samples were minced, and protein, water and intramuscular fat (IMF) content determined by near-infrared spectral analysis (NIR Systems 6500, Foss NIR System, Silver Spring, MD, USA) using internal calibrations *(Prevolnik et al., 2005).* Water holding capacity was determined as drip loss (after 24, 48 and 96 hours of storage) according to the EZ method *(Christensen, 2003),* thawing and cooking loss. For thawing loss, a LD chop

(8×5×3 cm) was weighed, vacuum packed and frozen at -20°C. After thawing (overnight at 4°C), the sample was gently drained with a paper towel and reweighed. The same sample was afterwards used for determination of cooking loss and shear force. For cooking loss, the samples were cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C, cooled and reweighed. After cooling, four 1.27-cm-diameter cores were excised and shear force was measured perpendicular to muscle fibres using a TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. For the determination of total collagen, hydroxyproline was determined according to ISO 3496 standard (1994). For insoluble collagen fraction, LD sample was heated (to 77 °C for 90 min) in Ringer's solution and centrifuged. The supernatant was discarded and the pellet was then further processed as in the case of total collagen. Soluble collagen was determined from the difference in total and insoluble collagen content. Lipid oxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by *Lynch and Frei (1993).* Briefly, LD samples were homogenised with 0.15 M KCl (with the addition of BHT), and incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH and 2.8% (w/v) trichloroacetic acid in a thermostatic heating block (100 °C) for 10 min. After cooling to room temperature, the pink chromogen was extracted into n-butanol and its absorbance was measured at 535 nm (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany). Protein oxidation of LD muscle was measured spectrophotometrically (BioSpectrometer Fluorescence, Eppendorf, Hamburg, Germany) according to the method of Oliver et al. (1987) as modified by *Mercier et al. (1998)* in myofibril isolates prepared according to the method of *Pietrzak et al. (1997).* Concentration of carbonyl groups was expressed in nmol/mg proteins.

Genomic DNA was extracted from pig ear tissue using Isolate II Genomic DNA kit (BIO-52067, Bioline), according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to screen for C/T SNP (C1843T) in the amplified 134 bp fragment of RYR1 gene, using the following primer pair; forward: 5'-GTGCTGGATGTCCTGTGTTCCCT-3' and reverse: 5'-ACCTCATCAACTATGTCACCAG-3' *(Brenig & Brem, 1992).* Thermocycler programme was as follows: 5 min at 95 °C, 30 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 20s, followed by final elongation step at 72 °C for 5 minutes. The reaction volume was 20 µl and contained 1 x PCR buffer, 1 µM primers, 150 µM dNTPs, 1.2 mM MgCl2, 0.5 U DNA Taq polymerase (Thermo Fisher Scientific), and approximately 50-200 ng template DNA.

The fragment was digested with restriction endonuclease HhaI (ER1851, Thermo Fisher Scientific) to obtain fragments of 50 and 84 bp in case of wild type allele. The restriction reaction consisted of 10 µl PCR product, 1.5 µl restriction buffer, 4.2 µl H2O, 0.3 µl (3U) of restriction enzyme, and was incubated for 3 h at 37 °C. Fragments after restriction were analyzed on 2.5 % agarose gel stained with ethidium bromide.

Data was submitted to analysis of variance using the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA). The model included the fixed effect of *RYR1* genotype (the pig of n/n genotype was excluded from the analysis). In the case of carcass traits, final live weight was included as covariate in the model. Differences between groups were considered significant if $P<0.05$. The results in the tables are presented as least square means (LS-means) with root-mean-square errors (RMSE). Effect size is presented as Hedges' *g* (difference between means of N/n and N/N divided by pooled SD).

Results and Discussion

The frequencies of *RYR1* genotypes were 1, 15 and 20 for n/n, N/n and N/N, respectively. Based on the present experiment, the incidence of recessive "n" allele in Krškopolje breed is estimated at 0.24, which is relatively high. The incidence of *RYR1* mutation is generally reported low in local southern European pig breeds *(Pugliese and Sirtori, 2012)*. However, a large variation (from 0 to 44%) was reported for French native breeds *(Labroue et al., 2001)*. The presence of *RYR1* mutation has also been attested for Portuguese Bísaro pig *(Santos e Silva et al., 2000)*. Even higher incidence was previously reported for Krškopolje pig *(Dovč et al., 1996; Čandek-Potokar et al., 2003)*. This indicates the introgression of this alelle from the modern breeds into the native breeds (in the case of Krškopolje pig probably from German Landrace or Pietrain) that most probably happened during the period of the severe reduction of the census of less performing local pig breeds *(Pugliese and Sirtori, 2012)*.

In the present study, 10.4 kg difference in live weight at slaughter between N/N and N/n pigs resulted in higher average daily gain in N/N than N/n pigs (Table 1). Majority of published studies on modern white breeds reported no differences in daily gain between N/N and N/n pigs *(Sather et al., 1991; Leach et al., 1996; Larzul et al., 1997; Tor et al., 2001)*. However, similarly as in the present study, lower daily gain of N/n vs. N/N genotype was reported for the lean line of Landrace and Large White pigs *(McPhee et al., 1992)*. For N/n also lower feed intake was reported *(McPhee et al., 1992; Leach et al., 1996)* which corroborates with lower growth rate reported for N/n pigs.

A strong effect of *RYR1* genotype was noted for carcass traits (Table 1). Carcasses of N/n pigs exhibited better muscularity than N/N pigs demonstrated as greater lean meat content (P=0.005) thicker muscle (P=0.002) and bigger loin eye area (P<0.001). N/n pigs had also thinner backfat than N/N pigs and smaller fat area measured over LD (P=0.087). The literature results are not consistent with regard to the differences between N/n and N/N pigs in muscle and fat tissue development. *Leach et al. (1996)* found no significant difference in backfat thickness between N/n and N/N pigs, but demonstrated superiority of N/n pigs in carcass yields. *Fisher et al. (2000)* found larger LD muscle thickness and area in N/n compared to N/N pigs, while other studies reported no significant differences in size of LD muscle between the two genotypes *(De Smet et al., 1996; Leach et al., 1996; Hamilton et al., 2000)*. There were no differences in chemically determined IMF or marbling scores (Table 2). N/n pigs exhibited higher protein content (P=0.018), which is in agreement with *Pommier et al. (1998)* who reported higher protein % (but also smaller fat %) in LD muscle of N/n compared to N/N pigs.

Table 1. Effect of *RYR1* genotype on carcass traits of Krškopolje pigs

	N/N (n=20)	N/n (n=15)	RMSE	P value	Hedges' *g*
Average daily gain (kg/day)	0.543	0.501	0.059	0.0476	-0.7
Warm carcass weight, kg	96.3	97.6	2.2	0.1211	-0.6
Dressing %	79.7	80.2	1.8	0.1011	0.7
Lean meat content, %	40.3	45.0	4.2	0.0050	1.2
Muscle thickness, mm	64.6	70.0	4.4	0.0021	0.3
Fat thickness over *gluteus medius*, mm	38.8	33.4	5.4	0.0096	-1.2
Fat thickness at last rib, mm	38.9	36.1	5.0	0.1278	-0.6
Carcass length a, cm	100.9	99.7	2.5	0.2190	-0.9
Carcass length b, cm	85.4	84.3	2.2	0.1604	-1.0
Loin eye area, cm^2	33.1	39.3	4.5	0.0008	0.8
Loin eye fat area, cm^2	28.8	26.5	3.5	0.0872	-0.9

RMSE – root-mean-square error

Regarding meat quality traits (Table 2) the results on *RYR1* gene effect are consistent with what has been previously demonstrated for modern white breeds. N/n pigs exhibited lower pH45 (pH measured 45 min post mortem) and no differences in pH24 (ultimate pH) which is consistent with many studies *(De Smet et al., 1996; Larzul et al., 1997; Monin et al., 1999; Fisher et al., 2000).* It is known that “n” allele influences the rate of pH fall by favouring calcium release in muscle cells *(Monin, 2004)* thus stimulating the activity of ATPase, while similar glycolytic potential, responsible for the amplitude of pH fall, is reported for N/n and N/N pigs *(Larzul et al., 1997).* No differences between genotypes on CIE colour parameters also corroborate with results of *Larzul et al. (1997)* who reported that the “n” allele appears to be almost completely recessive for meat colour. Water holding capacity of LD muscle was strongly affected by *RYR1* genotype, confirmed also by large values of effect size. Lower water holding capacity in N/n pigs is in agreement with numerous results using various measurement methods *(Sather et al., 1991; De Smet et al., 1996; Leach et al., 1996; Fisher et al., 2000; Hamilton et al., 2000; Škrlep et al., 2010).* Higher thawing and cooking losses of N/n pigs additionally confirm lower water holding capacity of N/n genotype. *RYR1* genotype did not show any effect on oxidation of lipids (TBARS) or proteins (carbonyl groups). Higher shear force (measured on cooked LD samples) in N/n than N/N pigs agrees with the literature showing either higher shear force values or lower sensory tenderness panel scores for stress-susceptible pigs *(Boles et al., 1991; McPhee and Trout, 1995).* The results of *Monin et al. (1999)* also indicated that the “n” allele of the *RYR1* was associated with pork texture and could be considered as detrimental to sensory acceptance. With regard to the effect on connective tissue, assessed as collagen content (total, insoluble and soluble) in LD muscle and its solubility, there was no effect of *RYR1* genotype noted. Although hydroxyproline content was shown as one of the main discriminant characteristics between Large White and highly muscular Pietrain pigs *(Sellier et al., 1971; Baland and Monin, 1987),* it was also suggested, that no clear relationship existed between muscular development and collagen content in pigs *(Baland and Monin, 1987).*

Table 2. Effect of *RYR1* genotype on meat quality (*longissimus dorsi* muscle)

	N/N (n=20)	N/n (n=15)	RMSE	P value	Hedges' *g*
pH45	6.60	6.41	0.21	0.0116	-0.9
pH24	5.63	5.55	0.17	0.2268	-0.4
Subjective colour (1-6)	4.38	4.30	0.64	0.7320	-0.1
Marbling (1-7)	4.05	3.97	1.03	0.8134	-0.1
Colour parameters CIE L*	51.2	52.8	3.2	0.1424	0.5
CIE a*	7.1	7.6	1.3	0.2543	0.4
CIE b*	1.5	2.0	1.2	0.2122	0.4
C*	7.3	8.0	1.5	0.1867	0.5
h°	11.3	13.9	7.5	0.3268	0.3
Chemical analysis					
Intramuscular fat, %	2.92	2.85	0.85	0.8113	-0.1
Protein, %	23.2	23.6	0.5	0.0176	0.8
Water, %	72.3	72.6	0.8	0.4147	0.3
Water to protein ratio	3.38	3.37	0.15	0.9743	-0.0
Water holding capacity					
Drip loss after 24 h, %	3.2	7.0	1.7	<0.0001	2.2
Drip loss after 48 h, %	4.8	9.0	2.0	<0.0001	2.1
Drip loss after 96 h, %	7.4	11.5	2.2	<0.0001	1.8
Thawing loss, %	10.4	14.8	3.0	0.0001	1.5
Cooking loss, %	27.2	29.6	3.1	0.0241	0.8
Hardness (WBSF, N)	53.1	59.7	8.2	0.0241	0.8
Collagen (mg/g)					
Total collagen	2.77	2.67	0.20	0.1924	-0.4
Insoluble collagen	2.25	2.18	0.16	0.1764	-0.5
Soluble collagen	0.52	0.50	0.09	0.6100	-0.2
Collagen solubility, %	18.6	18.6	2.7	0.9970	-0.0
Oxidation parameters					
TBARS, µg/kg	28.1	27.8	2.2	0.7480	-0.1
Carbonyl groups, nmol/g protein	1.43	1.38	0.29	0.5721	-0.2

RMSE – root-mean-square error, WBSF – Warner-Bratzler shear force, TBARS – thiobarbituric acid reactive substances

Conclusion

The incidence of mutated *RYR1* allele in the population of Krškopolje pig remains relatively high. In agreement with the results on modern white pig breeds, the presence of mutated allele at *RYR1* locus in the case of local pig breed Krškopolje negatively affects meat quality demonstrated as lower water holding capacity, and lower tenderness. An intensive breeding effort is needed to eliminate this mutation from the Krškopolje pig population in order to produce meat of better quality suitable for processing into high quality products.

Kvalitet mesa krškopoljskih prasadi u odnosu na *RYR1* genotip

Urška Tomažin, Nina Batorek Lukač, Martin Škrlep, Maja Prevolnik Povše, Jernej Ogorevc, Peter Dovč, Marjeta Čandek-Potokar

Rezime

Uticaj genotipa *RYR1* na kvalitet trupova i kvaliteta mesa istraživani su na grlima krškopoljske rase, jedine slovenačke lokalne rase svinja. Trideset šest kastrata poreklom iz 12 legala genotipizovano je za c. C1843T (str Arg615Cys) u *RYR1* lokusu (recesivni alel dalje označen kao "n" a wild-type alel kao "N"). Svinje sa mutiranim recesivnim alelom (N/n) imale su nižu stopu rasta i manje masne trupove (pokazali su veći sadržaj mesnatosti, debljinu mišića i područje slabine, tanju leđnu slaninu i manju prekrivnost LD mišića masnim tkivom). Izražen efekat *RYR1* na kvalitet mesa je primećen. Stopa opadanja pH u *longissimus dorsi* (LD) mišiću N/n svinja je bila brža (niži pH 45 min posle klanja), ali nije postojao efekat *RYR1* na krajnji pH. Zabeležen je manji kapacitet zadržavanja vode (veći kalo otapanje i kuvanja) mesa od N/n. Meso N/n svinja pokazalo je i veću otpornost na sile rasecanja (što ukazuje na slabiju mekoću mesa).

Ključne reči: krškopoljska svinja, RYR1 gen, porast, trup, kvalitet mesa

Acknowledgment

The study was conducted within the project TREASURE, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634476. The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains.

Authors would also like to acknowledge the core financers (Slovenian Agency of Research (grant P4-0133) and Ministry of Agriculture, Food and Forestry (grant V4-1417), and the breeders of Krškopolje pigs for their cooperation and support.

References

BALAND A., MONIN G. (1987): Relation between muscular development and muscle collagen content in pigs. Meat Science, 21(4): 295–299.

BATOREK LUKAČ N., TOMAŽIN U., ŠKRLEP M., PREVOLNIK POVŠE M., ČANDEK-POTOKAR M. (2016): Effect of production system (organic vs. conventional) and roughage supplementation in diet on performance of growing-finishing Krškopolje pigs. Book of Abstracts of 9th International Symposium on Mediterranean Pig, Instituto Politécnico de Portalegre, Portalegre, Portugal, p. 36.

BOLES J.A., PARRISH, Jr. F.C., SKAGGS C.L., CHRISTIAN L.L. (1991): Effect of porcine somatotropin, stress susceptibility, and final end point of cooking on the sensory, physical, and chemical properties of pork loin chops. Journal of Animal Science, 69, 2865–2870.

BRENIG B., BREM G. (1992): Molecular cloning and analysis of the porcine halothane gene. Archiv für Tierzucht, 35, 129-135.

CHRISTENSEN L.B. (2003): Drip loss sampling in porcine m. *longissimus dorsi*. Meat Science, 63, 469–477.

ČANDEK-POTOKAR M., ŽLENDER B., KRAMAR Z., ŠEGULA B., FAZARINC G., URŠIČ M. (2003): Evaluation of Slovene local pig breed Krškopolje for carcass and meat quality. Czech Journal of Animal Science, 48, 3, 120–128.

DOVČ P., ŠALEHAR A., KOVAČ M., KASTELIC M. (1996): Frequency of the *RYR1* n allele and its influence on fattening traits. Archiv für Tierzucht, 39(4), 441–446.

DE SMET S.M., PAUVELS H., DE BIE S., DEMEYER D.I., CALLEWIER J., EECKHOUT W. (1996): Effect of halothane genotype, breed, feed withdrawal and lairage on pork quality of Belgian slaughter pigs. Journal of Animal Science, 74, 1854–1863.

HAMILTON D.N., ELLIS M., MILLER K.D., McKEITH F.K., PARRETT D.F. (2000): The effect of the Halothane and Rendement Napole genes on carcass and meat quality characteristics of pigs. Journal of Animal Science, 78, 2862–2867.

FISHER P., MELLETT F.D., HOFFMAN L.C. (2000): Halothane genotype and pork quality. 1. Carcass and meat quality characteristics of three halothane genotypes. Meat Science, 54, 97–105.

KASTELIC A. (2001): Telesna sestava prašičev krškopoljske pasme. Graduation thesis, University of Ljubljana, Biotechnical faculty, Domžale, Slovenia.

LABROUE F., MARSAC H., LUQUET M., GRUAND J., MOUROT J., NEELZ V., LEGAULT C., OLLIVIER L..(2001): Performances of French local breeds. In: Pig genetic resources in Europe. Eds Ollivier L., Labroue F., Glodek P., Gandini G. and Delgado J.V. Wageningen Pers, Wageningen, Netherlands, 51–57.

LARZUL C., LE ROY P., GUEBLEZ R., TALMANT A., GOUGE J., SELLIER P., MONIN G. (1997): Effect of halothane (NN, Nn, nn) on growth, carcass and meat quality traits of pigs slaughtered at 95 kg or 125 kg live weight. Journal of Animal Breeding and Genetics, 114, 309–320.

LEACH L.M., ELLIS M., SUTTON D.S., MCKEITH F.K., WILSON E.R. (1996): The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. Journal of Animal Science, 74, 934–943.

LYNCH, S.M., FREI, B. (1993): Mechanisms of copper- and iron-dependent oxidative modification of human low density lipoprotein. Journal of Lipid Research, 34, 1745–1751.

McPHEE C.P., DANIELS L.J., KRAMER H.L., NOBLE J.W., TROUT G.R. (1992): The effect of the Halothane gene on performance, carcass and meat quality in a fat and a lean line of pigs. Proceedings of the Advancement of Animal Breeding and Genetics, 10, 76-79.

McPHEE C.P., TROUT G.R. (1995): The effects of selection for lean growth and the halothane allele on carcass and meat quality of pigs transported long and short distances to slaughter. Livestock Production Science, 42, 55–62.

MERCIER, Y., GATELLIER, P., VIAU, M., REMIGNON, H., RENERRE, M. (1998): Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. Meat Science, 48, 301–318.

MONIN G., LARZUL C., LE ROY P., CULIOLI J., MOUROT J., ROUSSET-AKRIM S., TALMANT A., TOURAILLE C., SELLIER P. (1999): Effects of the halothane genotype and slaughter weight on texture of pork. Journal of Animal Science, 77: 408–415.

MONIN G. (2004): Conversion of muscle to meat/Colour and texture deviations. In: Encycopedia of meat sciences. Eds Jensen W. K., Devine C. and Dikeman M. Elsevier Ltd., Oxford, UK, 323–330.

OJ EU L56/28 (2008): Commission Decision of 8th December 2005 authorising methods for pig carcasses in Slovenia.

OLIVER, C.N., AHN, B.W., MOERMAN, E.J., GOLDSTEIN, S., STADTMAN, E.R. (1987): Age related changes in oxidized proteins. Journal of Biological Chemistry, 262, 5488–5491.

PIETRZAK, M., GREASER, M.L., SOSNICKI, A.A. (1997): Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. Journal of Animal Science, 75, 2106–2116.

POMMIER, S.A., POMAR, C., GODBOUT, D. (1998): Effect of the halothane genotype and stress on animal performance, carcass composition and meat quality of crossbred pigs. Canadian Journal of Animal Science, 78, 257–264.

PREVOLNIK M., ČANDEK-POTOKAR M., ŠKORJANC D., VELIKONJA BOLTA Š., ŠKRLEP M., ŽNIDARŠIČ T., BABNIK D. (2005): Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. Journal of Near Infrared Spectroscopy, 13, 77–85.

PUGLIESE C., SIRTORI F. (2012): Quality of meat and meat products produced from southern European pig breeds. Meat Science, 90, 511–518.

SANTOS E SILVA J., FERREIRA-CARDOSO J., BERNARDO A., PIRES DA COSTA J.S. (2000): Conservation and development of the Bísaro pig. Characterisation and zootechnical evaluation of the breed for production and genetic management. In: Quality of meat and fat in pigs as affected by genetics and nutrition. Proc. of the joint session of the EAAP comissions on pig production, animal genetics and animal nutrition. Eds Wenk C., Fernández J.A. and Dupuis M. Wageningen Pers, Wageningen, Netherlands, 85–92.

SATHER A.P., MURRAY A.C., ZADAWSKI S.M., JOHNSON P. (1991): The effect of halothane gene on pork production and meat quality of pigs reared under commercial conditions. Canadian Journal of Animal Science, 71, 956–967.

SELLIER P., BOCCARD R., BOUTLER N., DANDO P., NICOLAS G. (1971): Étude du taux d'hydroxyproline d'un muscle et de ses relations avec certaines caractéristiques de composition corporelle dans les races large white et de piétrain. Annales de génétique et de sélection animale, INRA Editions, 3 (4), 433-448.

ŠKRLEP M., KAVAR T., ČANDEK-POTOKAR M. (2010): Comparison of *PRKAG3* and *RYR1* gene effect on carcass traits and meat quality in Slovenian commercial pigs. Czech Journal of Animal Science, 55, 149–159.

TOR, M., ESTANY J., VILLALBA D., CUBILÓ D., TIBAU J., SOLER J., SANCHEZ A., NOGUERA J.L. (2001): Within-breed comparison of RYR1 pig genotypes for performance, feeding behaviour, and carcass, meat and fat quality traits. Journal of Animal Breeding and Genetics, 118, 417–427.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

PHENOTYPIC VARIABILITY OF THE WEIGHT OF WARM CARCASS SIDE OF FATTENERS

Marija Gogić[1], Čedomir Radović[1], Milica Petrović[2], Radomir Savić[2], Aleksandar Stanojković[1], Maja Petričević[1], Vladimir Živković[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: gogic.marija@gmail.com
Original scientific paper

Abstract: In this study, the influence of sire breed, sires within the breed, gender of fatteners, gender within the sire breed and season of birth of fatteners on the variability of the following traits was studied: final weight of fatteners (MT), the weight of the warm carcass side (MTP_1) adjusted to the final weight (MT = 101.04 kg) and weight of the warm carcass side (MTP_2) adjusted to the average age at the end of fattening (UT, age at the end of the fattening = 204.91 days). The trial was conducted at the farm and experimental slaughterhouse of the Institute for Animal Husbandry, Zemun-Belgrade. Sires of fattening pigs were pure breed: Swedish Landrace (SL, n = 10), Large Yorkshire breed (LY, n = 3) and Pietrain (P, n = 3), while offspring (both sexes) belonged to the following genotypes: Swedish Landrace (SL), and crosses: Large Yorkshire x Swedish Landrace (LY × SL), Pietrain × Swedish Landrace (P × SL), [Pietrain × (Large Yorkshire x Swedish Landrace)] P × (LY × SL), [Swedish Landrace x (Large Yorkshire x Swedish Landrace)] × SL (LY × SL) and [Large Yorkshire x (Large White × Swedish Landrace)] × LY (LY × SL). At the end of the study it was established: that the fatteners gender within the sire breed (Sex : SB) showed no effect on the studied characteristics (P>0.05), the season of birth of offspring showed a statistically significant effect on the studied traits (P<0.001, P<0.01), the sire within the sire breed Pietrain (S: P) in Model 2 had a high impact (P <0.001).

Key words: fattening pig, genotype, sire breed, sex, season, weight of warm carcass side

Introduction

Due to the increased demand for meat in the world, preference is given to fast-growing species such as pigs, where pig farming becoming the leading livestock industry (*Zapryanova, 2017*). Pig meat production in the Republic of Serbia participates with over 50% in total meat production. The successful production of pigs requires adequate genotypes and applied production technology (*Uremović and Uremović, 1997*). The aim of the pig breeder is to increase the yield of meat in carcass sides of slaughtered pigs in order to achieve the highest price and efficiency of fattening (*Gogić et al., 2014*). In order to achieve this, efforts are being made to improve genetic potential by changing breed composition, the application of modern selection procedures, the introduction of new breeding technologies, etc. (*Stojanovski et al., 2011*). Changing the breed composition and genetic potential of pigs would lead to the intensified gain in fattening, as well as to the increase of the masses of certain parts of the body that are carriers of muscle mass, which has a significant place in meat production. The quality of carcass and pig meat varies under the influence of genetic and environmental factors (breed, sires, methods of rearing, individual animals, age and weight of animals, sex/gender, castration, nutrition, season, pre- and post-slaughtering procedures, etc., *Radović et al., 2007*). It is widely accepted that by increasing the age of pigs at slaughter, the sensory quality of the meat is improved, whereby this effect can be achieved by increasing pre-slaughter weight (*Lebret et al., 2001*). However, by increasing pre-slaughter weight, the pre-slaughter age increases, and thus a decrease in daily gain in fattening occurs. The capacity of the individual animal is very important, both in the fertile and meaty breeds, because it is in direct genetic correlation with the production performance of animals (*Vidović et al., 2013*). Systems of classification/categorization and payment for slaughter pigs play an important role in the process of intensifying the improvement of carcass quality (*Nakev et al., 2016)*. The weight of the warm carcass side represents the weight of the carcass side immediately after slaughter. The aim of this research is to demonstrate the necessity to use data from the slaughter line when assessing the breeding value of boars, the difference between sire breeds, sex of the offspring and the season of birth.

Materials and Methods

The trial was conducted at the farm and experimental slaughterhouse of the Institute for Animal Husbandry, Zemun-Belgrade. Sires of fattening pigs were pure breed: Swedish Landrace (SL, n = 10), Large Yorkshire breed (LY, n = 3) and Pietrain (P, n = 3), while offspring (both sexes, n=536) belonged to the following genotypes: Swedish Landrace (SL), and crosses: Large Yorkshire x Swedish Landrace (LY × SL), Pietrain × Swedish Landrace (P × SL), [Pietrain × (Large Yorkshire x Swedish Landrace)] P × (LY × SL), [Swedish Landrace x (Large Yorkshire x Swedish Landrace)] × SL (LY × SL) and [Large Yorkshire x (Large

White × Swedish Landrace)] × LY (LY × SL). There were 9 to 102 examined offspring per sire. The examined animals were born during four seasons (winter, spring, summer and autumn), so that the fattening was continuously carried out.

The final weight and the weight of the warm carcass side were taken on the appropriate weight scale, with an accuracy of ± 0.5 kg. Data processing was performed using the computer package "LSMLMW and MIXMDL, PC-2 VERSION" *(Harvey, 1990)*, using the method of least squares in order to determine the significance (P <0.05) of the systematic effects on the properties of the final weight (weight at the end of the fattening), the weight of the warm carcass side adjusted to the weight at the end of the fattening and the mass of the warm carcass side adjusted to the average age at the end of the fattening. The models include: sire breed (F), sires within the breed (B), gender/sex of fattening pigs (S), gender/sex of fattening pigs within the sire breed (G), season of birth of fattening pigs (Z), and final weight (weight at the end of the fattening) (linear impact) or age at the end of the fattening (linear impact). The phenotypic variability of the weight of the warm carcass side was examined using the following models (Model 1):

$$Y_{ijklm} = \mu + F_i + B_{j:i} + S_k + G_{k:i} + Z_l + b_1(x_1 - \overline{x}_1) + \varepsilon_{ijklm}$$

The used model for analysing the phenotypic variability of body mass characteristics at the end of the fattening and the weight of the warm carcass side had the following form (Model 2):

$$Y_{ijklm} = \mu + F_i + B_{j:i} + S_k + G_{k:i} + Z_l + b_2(x_2 - \overline{x}_2) + \varepsilon_{ijklm}$$

Results and Disscussion

Table 1 shows the effects of the sire breed and sires on the variation in body weight at the end of the fattening and the wight of the warm carcass side. By adjusting the body weight at the end of the fattening (MT) to the same age at the end of the fattening (UT = 204.91 days) it was determined that the animals had an average MT of 105.73 kg.

Table 1. The effect of sire breed and sires within breed (model 1 and 2) on final body weight and weight of warm carcass side (LSMean ± S.E.)

Variation source		MT[2)], kg	MTP_1, kg	MTP_2, kg
μ ± S.E.		105.73 ±0.68	81.78 ±0.21	85.81±0.61
SB[1)]	Sire No.			
Swedish Landrace	1	99.97±1.01	81.51±0.31	80.54±0.91
	2	99.76±1.03	81.34±0.32	80.26±0.92
	3	96.41±1.28	80.79±0.40	76.85±1.15
	7	112.93±2.61	80.09±0.81	90.35±2.34
	8	111.61±2.52	78.22±0.76	87.48±2.25
	9	108.82±2.63	80.69±0.81	87.41±2.36
	15	98.22±2.43	81.59±0.75	79.19±2.18
	16	99.69±2.26	81.34±0.70	80.21±2.02
	17	97.72±2.24	81.46±0.69	78.56±2.01
	18	94.28±2.08	81.20±0.65	75.43±1.86
	Average	101.94±0.91	80.82 ±0.28	81.63±0.81
Large Yorkshire	4	96.37±1.01	82.39±0.31	78.35±0.90
	5	97.46±1.15	81.61±0.35	78.50±1.03
	6	99.68±1.06	81.51±0.33	80.34±0.95
	Average	97.84±0.78	81.84±0.24	79.06±0.70
Pietrain	14	97.63±1.44	83.02±0.45	80.07±1.29
	19	121.39±2.75	82.15±0.85	99.75±2.46
	20	133.19±2.40	82.87±0.81	110.41±2.15
	Average	117.41±1.56	82.68±0.50	96.74±1.40
UT; MT (b)		0.307***	0.854**	0.271***

[1)]SB-sire breed. UT(b)- linear effect of the age at the end of the fattening; MT (b)- linear effect of the final body weight;

[2)]MT – final body weight; MTP_2- weight of warm carcass sides adjusted to the average age at the end of fattening (UT=204.91 days); MTP_1- weight of warm carcass sides adjusted to MT (MT=101.04 kg)

The offspring of sire P had the highest adjusted MT (117.41 kg) and the lowest offspring of sire LY (97.84 kg) at an average age of 204.91 days. Offspring of boars No. 7, 8 and 9 had higher body weight than the general average of all examined throats (μ = 105.73 kg). Contrary to them, females whose sires were LY breed had lower adjusted MT compared to the general average of 6.05 (sire 6) to 9.36 kg (sire 4). The difference in mean MT values between the offspring of sires 6 and 4 was 3.31 kg. The LSMean range for MT between the best sire of the SL breed (sire 7) and the worst (sire 18) was 18.65 kg at the same age. The largest established LSMean difference of the adjusted MT was between the boars 14 and 20 (35.56 kg) within the Pietrain breed.

The adjustment of the weight of the warm carcass side (MTP_1) to the same final body weight (weight at the end of the fattening), i.e. the final body weight

(MT = 101.04 kg), the highest average value of MTP_1 was obtained in offspring of sires P (82.68 kg) and the lowest in animals whose sires were SL (80.82 kg). With an increase in body weight at the end of the fattening by one kilogram, the weight of warm carcass sides was adjusted to the same body mass at the end of the fattening increased by 0.854 kg.

By the adjustment of the weight of warm carcass side (MTP_2) to the same average age at the end of the fattening (UT = 204.91 days), the highest average MTP_2 value was obtained for offspring of sires P (96.74 kg) and the lowest in animals whose sires were LY (79.06 kg). With an increase of the age at the end of the fattening by one day, the weight of warm carcass side adjusted to the same age at the end of the fattening increased by 0.271 kg.

Male castrated animals had higher body weight at the end of fattening (Table 2) compared to females at the same average age by 2.88 kg, and this difference was statistically significant (P = 0.0003). Observing this trait in relation to the birth season, it can be seen that the animals born in the spring had the lowest MT (97.86 kg) while the animals born in the winter, summer and autumn had almost identical MT (108.44 : 108.58 : 108.03 kg). At the same body weight at the end of the fattening (MT = 101.04 kg) females had higher weight of warm carcass side compared to male castrated animals (82.07 : 81.49 kg).

The research by *Radović et al. (2007)* shows that the average weight of the warm carcass side of offspring of Large Yorkshire and Swedish Landrace sires is 81.39 kg, at an average age of 216.87 days. In the research of *Ignjatović et al. (1998), Ignjatović and Petrović (1992), Brkić (2002)* and *Petrović et al. (2006),* the average weight of warm carcass sides of pure breeds, double and triple breed crosses ranged from 81.79 to 88.84 kg. A higher value for this feature is stated by *Kosovac et al. (2002)* 83.58 kg for the animals of Large Yorkshire breed, while *Radović et al. (2003)* state the lower values of the warm carcass side weight (74.17 kg) but with a higher slaughter age (236 days). Some studies included the offspring of one or both sexes (gilts and male castrated animals) and some did not mention the sex of the fatteners. *Ignjatovic et al. (1990)* state that the offspring of the Swedish Landrace sires have an average body weight at the end of the fattening of 98.5 kg, while the descendants of the Large Yorkshire sires reach the weight at the end of the fattening of 104 kg.

Table 2. The effect of gender/sex and season of birth (model 1 and 2) on final body weight and carcass side weight (LSMean ± S.E.)

Variation source		$MT^{2)}$, kg	MTP_1, kg	MTP_2, kg
Sex	M[1)]	107.17±0.76	81.49±0.24	86.76±0.68
	F	104.29±0.81	82.07±0.25	84.86±0.73
Season	Winter	108.44±1.87	83.04±0.58	89.41±1.67
	Spring	97.86±1.10	81.29±0.34	78.55±0.99
	Summer	108.58±1.10	81.59±0.34	88.07±0.98
	Autumn	108.03±1.03	81.20±0.32	87.22±0.93

[1)]M – male castrated animals. F – females; [2)]MT – final body weight;

MTP_2- weight of warm carcass sides adjusted to the average age at the end of fattening (UT=204.91 days);

MTP_1- weight of warm carcass sides adjusted to MT (MT=101.04 kg)

Male castrated animals had higher final body weight than females by 2.88 kg and MTP_2 by 1.90 kg, while MTP_1 was lower by 0.58 kg. The highest weight at the end of the fattening had animals born in summer and winter (108.58 and 108.44 kg), but they also had the highest MTP_1 and MTP_2.

Vidović et al. (2013) found that with the final body weight of males and females (104.07 and 102.04 kg), the weight of warm carcass side in males was 81.89 kg and females 80.67 kg, which is not in agreement with this research when it comes to female animals.

Table 3 shows the statistical significance of the included factors in models 1 and 2 for the properties of MT and MTP.

Table 3. Statistical significance (level of significance) of the influences included in the models in the analysis of the properties of MT and MTP

Variation source (influence)[1)]		MT[2)]	MTP_2	Variation source (influence)[1)]		MTP_1
Model 2	SB	***[3)]	***	Model 1	SB	***
	S:SL	***	***		S:SL	**
	S:LY	*	NS		S:LY	*
	S:P	***	***		S:P	NS
	Sex	***	**		Sex	*
	Season	***	***		Season	**
	Sex:SB	NS	NS		Sex:SB	NS
	UT (b)	***	***		MT (b)	**
	R^2	0.513	0.512		R^2	0.942

[1)] SB-breed of the sire. S : SL-sires within the Swedish Landrace breed; S : LY- sires within the Large Yorkshire breed; S: P- sires within the Pietrain breed; Sex: SB - offspring sex/gender within the sire breed; UT (b) - linear impact of age at the end of fattening; MT (b) - Linear impact of final body weight; R^2-coefficient of determination;

[2)] MT- final body weight; MTP_1- weight of warm carcass sides adjusted to MT (MT=101.04 kg); MTP_2- weight of warm carcass sides adjusted to the average age at the end of fattening (UT=204.91 days)
[3)] NS = P> 0.05; * = P <0.05; ** = P <0.01; *** = P <0.001

The results show that the MT, with the same mean age at the end of the fattening period (UT - linear influence, model 2) was under the influence (P<0.05 and P<0.001) of all included factors except for the interaction of gender of fattening pigs and sire breed (P> 0.05). Warm carcass side weight according to the MTP_2 (model 2) was not affected (P>0.05) by sires within the breed LY and sex of fattening pigs within the sire's breed. Varying of MTP_1 (model 1) also was not affected by the gender of fattening pigs within the sire breed and sires within the P breed (P>0.05).

The coefficient of determination shows that the effects included in the model 1 explained 94.2% of variation of MTP_1. The coefficient of determination shows that the effects included in the model 2 account for 51.3% variation of MT and 51.2% of variation of MTP_2.

In studies of carcass quality of five different genotypes (Swedish Landrace, F1 crosses of Swedish Landrace x Large White, and the three-breed and four-breed combinations of crosses of Large White, Swedish Landrace, Pietrain and German Landrace) with an average age at slaughter of 181.3 days, *Gorjanc et al. (2003)* determined the average weight of warm carcass sides of 77.70 kg.

Conclusion

On the basis of the results we can conclude that all factors included in Model 1 except for sires within the sire breed Pietrain and the sex/gender of fattening animals within the sire's breed, were statistically significant, very significant and highly significant (P<0.05; P<0.01; and P<0.001) influence on the trait of the weight of the warm carcass side adjusted to the same average final body weight. With the application of Model 2, all factors, except for the gender/sex of fattening animals within the sire's breed, were statistically significant and very significant (P <0.05 and P <0.001) influence on the pre-slaughter body weight; however, the weight of the warm carcass side adjusted to the average age was not under significant influence of factors sire within the breed Large Yorkshire as well as the sex/gender of fattening animals within the sire's breed.

Fenotipska varijabilnost mase tople polutke tovljenika svinja

Marija Gogić, Čedomir Radović, Milica Petrović, Radomir Savić, Aleksandar Stanojković, Maja Petričević, Vladimir Živković

Rezime

U radu je ispitan uticaj rase oca, očeva unutar rase, pol tovljenika, pol unutar rase tovljenika, sezona rođenja tovljenika na varijabilnost sledećih osobina: masa na kraju tova (MT), masa tople polutke (MTP_1) korigovane na masu na kraju tova (MT=101,04 kg) i masa tople polutke (MTP_2) korigovane na prosečni uzrast na kraju tova (UT, uzrast na kraju tova = 204,91 dana). Ogled je sproveden na farmi i eksperimentalnoj klanici Instituta za stočarstvo, Zemun-Beograd. Očevi tovljenika pripadaju čistim rasama: švedski landras (SL, n=10), veliki jorkšir (LY, n=3) i pijetren (P, n=3), dok potomci (oba pola) pripadaju sledećim genotipovima: švedski landras (SL), i melezi: veliki jorkšir×švedski landras (LY×SL), pijetren×švedski landras (P×SL), [pijetren×(veliki jorkšir×švedski landras)] P×(LY×SL), [švedski landras×(veliki jorkšir×švedski landras)] SL×(LY×SL) i [veliki jorkšir×(veliki jorkšir×švedski landras)] LY×(LY×SL). Na kraju istraživanja utvrđeno je: da pol tovljenika unutar rase oca (Pol:RO) ne pokazuje uticaj na ispitivane osobine (P>0,05), sezona rođenja potomaka pokazuje statistički značajan efekat na ispitivane osobine (P<0,001; P<0,01), otac unutar rase oca pijetren (O:P) u Modelu 2 ima visok uticaj (P<0,001).

Ključne reči: tovljenik, genotip, rasa oca, pol, sezona, masa tople polutke

Acknowledgment

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

References

BRKIĆ N. (2002): Ocena odgajivačke vrednosti priplodnih grla svinja primenom integrisanog selekcijskog indeksa. Doktorska disertacija, Poljoprivredni fakultet, Beograd-Zemun, 1-165.

GOGIĆ M., PETROVIĆ M., RADOVIĆ Č., ŽIVKOVIĆ B., RADOJKOVIĆ D., STANIŠIĆ N., SAVIĆ R. (2014): Variation of traits of fatteners under the impact of various factors. Biotechnology in Animal Husbandry 30, 4, 687-697.

GORJANC G., MALOVRH Š., KOVAČ M., GLAVAČ-VNUK M., ZRIM J. (2003): A note on the possibility to include carcass traits in the prediction of breeding values for pigs. Zbornik Biotehniške Fakultete Univerze v Ljubljani, Kmetijstvo Zootehnika, 82, 2, 89-96.

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

IGNJATOVIĆ I., PETROVIĆ M., OBRADOVIĆ J., DOBRIKOVIĆ K. (1990): Ispitivanje tovnih i klaničnih osobina švedskog landrasa i meleza F-1 generacije sa jorkširom i hempširom. X. skup svinjogojaca Jugoslavije, Pančevo, Zbornik radova, 177-181.

IGNJATOVIĆ, I., PETROVIĆ M. (1992): Tovne osobine i mesnatost polutki svinja različitih genotipova. Tehnologija mesa, 5, 33, 182-185.

IGNJATOVIĆ I., PETROVIĆ M., KOSOVAC O. (1998): Efekti ukrštanja različitih rasa svinja u cilju povećanja proizvodnje mesa. Biotehnologija u stočarstvu, 14, 1-2, 61-69.

KOSOVAC O., Petrović M., Živković B., Fabjan M., Radović Č. (2002): Evaluation of the quality level of carcass from the aspect of proportion of major carcass parts in pork carcass sides. Biotechnology in Animal Husbandry, 18, 3-4, 21-26.

LEBRET B., JUIN H., NOBLET J., BONNEAU M. (2001): The effects of two methods of increasing age at slaughter on carcass and muscle traits and meat sensory quality in pig. Animal Science, 72, 87-94.

NAKEV J., POPOVA T., IGNATOVA M., MARINOVA P., NIKOLOVA T. (2016): Seasonal and year dynamics in the quality characteristics in pig carcasses. Biotechnology in Animal Husbandry 32, 4, 341-352.

RADOVIĆ Č., PETROVIĆ M., JOSIPOVIĆ S., ŽIVKOVIĆ B., KOSOVAC O., FABJAN M. (2003): Uticaj različitih genotipova, očeva i sezone klanja na klanične osobine svinja. Biotehnologija u stočarstvu, 19, 1-2, 11-16.

RADOVIĆ Č., PETROVIĆ M., ŽIVKOVIĆ B., KOSOVAC O., RADOJKOVIĆ D., MIJATOVIĆ M., STOJANOVIĆ LJ. (2007): Effect of different fixed factors on pig carcass quality traits. Biotechnology in Animal Husbandry 23, 1-2, 71-80.

PETROVIĆ M., RADOJKOVIĆ D., MIJATOVIĆ M., ŽIVKOVIĆ B., RADOVIĆ Č., KOSOVAC O. (2006): The effect of genotype, herd and sex on the meat quality of pigs. Official journal of the British Society of Animal Science, 1, 164-165.

STOJANOVSKI M., KUZELOV A., RADEVSKA M. (2011): Participation of main parts in pig carcass sides from race daland. International Scientific Symposium of Agriculture – Agrosym Jahorina 2011, Impressum, 465-468.

UREMOVIĆ M., UREMOVIĆ Z. (1997): The effect of pigs genotype and body weight on meat quality. Agronomski glasnik 5-6, 419-428.
VIDOVIĆ V., LUKAČ D., ŠTRBAC LJ., PUNOŠ D., VIŠNJIĆ V., STUPAR M., DOKMANOVIĆ M. (2013): Selection criteria in relation to pork carcasses quality. Vet. glasnik 67, 3-4, 201-214.
ZAPRYANOVA I. (2017): Influence of the age of the first insemination on some reproductive indexes in sows. Biotechnology in Animal Husbandry 33, 1, 73-79.

REVIEWING THE POSSIBILITY OF THE SUBSTITUTION OF ANTIBIOTICS WITH PROBIOTICS IN DIET FOR WEANED PIGLETS

Vladimir Živković[1], Branislav Stanković[2], Bogdan Cekić[1], Miloš Marinković[1], Saša Obradović[3], Marija Gogić[1], Čedomir Radović[1]

[1]Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Republic of Serbia
[3]State University of Novi Pazar, Department of Chemical-Technological Sciences, Novi Pazar, Republic of Serbia
Corresponding author: Vladimir Živković, e-mail: vladimirzivkovic_87@yahoo.com
Original scientific paper

Abstract: The effects of the use of antibiotics and probiotics in the nutrition of weaned piglets were examined. The trial was conducted on 36 piglets divided into two groups during the entire investigation period. In the first study period, a feed mixture with 20% protein was used, while in the second period, a mixture with 18% protein. The first group, control, was fed with mixtures containing antibiotics in the amount of 0.2%, while the experimental group was fed with mixtures containing probiotic in the concentration of 0.03%. The obtained results showed that the use of probiotics, instead of antibiotics, resulted in the increase of gain by 4.09%, as well as food conversion ratio by 5.37% during the entire research period, while the cost was reduced by 6.48% per kilogram of gain.

Key words: piglets, nutrition, probiotic, antibiotic, production results

Introduction

In the last ten years, in the nutrition of domestic animals, there has been an indication of the negative consequences of the use of antibiotics in the diet of domestic animals. There is a legal pressure that is constantly growing to stop feeding animals feeds containing low levels of antibiotics as additives. Antimicrobials, when used as growth promoters, improve the daily gain by 3-9% and food utilization by 2-7%. For these reasons, they are commonly used even in farms with a high health status.

One of the first definitions of probiotics is that they are organisms and substances that contribute to the maintenance of intestinal microbial balance - eubiosis (*Sperti, 1971*). When using the probiotics, effects similar to antibiotics can be achieved, while avoiding possible side effects (residues, withdrawal, resistance, allergies, genotoxicity, etc.). They can increase their gain by stimulating the animal's immune system, and thus increase resistance to infectious agents. In addition, the disadvantage of antibiotics is that it takes longer, 5-10 days from the beginning of consumption, before their action starts.

In view of the increase in restrictions on the use of antibiotics as growth stimulants, more attention is directed to the use of alternative stimulants, such as probiotics that have a positive impact on animal health (*Reuter, 1997; Sinovec, 2001*).

The mechanism of probiotic action has not been sufficiently clarified to date. *Fuller et al. (1984)* consider that probiotics act similarly to normal intestinal microflora: neutralize toxins, suppress microflora growth, compete with adhesive sites, cause metabolic disorders of other bacteria, stimulate immunity, produce vitamins, and restore normal intestinal microflora after antibiotic therapy.

Several studies have shown that average daily gain and food conversion ratio are significantly improved after treatment with probiotics (*Živković et al., 2004; Živković et al., 2006).* The final effect acording to *Kalivoda (1983)* is a better use of food, increase of the gain, improvement of the meat, fat and water, milk and fertility ratios, etc. The aim of this paper is to evaluate the ability of probiotics to act as a substitute for antibiotic growth promoters in respect to health and productivity of weaned piglets.

Material and methods

The trial included 36 pigs of the same bred composition (F1) distributed in two feeding treatments (Table 1). Immediately after the piglets were weaned, on the basis of uniform initial weight, they were distributed to experimental groups, taking into account that in each group the sex ratio is the same. In the initial period of the experiment, animals were fed during 27 feeding days, with a starter mixture containing 20% of the crude protein, and in the final period of the experiment, which lasted 16 feeding days, the meals were formulated to contain 18% of the crude protein (grower).

The first group of piglets, control, was fed with mixtures based on the use of antibiotics, and the other group of piglets with mixtures where instead of antibiotics Digestarom for piglets was included at a concentration of 0.03% of the diet (Table 1). Food and water were *ad libitum*.

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

	Starter, Day 30-56		Grower, Day 57-72	
	%	%	%	%
Group	1 (control)	2 (trial)	1 (control)	2 (trial)
Probiotik Digestarom	+	-	+	-
Antibiotic Neodox	-	+	-	+
Crude protein, %	20.0	20.0	18.0	18.0
	%	%	%	%
Maize	42.3	42.47	-	-
Amiloproteks*	-	-	63.8	64.07
Barley	15.0	15.0	15.0	15.0
Triticale	-	-	5.0	5.0
Milk replacer	3.0	3.0	-	-
Ekofiš meal	5.0	5.0	3.0	3.0
Soybean meal	23.5	23.5	7.0	6.9
Oil	1.0	1.0	1.0	1.0
Premiks 1	10.0	10.0	-	-
Premiks 2	-	-	5.0	5.0
Antibiotic - Neodox	0.2	-	0.2	-
Digestarom	-	0.03	-	0.03
Total:	100.0	100.0	100.0	100.0
Din/kg	**42.05**	**41.75**	**33.91**	**33.59**

*Amiloproteks is mixture of maize and full fat soybean, in ratio 70:30, heat treated prior to mixing into diet

During the research period, the following production indicators were monitored: body weight, average daily gain, average daily food consumption, food conversion, by trial periods, and economic justification for the introduction of Digestarom over the kilogram of piglets gain in the trial. One-way analysis of variance was performed using the SPSS 20.0 software (IBM SPSS Statistics, Version 20, IBM Corp., USA).

Results and discussion

In the first period of the experiment, during feeding by a starter mixture, the control group of piglets achieved an average daily gain of 268 g (Table 2). The introduction of Digestor instead of antibiotics in the mixture has led to a deterioration in the gain, on average by 27 g or 10.08% compared to the control group of piglets. Somewhat lower food consumption per feeding day, by 0.023 kg or 4.84%, was achieved by the piglets of the experimental group. The use of Digestor in the mixture, caused the food conversion to deteriorate by 0.10 kg or 5.65% in relation to diet with a mixture based on antibiotics.

Table 2. Production results in piglets in the initial research period

Group	*1 (control)*	*2 (trial)*
Initial body weight of piglets, kg	9.77	9.63
Final body weight of piglets, kg	17.02	16.12
Trial duration, days	27	27
Average daily gain, g	268	241
Index, %	100.00	89.92
Average daily food consumption, kg	0.475	0.452
Index, %	100.00	95.16
Feed conversion ratio, kg	1.77	1.87
Index, %	100.00	105.65

Unlike the first trial period, in the final part of the experiment, when grower mixture was used, the the experimental group achieved on average by 81 g or 24.25% higher gain compared to piglets fed the mixture in which the antibiotic was incorporated. In addition to better gain, the second group consumed on average by 0.016 kg or 1.97% more food and had lower food conversion ratio compared to the control group of piglets (Table 3).

Table 3. Production results in piglets in the final research period

Group	*1 (control)*	*2 (trial)*
Initial body weight of piglets, kg	17.02	16.12
Final body weight of piglets, kg	22.36	22.76
Trial duration, days	16	16
Average daily gain, g	334	415
Index, %	100.00	124.25
Average daily food consumption, kg	0.813	0.829
Index, %	100.00	101.97
Feed conversion ratio, kg	2.43	2.00
Index, %	100.00	88.30

During the entire research period, piglets fed diet with the addition of antibiotics Neodox achieved average daily gain of 293 g, food consumption of 0.601 kg and feed conversion ratio of 2.05 kg per kilogram of gain (Table 4). The introduction of the tested Digestar instead of antibiotics led to improvement of gain on average by 12 g or 4.09%, decrease in food consumption by 0.09 kg or 1.50% on average, and improvement in food conversion on average by 0.11 kg or 5.37 %

relative to the control group fed the mixtures in which the Neodox antibiotic was used.

Table 4. Production results in piglets for total research period

Group	*1 (control)*	*2 (trial)*
Initial body weight of piglets, kg	9.77	9.63
Final body weight of piglets, kg	22.36	22.76
Trial duration, days	43	43
Average daily gain, g	293	305
Index, %	100.00	104.09
Average daily food consumption, kg	0.601	0.592
Index, %	100.00	98.50
Feed conversion ratio, kg	2.05	1.94
Index, %	100.00	94.63

Table 5. Economic analysis of the use of Digestar during the entire research period

Group	*1 (control)*	*2 (trial)*
Value of the food, din/kg	37.95	37.50
Value of the food, %	100.00	98.81
Food conversion, kg	2.05	1.94
Food conversion, %	100.00	94.63
Cost of gain of weaned piglets, din/kg	77.79	72.75
Cost of gain of weaned piglets, %	100.00	93.52

Table 5 gives indicators of the economic justification for the introduction of Digestar into mixtures for weaned piglets. Using the tested additive, on the basis of consumed food, the price of food is reduced by 0.45 dinars or 1.19%, and with better food conversion, the price of piglets that consumed Digestar was more favorable by 5.04 dinars or 6.48% compared to the control group. Statistical analysis showed that there were no statistically significant differences in the use of these two diets. In the case of weaned piglets, positive effects of probiotic compared to antibiotics were noted by *Garcia et al. (2003), Parrot and Rehberger (2004), Lawrence (2005) and Estienne et al. (2005).* Similar to these results, the addition of probiotics showed an improvement in the gain (*Yang et al., 1998; Burnham et al., 2004*), as well as food conversion (*Yang et al., 1998*).

The overall results obtained showed that it was recommended that Digestrom be used as a substitute of Neodox antibiotics in mixtures for weaned piglets.

Conclusion

The obtained results showed that the introduction of the examined Digestar in diets had following effects:

- lower gain by 10.1%, and unfavorable food conversion, by 5.65%, in piglets during the initial period of trial,
- in the second, final, period increase of gain by 24.2% and more favorable food conversion by 17.7%,
- during the entire trial period, Digestar additive showed positive effects on the gain, which was better by 4.09%, and the conversion of food was more favorable by 5.37% compared to the group of piglets fed with mixtures based on antibiotics,
- The Digestar in the mixture showed positive effects on the price of piglets growth which was more favorable by 6.48% compared to this indicator realized when animals were fed mixtures in which the Neodox antibiotic was incorporated.

Istraživanje mogućnosti aplikacije probiotika umesto antibiotika u ishrani zalučene prasadi

Vladimir Živković, Branislav Stanković, Bogdan Cekić, Miloš Marinković, Saša Obradović, Marija Gogić, Čedomir Radović

Rezime

Ispitivani su uticaji korišćenja antibiotika i probiotika u ishrani zalučene prasadi. Ogled je sproveden na 36 prasadi podeljenih na dve grupe tokom celog perioda istraživanja. U prvom periodu istraživanja korišćena je smeša hraniva sa 20% proteina, dok je u drugom korišćena smeša sa 18% proteina. Prva kontrolna grupa je hranjena smešama sa antibiotikom u količini od 0,2%, dok je ogledna grupa hranjena smešama sa probiotikom u koncetraciji od 0,03%. Dobijeni rezultati su pokazali da korišćenjem probiotika, umesto antibiotika, dolazi do poboljšanja prirasta za 4,09%, kao i konverzije hrane za 5,37% u toku celog perioda istraživanja, dok je cena koštanja smanjena za 6,48% po kilogramu prirasta.

Ključne reči: prasad, ishrana, probiotik, antibiotik, proizvodni rezultati

Acknowledgement

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

References

BURNHAM L.L., RICHARDSON C.R., GUYE A., NUNNERY G.A., SCHMITT R.A.M., KIM S.W. (2004): Growth performance and carcass characteristics of growing-finishing pigs fed either an antibiotic or a probiotic. J. Anim. Sci., 82 (Suppl. 1): XX, abstract.

ESTIENNE M. J., HARTSOCK T. G., HARPER A. F. (2005): Effects of Antibiotics and Probiotics on Suckling Pig and Weaned Pig Performance. Intern. Journal Applied Research Veterinarian Medicine, Vol. 3, Number 4, 303-308.

FULLER R., COLE C.B , COATESM E. (1984): The role of Streptococcus faecium in antibiotic relieved growth depression of chickens. In Antibiotics in Agriculture: Benefits and Malefits ed. Woodbine, M. pp. 395403. London: Butterworths.

GARCIA M. I., HANSEN S., SANCHEZ J., MEDEL P. (2003): Efficacy of addition of B. Licheniformis and b. Subtilis in pig diets from weaning to slaughter. Journal of Animal Science, 82, Suppl. 1, 26.

KALIVODA M. (1983): Koristi i rizici upotreba stimulatinih i drugih dodataka u stocnoj hrani / M. Kalivoda / Krmiva. 1983. - V. 1. - 2. - P.l-7.

LAWRENCE B. (2005): Swine solutions: Extensive Research Trial: Antibiotic-Free Alternatives Prove Promising. Hubbard Feeds Inc., 1 – 7.

PARROT D.S., REHBERGER T.H. (2004): Isolation of bacillus strains to inhibit pathogenic E.coli and enhance weanling pig performance. J. Anim. Sci. 83 (Suppl 1); 26 Abstr.

REUTER G. (1997): Ecology of two strains of probiotics in the instetine of zoung pigs. Microecology and Therapy, 26, 271-281.

SINOVEC Z. (2001): Stimulatori rasta, probiotici kao alternativna mogućnost. Živinarstvo, 36 (10), 195-202.

SPERTI G.S. (1971):Probiotics, Avi Publishing Co., West Point, Connecticut.

SPSS: IBM SPSS Statistics 20. 1 New Orchard Road, Armonk, IBM.

YANG S.J., HYON J.S., YANG C.B., KO S.M., CHOI H.H. (1998): Studies of the effects of feed additives fed to pigs: Effects of feeding probiotics on the growth performance and carcass quality in pigs. Corean Journal of Animal Science, 40 (1), 21-30.

ŽIVKOVIĆ B., MIGDAL W., FABJAN M., KOVČIN S., RADOVIĆ Č., KOSOVAC O., TODOROVIĆ M., JOKIĆ Ž. (2004): Nutritivna vrednost probiotika u ishrani svinja u tovu, Biotechnology in Animal Husbandry, 20, 1-2, 51-58.
ŽIVKOVIĆ B., NIKIĆ D., MIGDAL W., RADOVIĆ Č., FABJAN M., KOSOVAC O., PEJČIĆ S. (2006): Probiotik Beta Plus u ishrani krmača i prasadi, Biotechnology in Animal Husbandry, 22, 1-2, 109-117.

THE ECONOMICS OF MEAT PRODUCTION ON PIG FARMS IN SERBIA IN DIFFERENT FARMING SYSTEMS

Ivan Mičić[1], Zoran Rajić[1], Jelena Živković[1], Grujica Vico[2], Marko Mičić[3], Ivana Mičić[3], Marija Mičić[3]
I.
[1]Faculty of Agriculture, University of Belgrade, Republic of Serbia
[2]Faculty of Agriculture, University of East Sarajevo, B&H
[3]University of Niš, Republic of Serbia
Corresponding author: Ivan Mičić, divanlav@gmail.com
Original scientific paper

Abstract: The study deals with situational analysis and the basic problems development on pig meat production in Serbia, as well as an economical analysis of fattening pigs at farm production conditions. The research included small private farm “Gracanica” farm, in central Kosovo and Metohija, as well as a cooperative farm in December the first "Žitorađa" T Serbia. During the period in 2016, have been monitored the volume and application production technology of fattening pigs on both farms, and analyzed the resulting economic results. During the observing period, it was found that at private farm, their breeding pigs were 60 head, for the cooperative farm, their breeding pigs were 35,442 heads. The produced fattened pig in private farm costs 82 Euro’s, the pork meat carcasses 1.10 Euro’s / kg, and for thev farm, cost of fattened pigs is 132 Euro’s, while, the pork meat carcasses 1.84 Euro’s / kg. The average weight of carcass sides is uniform in both at 79 kg, whereas the share of pork sides varied from 80% to private farms and 78% cooperative farm.

Key words: development, pigs, pork carcass, price, economy justification

Introduction

Regardless of what the natural indicators in intensive, market oriented production of pigs are, it is very difficult to provide a detailed insight into the production cost of fattened pigs, which represents the research basis of the paper and proof that the pig production process is cost effective. *Mičić (2016a)* determines that with an increase in the number of piglets per sow from 10 to 20, the production cost per piglet reduced by 79.09%, while cost per sow increased by 11.67% per annum. Increase in the genetic basis of pigs represents a necessary precondition for the achievement of greater intensity in this branch of livestock production.So *Vidović et al. (2012)* report that the annual genetic progress for average daily growth was 8-11 gr, food conversion from 0.03 – 0.05 kg as well as 0.35 – 1.00% for the content of meat in sides. Based on previous research and results in practice there is opinion that betterresults can be expected in due time as follows: 30 fattened pigs per sow per year, conversion of food bellow 2 kg, less than 120 days of life to reach 100 kg of body weight, daily gain of live weight of about 2 kg. *Mičić (2016b)* states that the characteristic of breeds of pigs as well as individual characteristics of livestock to achieve greater daily weight gain, greater amount of meat and better carcass yield in same growing conditions are of great significance to successful and cost-effective pork meatproduction. By better use of these properties the fattening period is reduced and at the same time total production is increased. *Živković and Perunović (2012)* state that pork meatproduction in Serbia is characterized by the increasing participation of large farms (10,000 to 30,000, and more fattened pigs per year), and quality of pigs has significantly increased, especially on farms, and it can be said it is approaching the European average. Pork meatproduction is carried out by determining production cost of 1 kg, produced pork meatsides in first and second phase. Research of the economic parameters of producing fattened pigs deals with costs in the first phase of the production process and determining total cost of producing pork meat sides in the second phase, by the division calculation method. This allows for the given results to have common, rather than only local significance *Andrić (1998).*

Research goal: During research and proving, primarily a scientific method is used, whose basic application enables explanation and prediction of relations between individual relevant inputs, and results of achieved effects in production of pork meat. In accordance with the development strategy of producing pork meat, the research goal is to improve the quality of pork meatproduction which originate from noble pure breed, raised on farms A and B, by a large number of analyzed and realized economic parameters. The representation of the given results of plant production on farm A and pig production on both farms will be given. During preparation of this work, data from multiple sources was used. The data used were the production quantity, analysis of pig production and pork meatin long time period. The analysis of this data wouldn't be possible if it weren't approved by both farms and the data was processed by mathematical-statistical methods.

Materials and methods

The research conducted included family agricultural holding on farm A in Gračanica whose owner is Bojan Jovanović, address Kosovske Devojke 417 and a cooperative farm B, "1 Decembar" in Žitorađa. Both farms have a closed production cycle that includes pig production fattening. Farm A produces 60 fattened pigs and farm B around 35,442 a year. Within farm A 2 persons and 4 minors are employed and in farm B 80 people with corresponding qualifications are employed. Costs of producing fattened pigs on both farms are based on natural indicators determined based on research done in 2016 and all variable categories of costs in accordance with the production process. Material costs relate to consumption of nutrients and medicine used in the production process. Amortization costs are covering 2016 based on norms of necessary space and equipment, we approach the investment estimate, estimate of amortization costs based on which the fixed costs categories are calculated. When determining costs of producing costs we start from the price of a pig, weight of a pork meat side obtained by slaughter, variable costs of slaughter service and freezing.

Results of plant production of farm A and fattened pig production on both farms relates to one year period (2016). In order to make a conclusion, the production parameters were monitored: grain on farm A and food consumption of farms A and B per 1 kg ofgrowth, total growth and cost of food on both farms. Significance of results in production of fattened pigs and pork meatin 2016 was followed independently on farms A and B during one research year.

Results and discussion

Starting from the previously pointed facts and characteristics of pork meatside production strategy, we analyzed on both farms:

- Plant production on 10 ha on farm A,
- Pork meat side production on farms A and B,
- Characteristics of pork meatside quality on both farms.

Next to theoretical explanation and application on general examples, we showed efficiency of optimal feeding on an example of feeding mixture for fattened pigs on farm A. Production costs of fattened pigs on both farms are based on naturally determined indicators. Calculation of fixed and variable costs was calculated on both farms in accordance with the production process. Also costs are related to consumption of nutrients and medicine which are used in the production process, as well as amortization of the livestock, existing space and equipment which is done based on norms. It can also be seen that revenue from grain on farm A is 50 t, it moved from 3.5 t/ha to 7.5 t/ha and from total amounts of plant production farm A used 1/3 to feed the heard while 2/3 was sold on the market.

Pricelist of mixture for feeding pigs on farmA

During price calculation of the mixture from own produced grain for breeding pigs on farm, price of all products was taken into consideration, calculated in tons (t). More data about it is shown in Table 1.

Table 1.Prices of concentrate mixtures on farm A

PRICELIST OF FEED MIXTURE FOR PIGS ON A FARM IN KOSOVO AND METOHIJA IN GRAČANICA IN 2016	Price EUR/kg
Pre-starter mixture for feeding pigs up to 10kg (PS)	0.27
Grover mixture for feeding pigs from 15 to 25 kg (SS)	0.22
Starter mixture for feeding pigs up to 15 kg (SG)	0.24
Mixture fo feeding fattened pigs from 25 to 60 kg (TS-1)	0.20
Mixture for feeding fattened pigs from 60 to 100 kg (TS-2)	0.19
Mixture for feeding pregnant gilts and sows (SK)	0.17
Mixture feeding lactating sows and boars (SKD)	0.16

Source: Authors' calculation based on data from Mičić, 2016

From Table 1 we can see that the feeding mixture for all categories of pigs farm A produces by itself in powder form in their blenders at producers prices.
Price of the concentrate mixture on the farm was calculated by average exchange rate of the National Bank of Serbia in EUR/kg/120 RSD in 2016.

Productivity of sows and raising piglets on farm A

Farm had ten sows of landrace breed which had two farrowing a year with an average of ten pigletsper brood, i.e. 20 piglets per sow a year and one boar of the Yorkshire breed.

Piglets were weaned after 21 days with an average body weight of 5.24 kg. Their breeding lasted 38 days after that until they reached 25 kg, with the achieved daily growth of 0.52 kg/day (Table 2).

Table 2. Productivity of sows and raising piglets up to 25 kg in 2016 on farm A

Livestock number on farm 10	*Lowland region*
Racial compositionLandrace+Yorkshire	Input weight
Time for fattening in years (two rounds)	Exiting weight 25 kg
Average 20 piglets/sow a year	Weight of a piglet after weaning 5.24 kg
Age of piglets when weaning 21 day	Raising piglets 38 days x 0.52 kg/day

I	**Revenue**	**Amount**			**Price per unit €**	**Total €**
		Number of pigs	**kg/ livestock**	**Total kg**		
1	Raising piglets	200	25	5.000	2.80	14,000.00
2	IncentiveRS	200	-	-	-	-
3	IncentiveRS (sow)	10	-	-	-	-
4	Insurance reimbursement					-
A)	**T o t a l*(1 to 4)***					**14,000.00**
II	**EXPENSES**					
5	Feeding piglets					
6	-pre-starter 0.2 kg/day x10days x200 lives	200	2	400	0.2695	107.80
7	-SS (to 15 kg) (0.5 kg/day x 13days x200 l	200	6.5	1.300	0.2415	313.95
8	-SG (15-25 kg) 1.56 kg/day x15days x200	200	23.4	4.680	0.2190	1,024.92
9	Feeding sows 2.5 kg/day x365days x10 li	10	912.5	9.125	0.1666	1,520.23
10	Feeding boars 2.2 kg/day x 365days x 1li	1	803	803	0.1616	129.76
B)	**Total feed*(5 to 10)***					3,096.66
11	Losses in feeding 1%		50		2.40	120.00
12	Water and medicine – sow	10	-	-	10.00	100.00
13	Human labor (personal or someone else's)	kg/€		40	10.00	400.00
14	Amortization of the heard (400-100=300x20%)	10	-	-	60.00	600.00
15	Amortization of the facility and equipment			8.000	2.5%	200.00
16	Total direct costs***(5 to 15)***					4,906.66
17	Indirect costs of the farm					490.00
C)	**Total costs*(5 to 17)***					**5,006.66**
III	**GAIN/LOSS**					
18	**On a farm withour incentive (A – C)**					**8,993.34**
19	**Per pig without incentive (18 : 3)**					**899.33**
20	**Price of akg(C : 1)**					**1.00**
21	**Production efficiency (A : C)**					**2.79**
22	**Revenue profitability (18 : A) h 100**					**64.24%**

Source: Authors' calculation based on data from Mičić, 2016

From table 2 we can see that the average weight of piglets on the farm in Gračanica is 25 kg/livestock and that it ranged from 24 to 26 kg/ livestock, with the achieved average price of 1 €/kg and the value of 1 pig was 25.00 €. Total achieved gain for 200 piglets was 8,993.34 €, production efficiency 2.79 and revenue profitability 64.24 %.

In Table 3 there are 60 fattened livestock in 2016 on a family farm A.

Fattened pigs on farm A

Farm A fattens 60 pigs a year in 4 turns and sells them ex-loaded on the farm as well as excess piglets. Farm A breeds around 200 piglets a year of average weight of 25 kf and raises gilts for themselves. Farm A sells 80% of piglets free loaded after they have achieved 25 kg of mass at the price of 2.8 euros, in 2016 (Table 3).

Table 3.Achieved economic indicators in fattened pigs on farm A for 2016

No.	**Production year: 2016.**	**Amount**	**Feed conversion rate, kg:**			**2.68**	**kg growth**
1.	Fattening period: **Jan-Dec**	-	Unit of Measure	Mortality rate in feeding:		**2.43%**	
2.	Number of pigs placed in fattening:	**60**	livestock				
3.	Average weight of fattened:	**100**	kg/livestock				
4.	Fattening time:	**87**	days				
I	**Revenue**	-	-	**Price**	**Unit of Measure**	**Amount/€**	**Amount** (€/livestock)
5.	**Fattened pigs** (3 x 5)	40.00	livestock	1.58	EUR/kg	6.320.00	158.00 EUR
6.	**Manure**	20.00	t	4.00	€/t	80.00	2.00 EUR
7.	**Subventions per pig**	40.00	livestock	8.70	€/livestoc	348.00	8.70 EUR
A)	**Total revenue *(1 to 7)***	-				**6.748.00**	**168.70** EUR
II	**Expenses**	-	Unit of me	Price	Unit of mea	Amount/€	E/livestock
8.	Piglets (average livesto)	25.00	kg/livestoc				
9.	Piglets (2 x 8)	1,025.00	kg/livestoc	1.00	EUR/kg	1,025.00	25.00 EUR
10.	Own mixture of concentrate						
11.	TS1 (from 25-60 kg) (2.15kg/day x 41days x40 g.) 3.526			0.20	EUR/kg	705.00	17.63 EUR
12.	TS2 (from 60-100 kg) (2.45kg/day x46days x40 g.) 4.508			0.19	EUR/kg	856.50	21.41 EUR
13.	Average daily per pig	**2.31**	kg/EUR				
14.	Mechanical work (7 h14)		kg/EUR	2.00	EUR/kg	80.00	2.00 EUR
15.	Water per pig**(15x4) x 8:1.000**	10	L/day	1.25	EUR/m^3	43.50	1.08 EUR
16.	Veterinary services and medicine(7x16)			1.00	EUR/livestock	40.00	1.00 EUR
17.	Human labor**(3 x 5) x 17**			0.15	€/livestock	600.00	15.00 EUR
18.	Indirect costs**(7 x 18)**			1.40	€/livestock	56.00	1.40 EUR
19.	Amortization of facilities and equipment (7 x 19)			2.00	€/livestock	80.00	2.00 EUR
B)	**Total costs***(9 to 30)*					**3,486.00**	**86.52**EUR
III	**GAIN/LOSS**						
20.	On farm with incentive(A – B)					3.262.00	**81.55**EUR
21.	Price in kg B : (3 x 5)					0.87	
22.	Production efficiency (A : B)					1.94	
23.	Revenue profitabilit (20 : A) x 100					48.34%	

Source: Authors’ calculation based on data from Mičić, 2016

From Table 3. we can see that the value of one fattened pig was 87.00 EUR/pig. Total achieved gain for 40 fattened pigs is 3,262.00 EUR, production efficiency 1.94 and revenue profitability 48.34 %.

Pricelist of concentrate mixtures on farm B for feeding pigs of all categories is shown in Tab.4

Table 4.Price of concentrate mixture on farm B

PRICELEST OF MIXTURE FOR FEEDING PIGS ON A FARM ON KOSOVO AND METOHIJA IN GRAČANICA IN 2016		PRICE EUR/kg
Pre-starter mixture for feeding piglets to 10kg	(PS)	0.48
Grovermixture for feeding piglets from 15 to 25 kg	(SS)	0.34
Startermixture for feeding piglets to 15 kg	(SG)	0.33
Mixture for feeding fattened pigsfrom 25 to 60 kg	(TS-1)	0.28
Mixture for feeding fattened pigs from 60 to 100 kg	(TS-2)	0.26
Mixture for feeding pregnant gilts and sows	(SK)	0.25
Mixture for feeding lactating sows and boars	(SKD)	0.29

Source: Authors' calculation based on data from Mičić, 2016

The Farm has its own blenders that operates independently and is located by the entrance gate and by the above mentioned pricelist of mixture entrusts farm B.

Productivity of sows and upbringing of piglets on farm B

Farm B has 1,500 sows Landrace + Yorkshire which had two farrowing a year in the average of 10.3 raised piglets per breed, i.e. 20.6 piglets a year. Piglets are weaning after 28 days with the average body weight of 6.6 kg. Their upbringing lasted 34 days after that up to body weight of 25 kg, with the achieved daily growth of 0.54 kg a day.

More data on productivity of sows and raising piglets on farm B is given in Table 5.

Table 5. Productivity of sows and raising piglets up to 25 kg on farm B in 2016

Livestock Number on farm 1,500 *Lowland region*
Racial compositionLandrace +Yorkshire Entrance weight
Fattening weight in years (two cycles) Exiting weight 25 kg
Average 20.6 piglets/pig a year Weight of a piglet after weaning 6.6 kg
Age of piglets after weaning 28 days Raising piglets 34 days x 0.54 kg/day

I	**REVENUE**	**Number of livestock**	**kg/ livestock**	**Total, kg**	**Price unit**	**Total €**
1	Raising piglets put for fattening:	35,442				
2	Average end weight of piglets:	30,000	25	750,000	2.39	1,792,500.00
3	Manure (sows)total	1,500	500	750,000	0.01	7,500.00
A	**T o t a l (1 to 3)**					1,800,000.00
II	**EXPENSES**					
5	Feeding piglets/ mixture according to pricelist Table 8.					
6	-pre-starter (0.2 kg /day x10days x30,000 pigs)	30,000	2	60,000	0.48	28,800.00
7	-SP1 (to 15 kg) (0.6kg /day x11days x30,000 pigs)	30,000	6.6	198,000	0.34	67,320.00
8	-SP2 (15-25kg) (1.8 kg /day x13days x30,000 pigs)	30,000	23.4	702,000	0.32	224,640.00
9	Feed to sow (4.5kg/day x46days x1,500 pigs)	1,500	207	310,500	0.28	86,940.00
10	Feeding a sow (4.5 kg/day x365days x1,500 pigs)	1,500	1,642.5	2,463,750	0.26	640,575.00
11	Feeding a boar (4 kg/day x365days x25 pigs)	25	1,460	36,500	0.26	9,490.00
B)	**Total feed (5 to 11)**		3,341.5	3,770,750		**1,057,765.00**
12	Loss in fattening piglets 2%		-	-		36,000.00
13	Under vacuum	30,000			1.00	30,000.00
14	Water and medicine – sow and boars	1,525	-	-	20.00	30,500.00
15	Human labor (personal someone else's)	working day		365	400.00	146,000.00
16	Amortization of pigs (450 - 150=300x20%)	1,525	-	-	60.00	91,500.00
17	Amortization of facilities and equipment			1,449,275	3%	43,478.00
18	Total direct costs*(5 to 18)*					1,494,718.00
19	Indirect costs of the farm					93,559.00
C)	**Total costs***(18 + 19)*					**1,528,802.00**
III	**GAIN/LOSS**					
20	On a farm without incentive(A – C)					271,198.00
21	Per pig without incentive (20 : 3)					180.79
22	Price for kg (C : 2)					2.04
23	Production efficiency (A : C)					1.18
24.	Revenue profitability (20 : A) x 100					15.07%

Source: Authors' calculation based on data from Mičić, 2016

From Table 5 it is visible that breeding sows-piglets on farm B has the gain from 271,198 EUR, efficiency is 1.18 and revenue profitability is 15.07 %
Table 6 shows achieved economic indicators in fattened pigs on farm B in 2016.

Table 6. Achieved economic indicators in fattened pigs on farm B

1.	Production year: **2016**	Amount	Conversion rate of feed in kg:			**3.21**	kg/growth
2.	Fattening period:**Jan-Dec**	Unit of Measure	Mortality rate of fattened:			**2.0%**	
3.	Number of pigs put for fattening:	30,600	livestock				
4.	Average weight of a fattene pig:	**100**	kg / livestock				
5.	Length of fattening:	**98**	Days				
I	**Revenue**	**Unit of Measure**	**Unit of measure**	**Price**	**Unit of measure**	**Amount total**	**Amount€/** livestock
6.	**Fattened pigs*(4 x 6)***	30,000	livestock	1,58	EUR/kg	4,740,000.00	158.00 €
7.	**Manure**	15,000	t	4,00	EUR/t	60,000.00	2.00 EUR
8.	**Subventions per pig**	30,000	livestock	8,70	€/livestock	261,000.00	8,70 EUR
A)	**Total revenue *(1 do 8)***	-				**5,061,000.00**	**168.70** €
II	**Expenses**	-					
9.	Piglets (average/pig)	25.0	kg/livest.				
10.	Piglets***(3 x 9)***	765,000	kg/livest.	2,04	EUR/kg	1,560,600.00	52.02 EUR
11.	Farm is has the mixture according to priceli						
12.	TS1 (from 25- 60 kg) 2.35kg/dayx46days x30,000 pigs			0,28	EUR/kg	908,040.00	30.27 EUR
13.	TS2 (from 60-100 kg) 2.55kg/dayx52days x30,000 pigs			0,26	EUR/kg	1,034,280.00	34.48 EUR
14.	Average daily per livestock	**2.46**	kg/EUR				
15.	Mechanical work ***(6 x 15)***	-	kg/EUR	1,497	EUR/kg	44,901.00	1.50 EUR
16.	Water per livestock ***(16 x 5) x 6***	10	L /day	1,15	EUR/m^3	33,810.00	1.12 EUR
17.	Veterinary services and medicine***(6 x 17)***			0,80	€/ livestock	24,000.00	0.80 EUR
18.	Human labor***(4x6) x 18***		kg/EUR	0,18	€/ livestock	540,000.00	18.00 EUR
19.	Indirect costs ***(6 x 19)***		kg/EUR	1,00	€/ livestock	30,000.00	1.00 EUR
20.	Amortization of facilities and equipment ***(6 x 20)***			3,53	€/ livestock	105,900.00	3.53 EUR
B)	**Total costs*(9 to 20)***					4,281,531.00	**142.72** €
III	**GAIN/LOSS**						
21.	On farm with incentive ***(A – B)***					779,469.00	**25.98** EUR
22.	Cost perkg***B :(4 x 6)***					1.42	
23.	Production efficiency***(A : B)***					1.18	
24.	Revenue profitability (21 : A) x 100				%	15.40	

Source: Authors' calculation based on data from Mičić, 2016

From Table 6 we can see that total achieved gain for 30,000 fattened pigs is 779,469.00 EUR, production efficiency 1.18 and revenue profitability is 15.40 %.

Production efficiency of fattened pigs on both farms in 2016

In order to research production efficiency in fattening pigs, two farms from Serbia and Kosovo and Metohija were taken into consideration since they have the necessary conditions for such production. We researched farm A which has 60 fattened pigs and farm B which has 35,442 livestock. Average entrance weight of piglets for fattening was 25 kg and achieved exit weight was 100 kg. On farm B average fattening period of pigs was 98 days with the daily gain of 0.76 kg/livestock/day, while on farm A it lasted 87 days, with an average daily growth of 0.86 kg/livestock/day. Our results are in accordance with the results stated *(Vidović et al., 2012)* in the performance test of pure breeds of pigs, landrace and Yorkshire.

Calculation of income includes only the revenue from selling pigs while potential revenue from pig meat wasn't taken into consideration (Table 7).

Table 7. Economic indicators of pork meatproduction – pork meatside on farm A and B in 2016

I	**Indicator**	**Unit of measure**	**Farm A/**	**Farm B/**
A.	**Fresh pork meatsides**	livestock	**40.00**	**30,000.00**
B.	**Livestock weight of live pig** weight	**kg**	**100.00**	**100.00**
1.	**Total weight, pig(AxB)**	**kg**	4,000.00	3,000,000.00
2.	Pork sides/livestock	%	79.00	78.00
3.	Total pork/side**kg** (1 x 2) : 100	**kg**	3,160.00	2,340,000.00
4.	Price of pork sides	**€ /kg**	2.82	2.82
V.	**Total revenue (3 x 4)**	€	8,911.20	6,598,800.00
II	**EXPENSES**			
5.	Price of the slaughter service	€/livestock	8.70	8.70
6.	Direct costs of live pig weight	€	3,480.00	4,281,531.00
7.	Total pig slaughter service (Ax 5)	€	348.00	261,000.00
G.	**Total expenses(6 + 7)**	€	**3,828.00**	**4,542,531.00**
III	**GAIN/LOSS**	€		
8.	Pork sides from farms (V - G)	€	**5,083.20**	**2,056,269.00**
9.	Pork side livestock/EUR (8 : A)	€	127.08	68.54
10.	Pork side pricekg (G : 3)	€	1.21	1.94
11.	Meat production efficiency (V : G)		2.33	1.45
12.	Revenue profitability (8 : V) x 100	%	57.04	31.16

Source: Authors' calculation based on data from Mičić, 2016

From the shown data it can be seen that pork meat side price on farm A is 1.21 EUR/kg and that this production has the efficiency of 2.33 and revenue profitability of 57.04 %, while on farm B pork meat side price is 1.94 EUR/kg and that this production has the efficiency of 1.45 and revenue profitability of 31.16 %. It can further be seen that on both farms the calculation of slaughter expenses, cooling and processing of sides amounts to 8.70 EUR per fattened pig. Calculation was conducted in accordance with the achieved yield of slaughtered pigs, value of fattened pig with the mass of 100 kg with the price of 87.00 EUR + 8.70 EUR slaughter expenses, which amounts to 85.70 EUR on farm A. Slaughtered pigs have amounted to 79 kg of cooled side x 2.82 EUR price of a side, giving the value of 222.78 EUR, and when we take the price of pork meat (side) 95.70 EUR, we het the gain per livestock of 127.08 EUR/kg. It can be easily concluded that the price of a cooled side is 132.79 % bigger than the price of a fattened pig, which doesn't represent the usual relation on the market. Farm A achieved gain in the amount of 5,083.20 EUR. A calculation was also conducted of farm B in accordance with the yield of slaughtered pigs and the value of a fattened pig with the weight of 100 kg is 142 EUR + slaughter costs 8.7 EUR which amounts to 150.70 EUR, and since cooled pork meat side weighing 78 kg with the price of 2.82 EUR per kg, the value of the livestock (side) is 219.96 EUR. Slaughtering pigs they obtained pork meat sides with uniform mass, an average of 78 kg per livestock. Farm B realized a profit in the amount of 2,056,047 EUR. It can easily be concluded that the price of a cooled side is 45.96% bigger than the price of a fattened pig. We came to new scientific findings in the paper on the practical application of quality feeding in fattening pigs.

Advantages of such a method of raising pigs was examined and the scientific contribution to promoting development of producing quality pork meat, for which Serbia with its pig raising tradition has great geographic-ecological potentials, especially in its agricultural and livestock production. As a final conclusion of the research it can be recommended to pig breeding farms to organize production groups, cooperatives, clusters and francizes.

Conclusion

Based on the analysis of the state in agricultural-food production, specifically pork meat, we came to a conclusion when the approach in this type of production is in question. Another argument in the request for determining fattened pig price on the slaughter line is the given quality according to the share of meat, which is shown in the research on farm A, that it's best to close the entire production cycle from a field to fork. We primarily think on the market of fattened pigs, piglets and pork meat in Serbia in 2016. After the research, we answered a few very important questions in the paper, which are: the price level is not such that it stimulates pig breeders and the price influences production level. One of the reasons the there is a reduction and stoppage of slaughtering of big industries in the Republic of Serbia (facilities in Sombor, Subotica and Čoka). There is an expansion of big private slaughterhouses at the same time, which are far more flexible the industries, therefore their production is far more efficient. Pork meat consumption cyclically reduces due to relatively high prices and a decline in life standard.

Ekonomika proizvodnje mesa na svinjarskim farmama u Srbiji pri različitim sistemima uzgaoj

Ivan Mičić, Zoran Rajić, Jelena Živković, Grujica Vico, Marko Mičić, Ivana Mičić, Marija Mičić

Rezime

Studija se bavi analizom u razvoj proizvodnje svinjskog mesa u Srbiji, kao i ekonomska analiza tovljenika u proizvodnjim uslovimana na svinjogojskojskim farmama u Srbiji.. Istraživanje je obuhvatilo malu svinjogojsku farmu u "Gračanici" u centralnom delu Kosova i Metohije, i zadružnu farmu „1. Decembar“ u Žitorađi u Topličkom okrugu. U 2016. godini praćena je tehnologija proizvodnje tovljenika na obe farme, i analizirani su ekonomski rezultati u proizvodnji. Tokom posmatranog perioda, utvrđeno je da je na privatnoj farmi, uzgoj tovljenika bilo 60 grla, a na zadružnoj, farmi uzgoj tovljenika bio je 35.442 grla. Proizvedeni tovljenik od 100 kg na privatnoj farmi koštao je 82 eura, dok je svinjsko meso u polutama koštalo 1.10 eura/kg. Na Zadružnoj farmi, cena tovljenika od 100 kg koštala je 132 eura, dok je svinjsko meso u polutkama koštalo 1,84 euro/kg. Prosečna težina polutki bila je ujednačena oko 79 kg, dok je udeo svinjskih mesa u polutkama variro od 80% na privatnoj do 78% na zadružnoj farmi.

Ključne reči: razvoj, svinje, svinjske polutke, cena, ekonomska opravdanje

References

ANDRIĆ J. (1998): Troškovi i kalkulacije u poljoprivrednoj proizvodnji. Poljoprivrednifakultet – Zemun, Beograd.

Council Regulation (EEC) No 3220/84 of 13 November 1984 determining the Community scale for grading pig carcass, 1984. Official Journal ofthe European CommunitiesNo L 301,20/11/1984, No.1-3.

DŽINIĆ, N., PETROVIĆ, LJ., TOMOVIĆ, V., MANOJLOVIĆ, D., TIMANOVIĆ, S., VIDANRIĆ, D., KURJAKOV, N. (2004): Ocena kvaliteta polutki i mesa svinja rasa veliki jorkšir i švedski ladras. Biotehnologija u stočarstvu, Vol. 20, No. 1-2, 67-73.

VIDOVIĆ, V., KRNJAIĆ, J., LUKAČ, D**.,** VIŠNJIĆ, V., STUPAR, M. (2012): Growthintensity ofthe giltsfertile breedpigsinthe Nucleusfarm, Biotechnology in Animal Husbandry, Vol.28, No. 4, 787-796.

MIČIĆ I., UROŠEVIĆ D., VUJIĆ R., MIČIĆ I. I., MIČIĆ M., MIČIĆ I.M. (2016a): Operating costs of agricultural holdings with equal production possibilities. Economics of Agriculture Belgrade Serbia, Vol. LXIII, N^{o}2(353-740) EP 2016 (63) 2 pp.(407-428).

MIČIĆ I., RAJIĆ Z., ŽIVKOVIĆ D., NIKOLIĆ S., MIČIĆ I. I., MIČIĆ I.M. (2016b): Development strategy of economically justified pig meat production from farm to fork: agricultural economic analysis, ZAEM Association of Agricultural Economists of Republic of Macedonia, 10TH AAEM CONFERENCE, 148-155.

ŽIVKOVIĆ, D., PERUNOVIĆ, M. (2012): Poznavanje mesa, praktikum, Poljoprivredni fakultet, Univerzitetu Beogradu.

POSTER SECTION II

INFLUENCE OF GENOTYPES, AGE, NUTRITION AND INTERACTIONS GENOTYPE X NUTRITION ON MORTALITY OF BROILER CHICKENS

Nedeljka Nikolova[1], Dragoslav Kocevski[2], Rodne Nastova[1], Nikola Puvaca[3], Cyril Hrncar[4], Tosho Kostadinov[1]

[1]University "Ss. Cyril and Methodius", Insitute of Animal Science, Av. Ilinden 92/a 1000 Skopje, Macedonia
[2]University "Ss. Cyril and Methodius", Faculty of agriculture sciences and food, Av. Aleksandar Makedonski bb 1000 Skopje, Macedonia
[3]Centre for research and development in biotechnology, “BioTech” Zmaj Jovina 26 2100 Novi Sad, Serbia
[4]Slovak University of Agriculture in Nitra, Department of Poultry Science and Small Farm Animals,
Tr. A. Hlinku 2
949 76 Nitra, Nitranski Kray, Slovakia
Corresponding author: Nedeljka Nikolova, nikolova13@gmail.com
Original scientific paper

Abstract: The aim of this paper was to examine the influence of different genotypes, age, nutrition and interactions of genotype x nutrition, on mortality of broiler chickens. Research material was one-day chicken of Hubbard Classic and Cobb 500 genotypes, fattened 49 days in production conditions. The chickens were placed in 16 separate boxes with 150 birds in each box and used two tipe of nutrition. The first nutrition (mixture I) had higher level of energy and E:P ratio, while the second (mixture II) had higher level of proteins and lower values of E:P ratio in all phase of nutrition. The computer program STATISTICA 6 statistically processed all dates. Genotype had influence on mortality, chickens of Cobb 500 genotype had a statistically significant ($P<0.05$) higher total number and percentage of mortality by boxes, as well as a significantly higher number and percentage of mortality in the first week of age then the Hubbard genotype. The chickens fed with mixture I, had statistically significant ($P<0.05$) lower mortality then the chickens fed with mixture II, in all of broilers ages, and the chickens fed with mixture II had high mortality during the all of investigation period. The influence of mortality on genotype x nutrition interaction between examined treatments did not occur, only in the period from 35-42 day of fattening of broiler chickens, while in other periods there were statistically significant differences ($P<0.05$) related to mortality.

Key words*:* mortality, genotype, age, nutrition, broilers, interaction genotype x nutrition.

Introduction

The numerous studies of the influence of genotype, age and nutrition on the production characteristics of broiler chickens have confirmed the existence of statistical differences between the examined hybrids in aspect of their body weight, feed conversion, mortality, carcass weight, as well as the valuable parts of carcass, and percentage of abdominal fat.

Mortality is one of the main production traits in broiler fattening. According to many examinations, mortality is a low hereditary trait, so the efforts for its genetic improvement and selection practices have not yet shown a greater improvement of this production characteristic.

Salmon et al. (1983) determined the significant influence of different levels of proteins occurring in the starter and finisher on the mortality of male and female of some commercial broiler lines. Namely, in the starter with the highest amount of proteins, the highest level of mortality was observed, while the percentage of total mortality in chickens declined in the finisher with the decreased percentage of proteins.

Pavlovski et al. (1995) have been conducting experiments for a longer period of time concerning the production properties of broiler chickens belonging to different genotypes, and have reached the conclusion that the mortality and food conversion were similar in chickens from different genotypes.

Smith and Pesti (1998) have proven a high significance of the line and sex on the food intake in their research. The mortality of chickens was in average 3.6% on the 18th day and 10.5% on the 56th day of fattening.

The aim of the research performed by *Hopic et al. (1998)* was to examine the influence of the genotype and age in the most significant production traits. The genotype and age did not significantly affect on mortality of broiler chickens.

There is a connection between the early and late mortality in chickens, namely, the flock that lost many chickens during the early age will lose the same amount in the late age as well. Additionally, higher early mortality causes disturbances in the uniformity of flocks as well as disruption of the food conversion, which complicates the management of such flocks. *(Tabler et al., 2004).*

Corzo et al. (2005) compared the effects of the high and low-protein foods on the growth of different broiler lines. The broilers that were fed with a high-protein mixture had a greater body weight and lower food conversion in all ages. Mortality was not influenced by nutrition, line and sex.

Benyi et al. (2015) research showed that male chickens consumed more food, had better food conversion, better growth as well as weight, were heavier compared to the female ones at the 49th day of age, but in contrast had higher mortality. Significant interaction influence has been found between the genotype and sex in weight gain, weight estimated at the 49^{th} day of age, food consumption and mortality.

The aim of this research was to compare the influence of different genotypes, age and nutrition on the mortality of broiler chickens, as well as their genotype x nutrition interactions.

Materials and methods

The experiment was placed in a poultry farm with a floor system for fattening of broiler chicken, complete equipment and the necessary microclimate conditions for their conventional fattening. One-day chicks were used from the Cobb 500 and Hubbard hybrid lines. A total of 2400 birds were housed in 16 separated boxes with 150 chickens in each, fattened up to the 7^{th} week of age (49 days). Four different treatments were used (two genotypes and two types of nutrition) with four repetitions during one treatment. The chicks were fed *ad libitum* using two types of mixtures (mixture I and mixture II in table 1 and 2) different in their content of proteins, energy and ratio E:P (table 1 and 2).

Table 1. Chemical composition of mixture type I used for broiler chickens in the experiment

Chemical composition	Starter	Grover	Finišer 1	Finišer 2
ME, kcal/kg	3069,08	3197,20	3225,20	3212,30
Crude protein, %	23,03	22,04	21,06	19,20
Crude fiber, %	4,34	4,01	4,09	4,31
Crude fat, %	8,16	9,88	10,52	9,66
Ca, %	0,91	0,93	0,89	0,88
P (total), %	0,77	0,76	0,75	0,68
Metionin, %	0,61	0,69	0,58	0,64
Cistin, %	0,34	0,32	0,31	0,29
Metionin + Cistin,%	0,95	1.01	0,89	0,93
Lizin, %	1,30	1,32	1,10	1,00
Triptofan, %	0,32	0,30	0,28	0,26
Ratio: Energy/Protein	133,27	145,04	153,14	167,35

Table 2. Chemical composition of the mixture type II used for broiler chickens in the experiment

Chemical composition	Starter	Grover	Finisher 1	Finisher 2
ME, kcal/kg	3047,38	3107,70	3099,52	3100,58
Crude protein, %	23,54	22,55	22,02	21,95
Crude fiber, %	4,16	4,12	4,25	4,58
Crude fat, %	7,43	8,39	8,68	9,15
Ca, %	0,93	0,89	0,86	0,87
P (total), %	0,78	0,76	0,75	0,72
Metionin, %	0,74	0,72	0,58	0,68
Cistin, %	0,34	0,33	0,33	0,33
Metionin + Cistin,%	1,08	1.04	0,91	1,01
Lizin, %	1,51	1,42	1,27	1,27
Triptofan, %	0,32	0,30	0,30	0,30
Ratio: Energy/Protein	129,47	137,83	140,76	141,24

The first nutrition had higher level of energy and E:P ratio, while the second had higher level of proteins and lower values of E:P ratio in all phases of nutrition. The mixtures were given as a starter (during the first and second week), grower (during the third week), finisher 1 (weeks four to six) and finisher 2 (during the seventh week).

During the fattening, all of the production features were observed on a daily, weekly and monthly level. The mortality of chickens was controlled daily and estimated for every box. The received data bases are transferred into the computer program "Statistic" and in the subprogram "Basic Statistic" the mean-values and measures of variability are determined. In the ANOVA subprogram, variance analysis was performed in order to determine the characteristics with the highest statistically significant differences between the examined treatments. The influence of age on mortality was examined in eight treatments (days: 1, 7, 14, 21, 28, 35, 42 and 49 of age) so that the LSD test of 0.01% probability was used with the traits which showed statistical differences. The existence of interactions has been checked in chickens of the same age, by applying a variance analysis in the program Statistic - the subprogram ANOVA.

Results and Discussion

Mortality and genotype

In this paper, the mortality of chickens is represented as a number of dead chickens per box and as percentage (%) of dead chickens per box. The research included 16 boxes with 150 chickens which are used as a statistical unit during the variance analysis. The different nutrition factors and genotype are statistically analyzed with eight repetitions, while the influence of age is statistically tested with sixteen repetitions.

In table 3, the mortality of Cobb 500 and Hubbard chickens is shown during the research period.

Table 3. Mortality of broiler chickens Cobb 500 and Hubbard (number and percentage chickens per box)

Age (day)	Genotype				Statistically significant differences (F test)
	Cobb 500		Hubbard		
	X	SD	X	SD	
Mortality of chickens by number					
7	3,00	3,00	0,37	0,51	*
14	6,00	4,89	3,12	2,69	NS
21	1,50	1,41	1,75	1,49	NS
28	2,62	3,42	1,25	1,67	NS
35	2,25	2,49	0,62	0,51	NS
42	0,37	0,51	0,37	0,74	NS
49	0,50	0,75	0,37	0,51	NS
Total	16,25	12,90	7,87	4,18	*
Mortality of chickens percentage (%)					
7	2,00	2,00	0,25	0,34	*
14	4,11	3,37	2,09	1,79	NS
21	1,10	1,05	1,19	1,02	NS
28	1,98	2,62	0,87	1,19	NS
35	1,71	1,91	0,43	0,36	NS
42	0,27	0,37	0,26	0,51	NS
49	0,40	0,61	0,26	0,36	NS
Total	10,83	8,60	5,25	2,79	*

* - Statistically significant difference on level of 5% within the examined treatments
NS - Within the observed treatment does not exist statistically significant differences

A statistically significant difference in the number of dead chickens per boxes is visible from the hatching period until the 8th day of age. During this period, the Hubbard chickens had a significantly lower mortality rate, compared to the Cobb 500 chickens. Statistical significance appeared while examining the differences at the total level of the research. The Hubbard genotype had a significantly lower number of dead chickens per box (7,87) compared to the Cobb 500 chickens (16,25).

Statistically significant differences also occurred in the percentage representation of chickens mortality within their first week of age as well as the total percentage mortality of both genotypes. During this period, the Hubbard chickens (5,25%) experienced a lower percent of mortality then the Cobb 500 genotype (10,83%). In the remaining test periods, statistically significant differences were not found because of the small number of repetitions per treatment (eight boxes for every genotype) and the high variability of the traits being examined.

The variability of the mortality (number and % of dead chicks per box) is high in both genotypes, in almost every stage of the research. The variability expressed with the coefficient of variation resulted even above 100% in certain measurements. The mortality is a trait with high variability, so the received variations were expected. The high variability of mortality affected the non-fulfillment of statistically significant differences between the examined genotypes starting from the second until the seventh week of the fattening period.

Mortality and age

The lowest mortality rate is registered at the age of 35 to 42 and 42 to 49 days and it amounted 0,27% and 0,33% per box. Low mortality is also found in the period from the 28th to 35th day (1,07%), from hatching to the eight day (1,12%) and from the 14th to 21st day (1,15%). The highest mortality was observed during the period starting from the eight to fourteenth day and it amounted 3,10% (table 4). This mortality was statistically significant, while during the other adult weeks there were no statistically significant differences.

Table 4. Mortality of broiler chickens in different age (number and percentage chickens per box)

Age (day)	Mortality of chickens by number (1)		Mortality of chickens percentage (%) (2)		Statistically significant differences (Tukey test)	
	X	SD	X	SD	(1)	(2)
7	1,69	2,52	1,12	1,68	A	A
14	4,56	4,09	3,10	2,81	B	B
21	1,62	1,40	1,15	1,00	A	A
28	1,94	2,69	1,42	2,04	A	A
35	1,44	1,93	1,07	1,48	A	A
42	0,37	0,61	0,27	0,44	A	A
49	0,44	0,63	0,33	0,49	A	A

* - A-B - The average values in each column within the observed treatment are significantly different at the level of 5%

As with the examination of the genotype, a high variability has been established with the examination of mortality as well. The total mortality in the research is at a level of research results published in our country and abroad. The greater percentage of dead chickens in terms of technological norms is a result of research in production, and not in experimental conditions.

Mortality and nutrition

The chickens fed with mixture I, had lower mortality than the chickens fed with mixture II at all ages (table 5). Only during the period from the 35th to 42nd day, the number and percentage of dead chicks per box (0,37) was identical or similar (0,25% and 27%) in both groups of chicks.

Table 5. Mortality of broiler chickens fed with different mixtures (number and percentage chickens per box)

Age (day)	Mixtures				Statistically significant differences (F test)
	I		II		
	X	SD	X	SD	
Mortality of chickens by number					
7	0,62	0,74	2,75	3,24	NS
14	1,37	1,30	7,75	3,32	*
21	0,75	0,89	2,50	1,31	*
28	0,62	0,52	3,25	3,37	*
35	0,87	0,64	2,00	2,62	NS
42	0,37	0,51	0,37	0,74	NS
49	0,25	0,46	0,62	0,74	NS
Total	4,87	3,04	19,25	9,84	*
Mortality of chickens percentage (%)					
7	0,41	0,49	1,83	2,16	NS
14	0,92	0,87	5,28	2,30	*
21	0,51	0,60	1,80	0,92	*
28	0,42	0,35	2,43	2,56	*
35	0,60	0,44	1,54	2,00	NS
42	0,25	0,36	0,27	0,53	NS
49	0,17	0,32	0,49	0,59	NS
Total	3,25	2,02	12,83	6,55	*

* - Statistically significant difference on level of 5% within the examined treatments
NS - Within the observed treatment does not exist statistically significant differences

The chickens fed with mixture I experienced the highest death rate during the 8th to 14th day, with an established mortality of 0,92%, while the lowest mortality was registered within the period starting from the 35th to 49th day (0,17%

and 0,25%) from the raising. The chickens fed with mixture II had high mortality during the whole research period. The highest mortality was registered within the second week and it amounted 5,28%. A mortality rate below 1% was registered in the period from the 35th to 49th day (0,27 – 0,49%).

Taking into account the high variability of the mortality feature as well as the low number of repetitions after treatment, statistically significant differences were not found in other adult periods. The variability of mortality in different nutrition systems, as well as age and genotype, was quite high.

Interaction - genotype x nutrition

The mortality of chickens from different genotypes and nutrition is presented in table 6. The Cobb 500 chickens that were fed with mixture II had the highest mortality rate in the total period of research. The period from the 35th to 42nd day, when the highest mortality is noticed with the Hubbard chickens fed with mixture II, is an exception. From the 42nd to 49th day, no deaths occurred with the Cobb 500 chickens fed with mixture I and the Hubbard chickens fed with mixture II. The Hubbard chickens that were fed with mixture II had a lower death rate compared to the other groups that were examined during the first and fifth week of age. The Cobb 500 chickens, which were fed with mixture I, had a lower mortality as opposed to the other researched groups during the third and fourth week of age. The Hubbard chickens that were fed with mixture I, had a lower mortality then the remaining researched groups in the second and sixth week of age.

Table 6. Interaction genotype x nutrition in mortality of broiler chickens

Age/Influence	X	S.D.	Interactions
7. day			
Cobb 500-mixture I	0,50	0,64	b
Cobb 500-mixture II	3,50	1,83	a
Hubbard-mixture I	0,33	0,38	b
Hubbard-mixture II	0,17	0,33	b
Interaction genotype x nutrition		*	
14. day			
Cobb 500-mixture I	1,18	1,01	c
Cobb 500-mixture II	7,06	1,52	a
Hubbard-mixture I	0,67	0,77	c
Hubbard-mixture II	3,50	1,26	b
Interaction genotype x nutrition		*	
21. day			
Cobb 500-mixture I	0,17	0,34	c
Cobb 500-mixture II	2,04	0,36	a
Hubbard-mixture I	0,84	0,65	bc
Hubbard-mixture II	1,55	1,30	ab
Interaction genotype x nutrition		*	
28. day			
Cobb 500-hrana I	0,34	0,40	b
Cobb 500-hrana II	3,61	2,95	a
Hubbard-hrana I	0,51	0,34	b
Hubbard-hrana II	1,25	1,69	ab
Interaction genotype x nutrition		*	
35. day			
Cobb 500-mixture I	0,51	0,66	b
Cobb 500-mixture II	2,90	2,08	a
Hubbard-mixture I	0,68	0,01	b
Hubbard-mixture II	0,18	0,36	b
Interaction genotype x nutrition		*	
42. day			
Cobb 500-mixture I	0,34	0,39	NS
Cobb 500-mixture II	0,20	0,40	NS
Hubbard-mixture I	0,17	0,35	NS
Hubbard-mixture II	0,35	0,69	NS
Interaction genotype x nutrition		NS	
49. day			
Cobb 500-mixture I	0,00	0,00	b
Cobb 500-mixture II	0,81	0,66	a
Hubbard-mixture I	0,35	0,40	ab
Hubbard-mixture II	0,00	0,00	b
Interaction genotype x nutrition		*	

* - Statistically significant difference on level of 5% within the examined treatments
NS - Within the observed treatment does not exist statistically significant differences

Statistically significant differences concerning mortality between the researched treatments did not occur only within the 34th and 42nd day of fattening of the broiler chickens, while during the remaining periods; statistically significant differences connected with the mortality feature appeared. The Cobb 500 chickens, which were fed with mixture II had significantly higher mortality than the remaining researched groups during the period beginning with the first to seventh, as well as 28th to 35th day of experimentation. During days 14 to 21 and 21 to 28, the Cobb 500 chickens fed with mixture II had a significantly higher mortality compared to the Cobb 500 chickens, that were fed with mixture I and the Hubbard chickens, also fed with mixture I, but a statistical significance did not appear when compared to the Hubbard chickens fed with mixture II. At the age of 7 to 14 days, the Cobb 500 chickens, which were fed with mixture II, had a significantly higher mortality than the remaining researched groups, while the Hubbard chickens, fed with mixture II, had a significantly higher mortality than the Cobb 500 chickens and the Hubbard ones that were fed with mixture I.

The Hubbard chickens, which were fed with mixture II had a significantly higher mortality rate then the Cobb 500 chickens that were fed with mixture I during the 14th to 21st day of fattening. The Cobb 500 chickens fed with mixture II had a significantly higher mortality than the Cobb 500 chickens, which were fed with mixture I as well as the Hubbard chickens fed with mixture II from the 42nd to 49th day of age.

Mortality is a trait with an exclusively high variability. The small number of repetitions per treatment and the high mortality of the chickens that belong to the Cobb 500 hybrid line, which were fed with mixture II that contained higher level of proteins and lower values of E:P ratio in all phases of nutrition, affected the high variability in this research. In such statistic conditions, it was expected that statistically significant differences in mortality between the researched groups should not be present. However, the high mortality that occurred with the chickens from the Cobb 500 provenience, which were fed with mixture II, conditioned the largest number of statistically significant differences to occur when comparing this group of chickens with others during the research.

The genotype Cobb 500 had statistically and significantly higher ($P<0.05$) total number and percentage of dead chicks per boxes, as well as a significantly ($P<0.05$) higher number of dead chickens during their first week of age in relation to the Hubbard genotype, while in the remaining weeks of the fattening there were no statistically significant differences. The genotype factor did not have a significant influence on the mortality of chicks in the works of *Hopic et al. (1998)* and *Kapetanov et al. (1998)*, who have determined a fairly high variability in mortality, which is probably the reason for the failure to make a significant difference. *Farran et al. (1995, 2000)* and *Silversides et al. (1997)* did not find significant differences regarding food conversion and mortality in chicks with a different genotype, they state that the reason for this is the low number of repetitions per tretment. *Locniskar et al. (1982)* determined insignifficant differences in mortality and food conversion on the basis on numerous researches on the production traits in broiler chicks of a different genotype. Statistically significant differences in mortality were not established neither by *Bhardway and Mohapatra (1996).*

The influence of genotype on mortality and food conversion has been largely examined under various nutrition conditions. Statistically significant differences between genotypes in literature, occur fairly often connected with food conversion than mortality *(Bigili et al., 1992).* In this study, the previous finding was not confirmed, on the contrary, the genotype had a statistically significant influence on the production trait, mortality, while in the food conversion, the genotype was non-significant, which can be explained by a sufficient number of repetitions in the examination of this productive trait.

The chickens fed with nutrition I (with higher level of energy and E:P ratio) had significantly lower mortality then the chickens fed with mixture II (with higher level of proteins and lower values of E:P ratio) at all ages. The chickens fed with mixture II had higher mortality during the total research. Statistically significant differences ($P<0.05$) occurred from day 14 to 28 of age, as well as in the total number and percentage of dead chickens per box. Mixture II had a continuously higher level of proteins at all stages of the nutrition compared to mixture I, which is related to the occurrence of a somewhat higher mortality in chickens. In the research of *Salmon et al. (1983)* it is proved that the increase in total mortality was related to the high protein level in the starter mixture. However, the high protein level in the finisher caused a linear decrease in total mortality.

Mortality was significantly ($P<0.05$) under the influence of the interaction genotype x nutrition in all ages except in the sixth week of age of broiler chicks. Chickens from the Cobb 500 genotype had a generally higher mortality than the Hubbard chicks in both nutrition systems (mixture I and mixture II), although it can be concluded that the mortality was statistically and significantly higher with the nutrition containing mixture II.

The interactions genotype x nutrition do exist. However, they are responsible for no more than 2% of the total variations. In this manner we illustrate that the absolute influence of the interaction genotype x nutrition is rather weak, but it should be applied in most analysis.

In view of the fact that broiler production is made by different hybrid lines grown in different nutrition systems, it is necessary and understandable to undertake new researches regarding the interactions genotype x nutrition in order to ensure better understanding of this issue.

Conclusion

Regarding the research carried out as a means to determine the influence of mortality of broiler chickens on the genotype, age, nutrition as well as the possible interactions, the following conclusions were obtained:

- The chickens belonging to the genotype Cobb 500 had a statistically and significantly higher ($P<0.05$) total number and percentage of dead chickens estimated per boxes, as well as a significantly higher number and percentage of dead chickens in their first week of age (days one to seven) as opposed to the Hubbard genotype.

- The chickens fed with mixture I, which had higher level of energy and E:P ratio, had statistically significant ($P<0.05$) lower mortality compared with the chickens fed with mixture II, which on the other hand had higher level of proteins and lower values of E:P ratio in every adult category. The chickens fed with mixture II had high mortality during the total research.

- Significant influences of the mortality on the interaction genotype x nutrition between the researched treatments did not occur only in the period from 35-42 day of fattening of broiler chickens; statistically significant ($P<0.05$) differences connected with the mortality feature were present within the remaining periods.

Uticaj genotipova, starosti, ishrane i interakcije genotip x ishrana na smrtnost brojlerskih pilića

Nedeljka Nikolova, Dragoslav Kocevski, Rodne Nastova, Nikola Puvaca, Cyril Hrncar, Tosho Kostadinov

Rezime

Cilj ovog rada je bio da se ispita uticaj različitih genotipova, starosne dobi, ishrane i interakcije genetop x ishrana, na smrtnost brojlerskih pilića. Istraživački materijal su bili jednodnevni pilići Hubbard Classic i Cobb 500 genotipova, uzgajanih 49 dana u proizvodnim uslovima. Pilići su smešteni u 16 odvojenih bokseva sa 150 ptica u svakom boksu i koristili su dva tipa ishrane. Prvi tip hrane (smeša I) imala je viši nivo energije i odnos E : P, dok je drugi (smeša II) imao veći nivo proteina i niže vrednosti odnosa E: P u svim fazama ishrane. Računarski program STATISTICA 6 je korišćen za statističku obradu svih podataka. Genotip je uticao na mortalitet, pilići genotipa Cobb 500 imali su statistički značajan (P<0,05) veći ukupan broj i procenat smrtnosti po boksevima, kao i znatno veći broj i procenat smrtnosti u prvoj nedelji, a zatim Hubbard genotip. Pilići hranjeni smešom I, imaju statistički značajnu (P<0.05) nižu smrtnost, a pilići hranjeni smešom II, u svim starosnim dobima broilera, a pilići hranjeni smešom II imali su visoku smrtnost tokom celog istraživanja. Uticaj smrtnosti na interakciju genotip x ishrana, između ispitivanih tretmana, nije zabeležen, samo u periodu od 35-42 dana tova brojlerskih pilića, dok su u drugim periodima statistički značajne razlike (P <0,05) povezane sa mortalitetom.

Ključne reči: mortalitet, genotip, starost, ishrana, brojleri, interakcija genotip x ishrana.

References

BENYI K., TSHILATE T.S., NETSHIPALE A.J., MAHLAKO K.T. (2015): Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. Trop Anim Health Prod. 2015 Oct; 47(7):1225-31.
BHARDWAY J.K., MOHAPATRA S.C. (1996): Influence of sex, genotype, density and their interactions on broiler performance traits. XX World's Poultry Congress, New Delhi, India, 2-5 September 1996, IV, 100.
BIGILI S.F., MORAN E.T., ACAR N. (1992): Strain-cross response of heavy male broilers to dietary lysine in the finisher feed: Live performance and further processing yields. Poultry Sci., 5, 850-858.
CORZO A., KIDD M.T., BURNHAM D.J., MILLER E.R., BRANTON S.L., GONZALEZ-ESQUERRA R. (2005): Dietary amino acid density effects on growth and carcass of broilers differing in strain cross and sex. Jour. of Applied Poultry Research 14, 1-9.
FARRAN M.T., KHALIL R.F., UWAYJAN M.G., HAJJ R.N., ASHARIAN V.M. (2000): Performance and carcass quality of commercial broiler strains. J Appl. Poultry Res. 9:252–257.
FARRAN M.T., UWAYJAN M.G., KHALIL R.F., ASHARIAN V.M. (1995): Comparative performance and carcass composition of three sexed broiler strain. 16th Annual Meeting of Southern Poultry Science Society, Poultry Sci., Abstracts, S-45, 189.

HOPIC S., PAVLOVSKI Z., CMILJANIC R., VRACAR S., SKRBIC Z., LUKIC M. (1998): Uticaj genotipa i godine na proizvodne osobine brojlerskih pilića. Nauka u živinarstvu, 1-2, 45-51.

KAPETANOV R., TESIC M., PALIC T., OGLIC D., MASIC Z., KAPETANOV M. (1998): Analysis of broilers mortality structure and its causes at cooperatives farms in the period of economical sanctions. 10th European Poultry Conference, Jerusalem, Israel, June 21-26, 1998, Abstracts, 82.

LOCNISKAR F., POHAR J., HOLCMAN A., KMECL A. (1982): Selekcija živine. Peradarstvo, 6, 17-21.

PAVLOVSKI Z., CMILJANIC R., MASIC B., DZURDZEVIC Z., HOPIC S. (1995): Uticaj produženog tova pilića na osobine brojlera sa posebnim osvrtom na kvalitet mesa. Tehnologija mesa, 2-3, 93-95.

SALMON R.E., CLASSEN H.L., McMILAN R.K. (1983): Effect of starter and finisher protein on performance, carcass grade and meat yield of broilers. Poultry Sci., 62, 837-845.

SILVERSIDES F.G., LEFRANCOIS M.R., VILLENEUVE P. (1997): The effect of strains of broiler on physiogical parameters associated with the ascites syndrome. Poultry Sci., 5, 663-667.

SMITH E.R., PESTI G.M. (1998): Influence of broiler strain cross and dietary protein on the performance of broilers. Poult. Sci., 77, 276-281.

TABLER G.T., BERRY I.L., MENDENHALL A.M. (2004): Mortality Patterns Associated with Commercial Broiler Production. University of Arkansas's Avian Advice, Spring 2004 - Volume 6, Number, http://www.thepoultrysite.com/articles/253/mortality-patterns-associated-with-commercial-broiler-production .

OFFERING OF SUCROSE IN THE DRINKING WATER AND ITS EFFECT ON THE MAJOR ZOOTECHNICAL PARAMETERS IN MALE LAYER-TYPE CHICKENS

Evgeni Petkov[1]*, Maya Ignatova[1,2], Teodora Popova[1]

[1]Institute of Animal Science, 2232 Kostinbrod, Bulgaria
[2]Agricultural Academy, 1373, Sofia, Bulgaria
Corresponding author:Evgeni Petkov, mechovet@abv.bg
Original scientific paper

Abstract: The study was carried out in the experimental poultry farm of the Institute of Animal Science-Kostinbrod, Bulgaria with two groups of male layer-type chickens, each containing 50 birds of the Lohmann Brown Classic hybrid. The chickens were reared in deep litter with controlled parameters of the microclimate. Both groups were fed *ad libitum* starter, grower and finisher diets, as the chickens of the experimental group received sucrose added to the drinking water in amount 50g/l. The intake of the sucrose solution and feed was recorded daily and weekly respectively. The results of the experiment showed that the addition of sucrose to the drinking water of male layer-type chickens had positive effect on the live weight of the birds from 6 until 12 weeks of age. Furthermore, the feed intake and the utilization per kg weight gain were decreased (P<0,001), while weight gain was increased in the group receiving sucrose, mostly on the 9th, 10th and 11th weeks of the trial (P<0,001).

Keywords: male layer-type chickens, sucrose, zootechnical parameters

Introduction

Slow growing male layer-type chickens need higher energy levels in feed so that their rearing becomes more efficient (*Morris and Njuru, 1990*). According to *Hocking et al.* (*2001*) such nutritional requirements could be explained by the type of the nervous system in these birds and its influence on their metabolism. Negative effect of the intake of easily utilized carbohydrates during stress has been mentioned in the manual of Lohmann-Brown Classic. According to *Morris and Njuru (1990)* and *John (2008)* such effect is a function of the preference of the chickens to eat the easily absorbable additives rich in energy (sucrose). The negative influence does not exist when the chickens are reared in controlled conditions due to the lack of stress, and the part of the total energy intake ensuring the process of digestion in the birds decreases (*John, 2008;Faber et al., 2012*). Chickens spend between 25-75% of the total energy intake of feed to ensure the digestion according to *Morris and Njuru* (1990). Research show that addition of sugar into the drinking water in controlled conditions has positive effect on the major productive parameters in birds (*Faber et al., 2012; Hashim et al., 2013; Ahmed, 2015; Hussein et al., 2016*).

As stated by *Morris and Njuru* (*1990*), in order to maintain their live weight, birds with slower growth need 1/3 higher levels of metabolizable energy compared to the commercial fast growing broilers. According to *John* (*2008*), at least 60-65% of the metabolizable energy should be supplied by carbohydrates such as sucrose rather than fats. Also, *Thurman* (*2010*) pointed that in order to obtain the necessary energy, the organism of birds utilizes preferentially the carbohydrates in the diet as such protection of the fats by carbohydrates is mentioned by *Hashim et al.* (*2013*).

The increase of the energy in the feed can be done through addition of different high energy components such as animal fats, vegetable oils and carbohydrates, as described in experiments from hot climate countries (*Hashim et al., 2013; Ahmed, 2015; Hussein et al., 2016*). According to *Peebles et al.* (*1999*), fats added in amounts of 5.5 % or more are not as efficient as an energy source and their higher concentrations make the feed absorption difficult. The partial replacement of fats by carbohydrates as an energy source in the drinking water of birds (*Faber et al., 2012*) favors and improves their absorption in the organism (*Bach and Babayan, 1982*). The administration of sucrose to the water appeared to have stronger positive effect compared to the feed where the main energy source are fats (*Chamberlain et al., 1993*). Such positive influence has been found by *Faber et al.* (*2012*) in 10 days old broiler chickens receiving 2-4% sucrose in the drinking water. On the other hand, *Thurman* (*2010*) observed similar results after administration of sucrose and maltose to the feed. Hence, the aim of this work is to assess the effect of the intake of carbohydrates in the drinking water on the productive performance in male layer type chickens.

Material and methods

The experiment was carried out in the poultry farm of the Institute of Animals Science- Kostinbrod with male Lohmann Brown Classic chickens divided in two groups each containing 50 birds. The 1-day old chickens of both groups were individually marked and weighed. The birds were kept on a deep litter facility with controlled parameters of microclimate responding to the requirements of Council Directive 2007/43/EC.

Both groups were fed starter (ME 3160 kcal/kg, protein content 19,00%), grower (ME 2950 kcal/kg, protein 21,00%) and finisher diet (ME 3048 kcal/kg and protein 21,00 %). The birds received their feed *ad libitum* and the sucrose was added to the drinking water of the experimental group in amount 5% (50 g/l). The parameters studied included feed intake (g/bird), metabolizable energy intake (kcal/kg), average live weight (g), average weight gain (g), feed utilization per kg of the average weight gain (g/kg), metabolizable energy per kg weight gain (kcal/kg). Data were statistically evaluated through SPSS v.19 software package.

Results and discussion

The control group had higher feed intake compared to the treated one as a consequence of the addition of sucrose in the drinking water (Fig. 1). No such trend, however, was observed at 5th, 8th, 10th and 11th week of the trial.

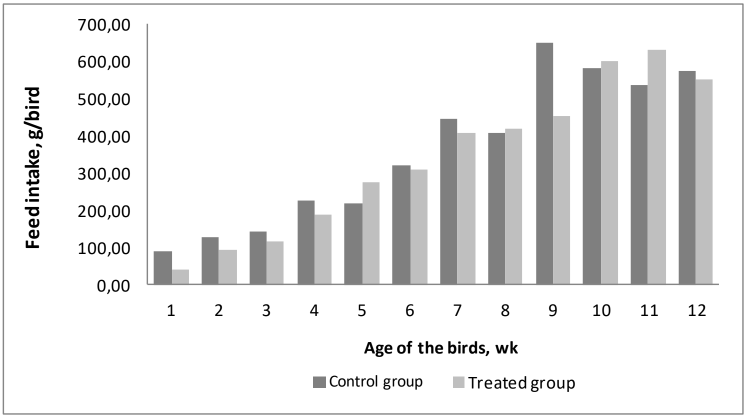
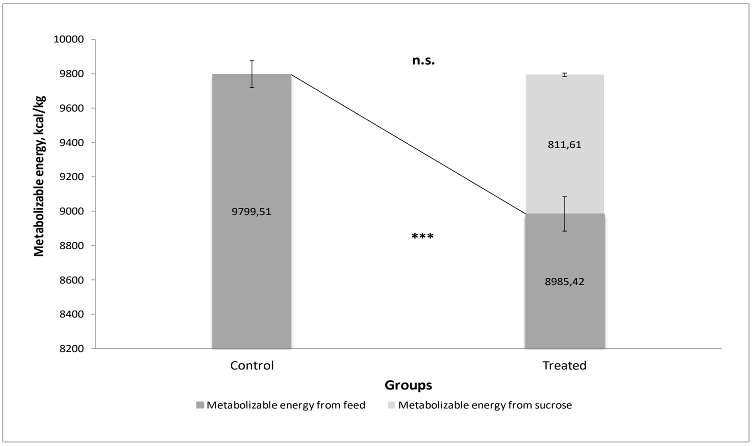


Figure 1. Feed intake (g/bird) in male layer-type chickens

The feed intake could be negatively affected after the 4th week of age by the stress of the transition from one type of feed to another (*Morris and Njuru, 1990*), accompanied by decreased feed intake of the second type of feed during the first days. This, however, was not observed in the treated birds in our experiment. The available literature does not provide data about the effect of the offered carbohydrates on the stress caused by the transition between feeds, but evidence exist about the positive influence of this transition (*Faber et al., 2012*). Some researchers (*Lichovnikova et al., 2009; Wang et al., 2009*) confirmed abnormalities in the curve concerning the feed intake at the 7th week of age, explaining this by the hormonal changes in the male chickens related to the male sexual hormones and the formation of the secondary sexual traits (*Onagbesan et al., 2006*) as well as the competition among the males (*Hocking et al., 2001*). Similar results have been reported also for later age (*Onagbesan et al., 2006; Hocking et al., 2001*).

The total metabolizable energy intake was the same for both groups, however the final total intake of metabolizable energy supplied by the feed (Fig.2) was lower in the treated group (8 985 kcal/kg live weight), in comparison to the control chickens (9 800 kcal/kg live weight).

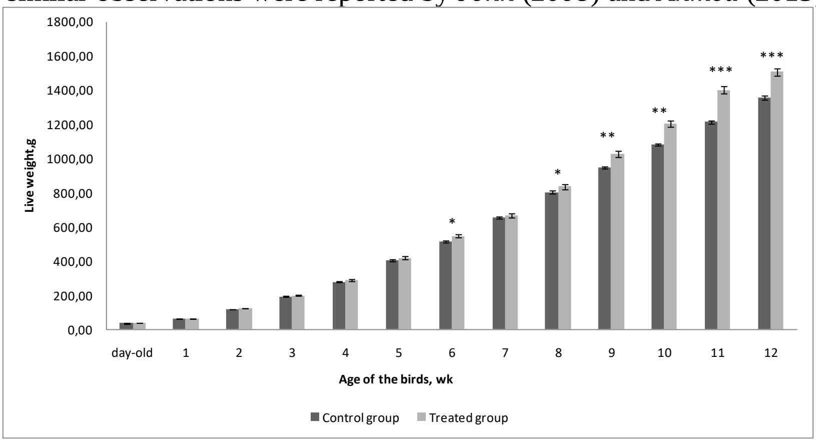


*** P<0,001; n.s.- non-significant

Figure 2. Metabolizable energy intake in male layer-type chickens

Faber et al. (*2012*) also observed decrease in the amount of the energy consumed with feed which was due to 3,2 % decrease in feed intake. According to *John* (*2008*) the lower feed intake was observed when sucrose solutions with different concentration were given to birds.

During the trial period the group receiving sucrose in the water displayed higher average live weight (Fig. 3) compared to the control birds, with the only exception registered at the first week of age (62,86 *vs*. 64,22 g, for the birds receiving sucrose and the control group respectively). However, these differences remained insignificant during the first 5 weeks, and also on the 7th week of the trial. The most considerable differences between groups were observed in the 9th (P<0,01), 10th (P<0,01), 11th (P<0,001) and 12th week (P<0.001). According to *Lott et al*. (*1992*) the higher live weight is due to the increased energy content of the diet, as similar observations were reported by *John* (*2008*) and *Ahmed* (*2015*).

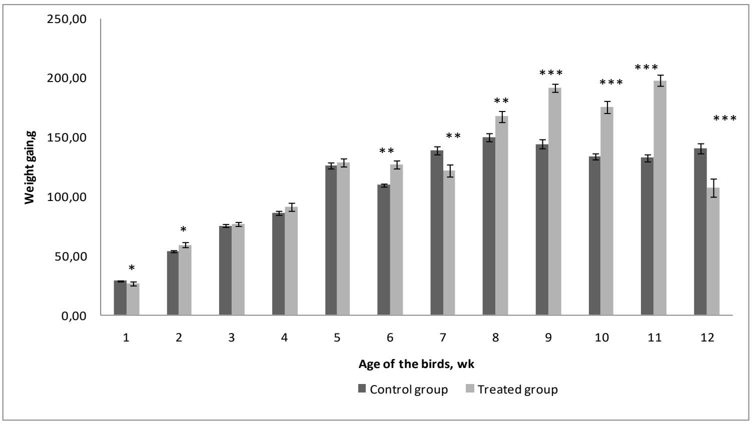


*P<0,05; **P<0,01; ***P<0,001

Figure 3. Changes in the average live weight of male layer-type chickens in response to the addition of sucrose to the drinking water

Our results are in line with those presented by *Turner et al.* (*1999 a,b*). According to these authors, this is not of critical importance and is normal in view of the considerable difference in the average live weight and the close values of the feed utilization per kg live weight. Such tendency is confirmed by *Batal and Parsons* (*2004*) and *John* (*2008*) in an experiment with different concentrations of sugar syrup replacing water, and also by *Ahmed* (*2015*).

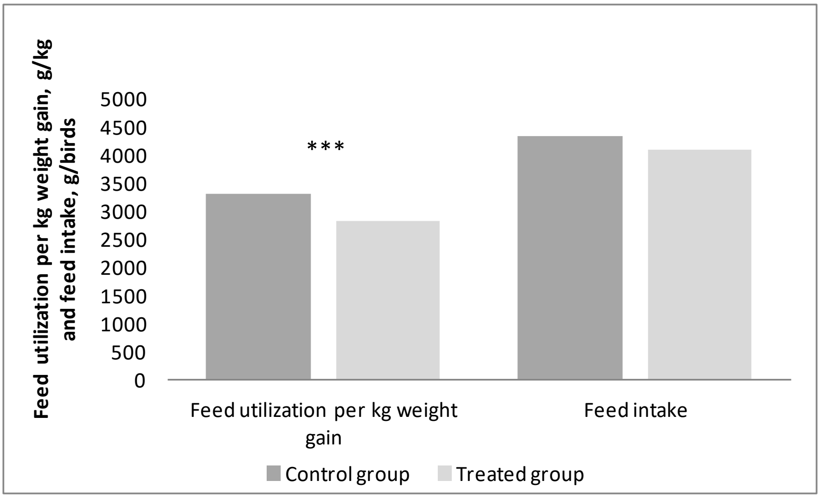
With the exception of the 1-st, 7th and 12th week of age, the weight gain was higher in the treated group when compared to the control (Fig. 4). The most considerable were the differences between both groups observed during the last four weeks of the trial period (P<0.001). *Turner et al.* (*1999 a,b*) reported increase in the weight gain in the first two weeks after hatching in chickens receiving 3,5% dextrose in feed. According to *Batal and Parsons* (*2004*) the addition of carbohydrates during the first three weeks increased considerably the weight gain for this period in broilers. On the other hand, *Hussein et al.* (*2016*) did not observe any significant difference between birds fed graded sugar syrup and control group. It could be suggested, that the lower weight gain in the group receiving sucrose that we registered in the 7th and 12th weeks of age might be due to the physiological changes that the organism of the male chickens undergoes and consequently reduced feed intake (*Tyler and Gous, 2008*).



** P<0,01; ***P<0,001

Figure 4. Changes in the weight gain in male layer-type chickens in response to the sucrose in the drinking water

The result of these physiological changes was expressed by the big differences in the mean values of the weight gain, but with an opposite tendency between both studied groups (at the age of 6 and 7 weeks), which is a consequence not of a higher energy intake but of decreased feed intake during the same weeks (6 weeks of age- 309g for the treated group, and 321g for the control; 7 weeks – 407 g and 441g for the birds receiving sucrose and the control group respectively), which caused decreased protein intake from the feed. This limited the growth during the age when increased energy needs were normal due to formation of hierarchy in the flock as stated by *Hockin et al. (2001*). Similarly according to *Morris and Njuru* (*1990*) the higher energy intake with feed blocked the protein utilization and retarded the growth in the chickens.

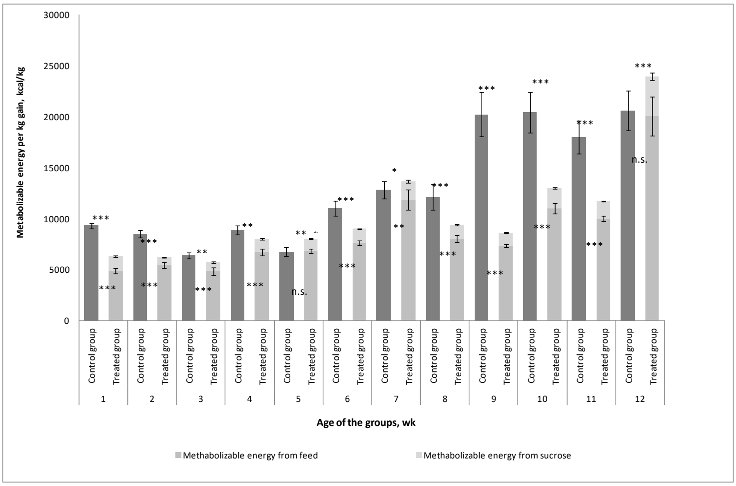


***P<0,001

Figure 5. Feed utilization per kg weight gain in male layer-type chickens as affected by the sucrose intake

At the end of the trial period we found considerable difference in the feed utilization per kg weight gain between the two groups (Fig.5). The values of the feed utilization per kg weigh and feed intake are higher in the birds of the control group that those receiving sucrose – 3291,69±27,05 g/kg vs. 2811,76±45,68g/kg and 4331g/bird vs. 4096g/bird for the feed utilization and feed intake respectively. Such differences in the feed intake were observed by *John* (*2008*), who explained that the birds correct the energy first (*Morris and Njuru,1990*) and also that as an energy source the birds utilize first the simple carbohydrates since they are easily absorbed (*John, 2008; Hashim et al., 2013*). Such trends were reported by *Turner et al.* (*1999a,b*) and *Batal and Parsons* (*2004*).

During the experiment we observed considerably lower metabolizable energy intake from the feed and total energy intake from both sources (feed and sucrose). Feed intake was lower in the treated group compared to the control. In line with us, *Faber et al. (2012)* also observed and explained this as a characteristic of the easily absorbed carbohydrates which can fast and easily supply energy for the chicken organism during the first weeks when the chicken are in transition from yolk feeding to using of gastro-intestinal tract. During the first two weeks, we could clearly notice big difference due to this transition. In the course of the rearing these discrepancies decreased more and more (Fig. 6). This could be explained with considerable differences in the feed intake during the first week of the birds decreasing with the age (50,04 g/bird to 36,76 g/bird respectively for the first and 4th week of the trial). Such trends were observed in other research as well (*John, 2008; Faber et al., 2012; Ahmed, 2015*).



** P<0.001; *** P<0.001; n.s. – non-significant, comparisons are made between control and treated group in regard to metabolizable energy from feed and from sucrose

Figure 6. Metabolizable energy per kg weight gain in male layer-type chickens

As a whole, the tendency during the rest 8 weeks of the trial period showed that the total metabolizable energy intake in the control group increased compared to the treated group except in the birds at 5, 7 and 12 weeks of age. These exceptions were due to stress factors such as changes in the feed after 4 weeks of age (*Morris and Njuru, 1990; Hocking et al., 2001*) and the normally observed hormonal peaks at the age of 7 and 12 weeks (*Onagbesan et al., 2006; Hocking et al., 2001*). Such peaks were registered in other experiments at the age of 4 weeks, between 6 and 8 weeks and after 11 weeks (*Morris and Njuru, 1990; Fanatico et al., 2008; Wang et al., 2009; Lichovnicova et al., 2012*). Negative impacts might be due to the features of the nervous system in birds observed by *Hocking et al. (2001)*, combined with the natural stress factor such as hormonal changes (6-8 weeks and after 10 week of age). In addition, during rearing the changes in the type of the feed consequently during with the age (4 weeks of age) as stated by *Onagbesan et al. (2006)* are also a main reason for neutralization of the positive effect of the carbohydrates on the energy balance in the chicken organism (*Morris and Njuru, 1990; Hocking et al., 2001*).

Conclusions

The results of the study showed that the addition of sucrose in the drinking water of male layer-type chickens had a significant positive effect on the average live weight after 6 weeks of age. Furthermore, at the end of the trail period decreased the feed intake and feed utilization per kg/live weight in birds but increased their weight gain mostly at 9th, 10th and 11th weeks of age.

Dodatak saharoze u vodu za piće i njen uticaj na glavne zootehničke parametre kod muških pilića lakog tipa

Evgeni Petkov, Maya Ignatova, Teodora Popova

Rezime

Studija je sprovedena na eksperimentalnoj farmi živine Instituta za stočarstvo - Kostinbrod, Bugarska sa dve grupe muških pilića lakog tipa. U svakoj grupi je bilo 50 grla hibrida Lohmann Brown Classic. Pilići su odgajani na dubokoj prostirci sa kontrolisanim parametrima mikroklime. Obe grupe su bile hranjene ad libitum starter, grover i finišer obrocima, a pilići eksperimentalne grupe su dobijali saharozu koja je bila dodata u vodu za piće u količini od 50 g/l. Unošenje saharoznog rastvora i hrane se beležilo dnevno i nedeljno. Rezultati eksperimenta pokazali su da dodavanje saharoze u vodu za piće muških pilića lakog tipa je pozitivno uticalo na živu težinu ptica od 6 do 12 nedelja starosti. Osim toga, konzumiranje hrane i njeno iskorišćenje po kg povećanja telesne težine je smanjeno ($P < 0,001$), dok je prirast telesne mase povećan u grupi koja je primila saharozu, uglavnom u 9-toj, 10-toj i 11-oj nedelja ispitivanja ($P < 0,001$).

Ključne reči: muški pilići lakog tipa, saharoza, zootehnički parametri

Acknowledgements

We would like to thank Mr. Ivailo Galabov of “Bulagro-97” for the free experimental material (1 day old male Lohmann Brown Classic chickens) needed for the study.

References

AHMED S. Y. (2015): Growth and water intake of boiler chickens offered sucrose in the drinking water and the effect of nutrient density under hot arid conditions, MSc Thesis, University of Khartoum, Faculty of Animal Production, Department of Poultry Production.

BACH A. C., BABAYAN V. K. (1982): Medium-chain triglycerides: An update. American Journal of Clinical Nutrition, 36, 950–962.

BATAL A. B., PARSONS C. M. (2004): Utilization of various carbohydrate sources as affected by age in the chick. Poultry Science, 83, 1140-1147.

CHAMBERLAIN D. G., ROBERTSON S., CHOUNG. J. J. (1993): Sugars versus starch as supplements to grass silage: Effects on ruminal fermentation and the supply of microbial protein to the small intestine, estimated from the urinary excretion of purine derivatives in sheep. Journal of Science of Food and Agriculture, 63, 189–194.

COUNCIL DIRECTIVE 2007/43/EC of 28 June 2007 laying down the minimum rules for the protection of chickens kept for meat production. Official Journal of the European Union, L182/19-28.

FANATICO A. C., PILLAI P. B., HESTER P. Y., FALCONE C., MENCH J. A., OWENS C. M., EMMERT J. L. (2008): Performance, livability, and carcass yield of slow- and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poultry Science, 87, 1012-1021.

FABER T. A., DILGER R. N., IAKIVIAK M., HOPKINS A. C., PRICE N.P., FAHEY JR. G. C. (2012): Ingestion of a novel galactoglucomannan oligosaccharide-arabinoxylan (GGMO-AX) complex affected growth performance and fermentative and immunological characteristics of broiler chicks challenged with Salmonella typhimurium. Poultry Science, 91, 2241–2254.

JOHN P. G. K. (2008): Sugar syrup: A new energy feed for poultry. World Poultry, 24, 12–13.

HASHIM I. B., HUSSEIN A. S., AFIFI H. S. (2013): Quality of breast and thigh meats when broilers are fed rations containing graded levels of sugar syrup. Poultry Science, 92, 2195-2200.

HUSSEIN A. S., GHURAIR J. A., JOHN P.G. K., HOSAM M. H., SULAIMAIN M. (2016): Graded levels of sugar syrup in broiler rations and its effect on growth performance and blood biochemical parameters. Animal Nutrition, 2, 180-185.

HOCKING P. M., MAXWELL M. H., ROBERTSON G. W., MITCHELL M. A. (2001): Welfare assessment of modified rearing programmes for broiler breeders. British Poultry Science, 42, 424-432.

IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

LICHOVNIKOVA M., JANDASEK J., JUZL M., DRACHKOVA E. (2009): The meat quality of layer males from free range in comparison with fast growing chickens; Czech Journal of Animal Science, 54, 490–497.

LOTT B. D., DAY E. J., DEATON J. W., MAY J. D. (1992): The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poultry Science, 71, 618–624.

MORRIS T. R., NJURU D. M. (1990): Protein requirements of fast- and slow-growing chicks. British Poultry Science, 31, 803–809.

ONAGBESAN O. M., METAYER S., TONA K., WILLIAMS J., DECUYPERE E., BRUGGEMAN V. (2006): Effects of genotype and feed allowance on plasma luteinizing hormones, follicle-stimulating hormones, progesterone, estradiol levels, follicle differentiation, and egg production rates of broiler breeder hens. Poultry Science, 85, 1245–1258.

PEEBLES E.D., DOYLE S.M., PANSKY T., GERARD P.D., LATOUR M.A., BOYLE C.R., SMITH T.W. (1999): Effects of breeder age and dietary fat on subsequent broiler performance. 2. Slaughter yield. Poultry Science, 78, 512-515.

TURNER K.A., APPLEGATE T.J., LILBURN M.S. (1999a): Effects of feeding high carbohydrate or high fat diets. 1. Growth and metabolic status of the posthatch poult following immediate or delayed access to feed. Poultry Science, 78, 1573-1580.

TURNER K. A., APPLEGATE T. J., LILBURN M.S. (1999b): Effects of feeding high carbohydrate or fat diets. 2. Apparent digestibility and apparent metabolizable energy of the posthatch poult. Poultry Science, 78, 1581-1587.

THURMAN K. (2010): A new carbohydrate concept for broilers. http://www.allaboutfeed.net/Home/General/2010/11/A-new-carbohydrate-concept-for-broilers-AAF011531W/

TYLER N. C., GOUS, R.M. (2008): The effect of constant photoperiod on testis weight and the use of comb area to predict testis weights in broiler breeder males. South African Journal of Animal Science, 38, 153-158.

WANG K. H., SHI S.R., DOU T.C., SUN H. J. (2009): Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. Poultry Science, 88, 2219–2223.

THE INFLUENCE OF THE ADDITION OF NETTLE IN BROILER FOOD AT THE FINAL STAGE OF THE FATTENING

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

Institute for Animal Husbandry, Autoput 16. P. Box 23, 11080 Belgrade-Zemun, Serbia
Corresponding author: miloslukic.izs@gmail.com
Original scientific paper

Abstract: The aim of the research is to determine the influence of dried and fresh nettle, as an alternative nutrient added to food for broilers in the last week of fattening, on the production results and slaughter quality of the product. The trial was performed on a total of 600 ROSS 308 one - day chickens of both sexes, divided into 3 groups (C - Control group; FN - Fresh Nettle group; DN - Dry Nettle group), and each group with 4 repetitions/50 poultry boxes. Chickens are fed same diet from age of 1to 42 days, except for the last seven days of fattening, when FN broiler group was added to the feeder *on top* 20g/chicken daily freshly grown and blanched nettle, and in case of DN group of broilers, 10g/kg finely ground dry nettle was mixed into a finisher. Adding nettle in broiler food for the last seven days did not significantly affect the broiler production performance. At the same time, a significant increase in liver mass in chickens fed with fresh nettle ($p<0.05$) was observed, as well as a certain trend in abdominal fat loss without decrease in carcass mass. Nutrition of broilers with nettle has a certain potential that requires further research.

Key words: nutrition, nettle, broilers, fattening, slaughter performance

Introduction

Finding effective alternative nutrients that would enrich the choice of standard natural components of poultry meals, which would be nutritionally rich, health-beneficial and widely available, has been the focus of research in recent years. One of these nutrients may potentially be nettle (*Urtica dioica* L.).

The nettle is common and well known as food and medicine in folk medicine and traditional human and poultry nutrition. Despite the long tradition and the former practice of using nettle in the poultry diet, especially in the breeding of chickens on small farms, in modern conditions and methods of feeding poultry it is no longer used. Also, there are relatively few new studies that would investigate the ways and effects of nutrition with nettle of modern poultry types.

As a nutrient, the chemical composition and nutritive quality of the nettle can vary greatly, especially depending on the age of the plant in terms of protein content (20-25%) and cellulose (10-20%). It is rich in minerals, especially iron, potassium, manganese and calcium, vitamins A, C and D, as well as lutein that naturally enhances the yellow colour of the skin and yolk in poultry (*Loetscher et al., 2013*). Some studies indicate that the use of nettle in poultry nutrition can affect the metabolism of lipids and proteins (*Đukić Stojčić et al. 2013; Loetscher et al., 2013; Ghasemi et al., 2014).*

In the capacity of phyto-additive, mainly dried or extracted leaf or nettle root is used. It is thought to possess antibiotic, antioxidant and anti-inflammatory properties (*Nasiri et al., 2011; Keshevarz et al., 2014*).

The objective of our research was to determine the effect of added dried and fresh nettle in nutrition for broilers in the last week of fattening on the production results and slaughter quality of the product. The addition of dried nettle is a possible model of use in the industrial methods of broiler fattening, and fresh, or blanched, in alternative livestock production systems.

Materials and Methods

The trial was carried out on a total of 600 one - day chicks of both sexes of ROSS 308 provenience, at the experimental farm for fattening chickens of the Institute for Animal Husbandry, Belgrade - Zemun.

Chickens were divided into 3 groups (C - Control group, FN - Fresh Nettle group, DN - Dry Nettle group), and each group was divided into 4 repetitions/boxes per 50 chicks. They were housed in boxes in the floor housing system, on chopped straw litter with ad libidum access to food and water. The recommendations of the hybrid manufacturer regarding the required light, ventilation, temperature, and other parameters related to fattening technology were fully respected (*Aviagen, 2014*)

All groups of chickens were fed during the experiment with four complete feed mixtures based on maize, soybean products, wheat bran and standard additives, in flour form, formulated to satisfy all nutritional needs of broilers: pre-starter mixture until 10 days of fattening with 12.6 MJ/kg metabolic energy (ME) and 21.0% crude protein (CP); starter mixture, from day 11 to 22 of fattening, with 12.9 MJ/kg ME and 19.5% CP; grower mixture, from day 23to 35 of fattening, with 13.1 MJ/kg ME and 19% CP and finisher mixture from day 35 to 42 of fattening with 13.3 MJ/kg ME and 17.3% CP. In the last, sixth week of fattening and trial (from 35th to 42nd day of fattening), the diet of experimental FN and DN groups of broilers compared to the nutrition of the control group differed only in the addition of dried, that is, freshly blanched nettle to finisher foods. The FN group of broilers was added once a day directly to round group feeders from which experimental chickens were fed, *on top* 20g per chicken of freshly harvested nettle, previously blanched for two minutes in hot water and roughly chopped.

The DN group of broilers was fed diet mixed with 1% finely ground dry nettle during the preparation of finisher mixture, wherein, in the composition of the mixture for this group of broilers, the share of wheat bran was decreased for the same percentage.

The dried nettle, as well as the nettle used in the fresh state, were harvested from the same locations and according to the same principle, taking 15-20 cm peak parts of plants from nettles not higher than 1 m. The basic chemical composition and microbiological correctness of dry nettle and all the complete feed stuffs used in the experiment were determined by standard, accredited methods.

During the trial, the health condition of chickens was monitored, the body mass of the broiler (individual) measured and calculated, also the gain, consumption and the conversion of food (at the level of the box) were observed.

After the experiment, the random sample of 6 male and 6 female chickens were taken from each group. After 12-hour starvation, stunning, slaughtering and 24-hour cooling of carcasses, the carcass mass, mass of carcass parts and broiler organs were determined.

The obtained results were processed with statistical software package STATISTICA, version 8, StatSoft, Inc. (www.statsoft.com). Methods of descriptive statistics, corresponding variation analysis tests (ANOVA) and corresponding *post hoc* tests were used, and statistically significant differences were considered and presented where the value was $p \leq 0.05$.

Results and Discussion

The broilers from the control and experimental group were healthy, vital and without mortality. The realized body masss of the broilers at the beginning of the introduction of nettle into broiler diet and at the end of the trial are shown in Table 1.

Table 1. The average body mass of chickens at the beginning and at the end of additional feeding with the nettle in the final stage of fattening

	Body mass, g							
	n	Mean	SD	SE	n	Mean	SD	SE
Control	200	1660	267	18.9	200	2280	335	23.7
Fresh Nettle	200	1644	288	20.4	200	2239	347	24.6
Dry Nettle	200	1666	242	17.1	200	2291	329	23.2
p value	p>0.05				p>0.05			

SD-standard deviation; SE-standard error

The realized mass gains of chicks, consumption and food conversion during the final stage of fattening are shown in Table 2.

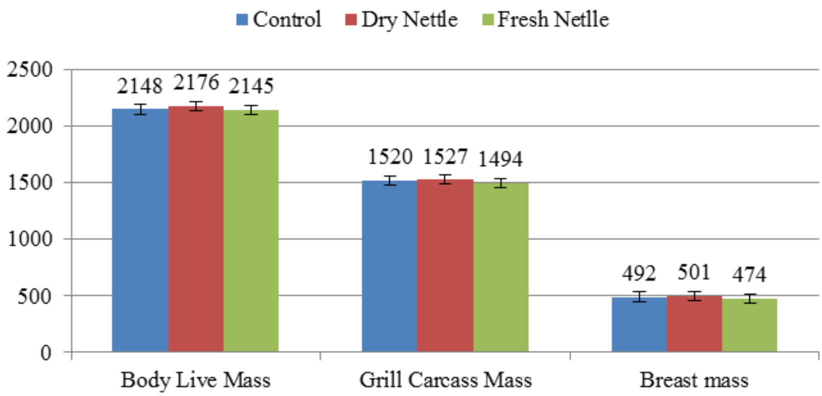
Table 2. The daily mass gains (WG), feed intake (FI) and feed conversion ratio (FCR) of broilers in observed trial period (35-42 day of fattening)

	Production parameters											
Group	WG, g/head/day				FI, g/head/day				FCR, kg feed/kg gain			
	n	Mean	SD	SE	n	Mean	SD	SE	n	Mean	SD	SE
Control	4	88.56	4.40	2.19	4	180	6.63	3.32	4	2.02	0.07	0.03
Fresh Nettle	4	84.95	4.62	2.31	4	179.5	5.45	2.72	4	2.13	0.11	0.06
Dry Nettle	4	89.26	3.46	1.73	4	181.5	5	2.5	4	2.04	0.09	0.04
p value	p>0.05				p>0.05				p>0.05			

Based on the trial results, it can be noticed that the addition of nettle in broiler food in the last seven days did not significantly affect the broiler production performance.

The use of nettle in nutrition of broilers also did not lead to a change in production results in fattening in studies carried out by *Nasiri et al. (2011), Loetscher et al. (2013),* as well as *Keshevarz et al. (2014).* On the other hand, *Ghasemi et al. (2014)* reported mass gain and reduced food conversion in chickens fed with over 2% dry nettle leaves in a meal throughout the fattening.

Slaughter results determined at the end of the experiment are shown in Graph 1, and the weight of individual edible organs and abdominal fat in Table 3.



Graph 1. Pre-slaughter mass, the mass of carcass "Ready to roast" and breast mass of trial broilers, g

Table 3. Mass of some edible organs and abdominal fat in trial chickens, g

Group	Body organs											
	Gizzard				Liver				Abdominal fat			
	n	Mean	SD	SE	n	Mean	SD	SE	n	Mean	SD	SE
Control	12	41.3	7.79	2.25	12	34^{b}	10.47	3.02	12	22.13	5.43	1.57
Fresh Nettle	12	40.94	6.13	1.77	12	40.94^{a}	4.39	1.27	12	18.98	6.35	1.83
Dry Nettle	12	43.8	9.28	2.68	12	38.42^{ab}	3.88	1.22	12	21.22	8.14	2.35
p value	$p>0.05$				$p<0.05$				$p>0.05$			

a-b - values between groups with different letters are significantly different ($p<0.05$)

The trial results shown in Graph 1 indicate that there were no statistically significant differences in the quality of carcasses of broilers fed in the last week of fattening with or without the addition of nettle.

Based on data on mass of abdominal fat in the carcasses, a certain reduction trend can be observed when adding nettle to broilers' diet, especially in feeding with fresh nettle where broilers had 14% less carcass fat compared to the control group, but the differences were not statistically significant.

There was a notable increase in liver weight in chickens with nettle in the diet, in particular a statistically significant increase in liver weight in chickens with fresh nettle in food, indicating the possible effect of this nutrient on the total metabolism and health of broilers.

The positive effect of nettle nutrition in broilers on slaughter traits and liver and stomach mass was demonstrated in the research by *Nasiri et al. (2011)*, on the mass of individual internal organs by *Keshevarz et al. (2014)*, while *Đukić*

Stojčić et al. (2013) report of the more favourable fatty acid composition of meat of broilers fed with fresh nettle.

Conclusion

The use of nettle in the nutrition of broilers in the last week of fattening, whether fresh or dried, in our experiment, did not show any positive or negative effect on the achieved production results, but there is a certain positive effect on certain slaughter results. It seems that nutrition by nettle, as a path to healthier broilers and higher quality of products, even in modern conditions of production, has a certain potential that requires further research.

Uticaj dodavanja koprive u hranu brojlera u završnoj fazi tova

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

Rezime

Cilj istraživanja je da se utvrdi uticaj sušene i sveže koprive, kao alternativnog hraniva dodatog hrani za brojlere u poslednjoj nedelji tova, na prozvodne rezultate i klanični kvalitet proizvoda.

Ogled je izveden na ukupno 600 ROSS 308 jednodnevnih pilića oba pola, podeljenih u 3 grupe (C- Control group; FN- Fresh Nettle group; DN- Dry Nettle group), a svaka grupa na 4 ponavljanja/boksa od 50 pilića. Pilići su hranjeni od 1-42. dana istim obrocima, osim poslednjih sedam dana tova, kada je FN grupi brojlera dodavano u hranilica *on top* po 20g/piletu dnevno sveže urbane i blanširane koprive, a DN grupi brojlera umešavano u finišer smešu 1% fino mlevene suve koprive.

Dodavanje koprive u hranu brojlera poslednjih sedam dana tova nije značajnije uticalo na praćene proizvodne rezultate brojlera. Istovremeno je uočeno značajno povećanje mase jetre kod pilića sa svežom koprivom u ishrani ($p<0.05$), kao i izvestan trend smanjenja abdominalne masti bez gubitka mase trupova. Ishrana koprivom brojlera ima izvestan potencijal koji zahteva dalja istraživanja.

Ključne reči: ishrana, kopriva, brojleri, tov, klanični rezultati

Acknowledgment

The study is part of the project TR 31033 financially supported by the Ministry of Education and Science of the Republic of Serbia.

References

AVIAGEN (2014): ROSS 308 Broiler Management Handbook.

ĐUKIĆ STOJČIĆ M., LEVART A., SALOBIR J., PERIĆ L., MILOŠEVIĆ N. (2013): The effect of nettle (*Urtica dioica*) on the fatty acid profile of breast meat of Red Bro chicken. Word's Poultry Science Journal, Volume 69, Supplement (Proceedings from Egg Meat Symposia, Bergamo, 15-19 September 2013).

GHASEMI H.A., TAHERPOUR K., HAJKHODADADI I., AKHAVAN-SALAMAT H. (2014): Comparative effects of nettle *(Urtica Dioica*) and commercial feed additives on productive performance and blood lipid profile of broiler chickens. Journal of Animal Science Advances 4, 1, 633-640.

KESHAVARZ M., REZAEIPOUR V., ASADZADEH S. (2014): Growth performance, blood metabolites, antioxidant stability and carcass characteristics of broiler chickens fed diets containing nettle (*Urtica dioica L.*) powder or essential oil. International Journal of Advanced Biological and Biomedical Research, 2,9, 2553-2561.

LOETSCHER Y., KREUZER M., MESSIKOMMER R.E. (2013): Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. Poultry Science 92, 2938-2948.

NASIRI S., NOBAKHT A., SAFAMEHR A. (2011): The effects of different levels of nettle *Urtica dioica L.* (Urticaceae) medicinal plant in starter and grower feeds on performance, carcass traits, blood biochemical and immunity parameters of broilers. Iranian Journal of Applied Animal Science. 1, 3, 177-181.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

IMPACT OF CALCIUM ADMINISTERED THROUGH DRINKING WATER ON EGG PRODUCTION OF LAYING HENS

Ksenija Nešić[1], Snežana Ivanović[1], Vladimir Radosavljević[1], Jasna Kureljušić[1], Nikola Rokvić[1], Aleksandar Stanojković[2], Milan Radović

[1]Institute of Veterinary Medicine of Serbia, Autoput 3, 11070 Belgrade, Serbia
[2]Institute of Animal Husbandry, Autoput 16, 11080 Zemun, Serbia
Corresponding author: ksenija_n@yahoo.com
Original scientific paper

Abstract: Having on mind the estimation regarding the world's population enlargement of 25 % after 2050, and therefore 60 % higher demand for food production, the most realistic solution to provide necessary animal protein for human consumption is intensification of poultry breeding. Among many other efforts to accomplish this goal, attempts to improve production capacities of laying hens and to increase the egg production is one of the primaries. The aim of this paper is to present the results of additional calcium administered in the drinking water on the egg production of laying hens. The nutritional role of calcium is closely linked to that of phosphorus and to the effect of vitamin D, so two nutritive supplements were used. Statistically very significant difference in number of produced eggs was observed in the treatment with the product containing calcium and phosphorus. On the other hand, product consisting of calcium and vitamin D3 had no beneficial impact on the examined production parameter. Based on the obtained results, it could be concluded that amount and source of calcium, as well as phosphorus, in the diet of laying hens is sensitive and complex issue. Therefore, it requires careful handling in practice and opens up many opportunities for further research.

Key words*:* calcium, phosphorus, egg production, laying hens

Introduction

In the next decades, until middle of this century, the world's population will enlarge by 25 %, so there will be ultimate need to increase food production by 60 % (*FAOSTAT, 2013*). One of the most affordable sources of animal protein are eggs. Therefore, the number of laying flocks is rapidly increasing in developing countries. In Europe, the priority is to increase egg production, as well as egg quality (*Bain et al., 2016*).

Egg production is influenced by many factors, like breed and age of laying hens, inheritance, the environmental conditions or diseases, but feed is one of the major. During the laying period, the first challenge is to adjust the energy and protein requirements to optimize egg output and to carefully control body weight. The crude protein concentration and amino acids in the diet for layers are also important, methionine being the main limiting amino acid. A deficiency of calcium in the diet of poultry cause declining egg production. The laying hens requirement for dietary calcium within the diet for different ages is in the order of 0.9 to 1.2% during the growth period of the pullet, increasing to 2 to 2.5% just prior to the onset of lay and 3.5 to 4.5% once lay is established (*Bouvarel* et al., *2011*). The optimum level of dietary calcium for high egg production and hatchability appears to be 2.5 to 3 percent, while higher level caused reduced hatchability. *Sultana et al. (2007)* emphasized that among three calcium levels of 2, 3.4 and 3.75 only calcium level 3 showed better result in increasing egg production.

Pelicia et al. (2009) in their trial investigated influence of calcium and phosphorus levels in the diet of laying hens. They studied the effect of four calcium (3.0, 3.5, 4.0, and 4.5%) and four available phosphorus levels (0.25, 0.30, 0.35, and 0.40%) in the feed of semi-heavy commercial layers after molting, but no significant effect ($p>0.05$) of the interaction among factors (Ca and P levels) was observed on layer performance. On the other hand, *Hartel (1990)* evaluated dietary interactions between Ca and P in high production layers and observed that there were significant performance depression and high mortality when low P content was combined with high Ca in the diet, and that these effects were compensated when dietary P content was increased.

Having many different literature data on mind, the aim of this study was to determine the influence of two nutritive supplements, administered in the drinking water, but of different composition (one containing calcium and phosphorus and another consisting of calcium and vitamin D) on egg production of laying hens.

Materials and Methods

The experiment was conducted on the farm of 34200 Lohmann Brown laying hens, 78 weeks old. All of them were fed a mixture of standard ingredient (Table 1) and chemical composition (Table 2).

Table 1. Ingredient composition of feed mixture

INGREDIENTS	COMPOSITION [%]
Maize	56.00
Sunflower meal	12.50
Soybean cake	8.75
Soybean oil	0.75
Cereal meal	7.50
Mineral chalk	3.75
Grit 2-4 mm	6.25
Yeast	2.50
Premix	2.00
TOTAL	100.00

Table 2. Chemical composition of feed mixture

CHEMICAL	COMPOSITION [%]
Crude proteins	14.30
Moisture	12.00
Cellulose	5.50
Ash	12.00
Calcium	3.95
Phosphorus	0.59
Metabolizable energy	11.4 MJ

The system was cage rearing. The facility contained three batteries, each consisting of 184 cages, and the layers were thus divided into 3 experimental groups with about 11400 individuals in each (R, C and L). Each group had its own dispenser of drinking water. The experimental group R in the period from 07:00 to 14:00 h was given a product which contained calcium 5 g/l and phosphorus 144 g/l, in the dosage of 1 l per 1000 l of drinking water, while at the same time control group C and the experimental group L got pure water. In the period from 14:00 to 22:00 h, experimental group L received another product containing calcium 75 g/l and vitamin D3 300 000 IU/l, in a dosage of 1 l per 1000 l of drinking water, while for other two groups (C and R) only pure water was simultaneously provided. The experiment lasted 15 days, during which the egg production was recorded.

Statistical analysis was done using Microsoft Excel 2007 and differences of measurements between the groups were tested by paired two-tailed t-test.

Results and Discussion

Results for egg production are given for each group separately and specified for six individual days as: Egg production in control group C, given plane water (Table 3), Egg production in experimental group R, given a product which contained calcium and phosphorus (Table 4) and Egg production in group L, given a product containing calcium and vitamin D3 (Table 5).

Table 3. Egg production in control group C

	4th day	6th day	8th day	10th day	12th day	15th day
Number of eggs	9916	10004	10138	9971	9919	10921
% of production	86.98	87.75	88.93	87.46	87.01	95.80

Table 4. Egg production in experimental group R

	4th day	6th day	8th day	10th day	12th day	15th day
Number of eggs	10264	10278	10386	10185	10114	11180
% of production	90.04	90.16	91.11	89.34	88.72	98.07

Table 5. Egg production in experimental group L

	4th day	6th day	8th day	10th day	12th day	15th day
Number of eggs	9927	10083	10132	9886	9780	10521
% of production	87.08	88.45	88.88	86.72	85.79	92.29

Different data regarding calcium sources in breeding hens could be found in literature. There are authors who noted no benefit of adding more calcium (*Keshavarz and Nakajima, 1993*; *Tunc and Cufadar, 2015),* as well as those who recorded that lower Ca solubility and higher Ca intake have positive influence (*Pavlovski et al., 2003; Vitorovic et al., 2004; Sultana et al., 2007; Ahmed et al, 2013; Zhang et al., 2017).*

During two weeks of our experiment, statistically very significant difference (< 0.01) comparing to the control group C was noted in egg production of the experimental group R, which was given product containing calcium and phosphorus. Additional calcium from liquid source consisting of calcium and vitamin D3, given through drinking water of group L, had no significant impact on examined parameter. It possibly means that the maximal exploitation of hens, in these circumstances, was achieved through adequate nutrition. In the used complete mixture all nutrients were balanced and aligned with the needs of Lohmann Brown breed, while feed calcium was at the level 3.95 %. For adequate egg production amino acids, in particular methionine and lysine, are also of great importance (*Bain* *et al., 2016*). Improvement is observed in the treatment with the product containing calcium and phosphorus, which indicates the significance of both minerals and demonstrates the importance of balancing Ca-P ratio in the diet of laying hens *(Sefer and Sinovec, 2008)*. It is in agreement with conclusion of Hartel (1990) who evaluated dietary interactions between Ca and P in high production layers and observed that there were significant performance depression and high mortality when low P content was combined with high Ca in the diet, and that these effects were compensated when dietary P content was increased. But, there is also statement of *Pelicia et al. (2009)* that no significant effect ($p>0.05$) of the interaction among factors (Ca and P levels) was observed on layer performance.

The establishment of Ca and P requirements of commercial layers is a continuous challenge for poultry nutritionists and egg producers as the needs for these two minerals seem to constantly change.

Conclusion

Taking into account demand to increase food production for 60% by the middle of this century and capabilities of poultry breeding to provide animal proteins, all research efforts to improve production of laying hens are necessary and important. Based on the obtained results in this study it could be concluded that amount and source of calcium, as well as phosphorus, have an impact on egg production of laying hens. But, it is still very complex and not fully solved issue which requires careful handling in practice and opens up many opportunities for further research.

Uticaj kalcijuma dodatog u vodu za piće na proizvodnju jaja kokoši nosilja

Ksenija Nešić, Snežana Ivanović, Vladimir Radosavljević, Jasna Kureljušić, Nikola Rokvić, Aleksandar Stanojković

Rezime

Imajući u vidu procenu porasta stanovništva na planeti za 25 % do 2050. godine, te zbog veće potražnje i posledičnu potrebu za 60 % obimnijom proizvodnjom hrane, najrealnije rešenje da se obezbede neophodni proteini životinjskog porekla za ljudsku ishranu jeste intenziviranje živinarstva. Među mnogim drugim naporima da se ostvari taj cilj, pokušaj da se poboljšaju proizvodni kapaciteti kokoši nosilja i da se poveća proizvodnja jaja je jedan od osnovnih.

Cilj ovog rada je da prikaže uticaj dodavanja kalcijuma kroz vodu za piće na proizvodnju jaja kokoši nosilja. Nutritivna uloga kalcijuma je usko povezana sa funkcijom fosfora i vitamina D, te su tokom dve nedelje ogleda na 34200 nosilja, podeljenih u tri grupe, korišćena dva nutritivna dodatka različitog sastava. Statistički veoma značajna razlika u nosivosti u odnosu na kontrolnu grupu uočena je u eksperimentalnoj grupi koja je kroz vodu konzumirala proizvod na bazi kalcijuma i fosfora. Dodatak u čijem su sastavu primarne komponente kalcijum i vitamin D3 nije ispoljio pozitivan efekat na ispitivani proizvodni parametar.

Na osnovu dobijenih rezultata, može da se zaključiti da količina i izvor kalcijuma, kao i fosfora, u ishrani koka nosilja utiče na proizvodnju jaja, a predstavlja veoma osetljivo i složeno pitanje. Zbog toga, ono zahteva pažljivo postupanje u praksi i otvara mnoge mogućnosti za dalja istraživanja.

Ključne reči: kalcijum, fosfor, proizvodnja jaja, kokoš nosilja

Acknowledgment

This paper is published as part of the project of the Ministry of Education, Science and Technological Development of Republic of Serbia III46009.

References

AHMED N. M., ABDEL ATTI K. A., ELAMIN K. M., DAFALLA K. Y., MALIK H. E. E., DOUSA B. M. (2013): Effect of Dietary Calcium Sources on Laying Hens Performance and Egg Quality. Journal of Animal Production Advances, 3(7): 226-231.

BAIN M.M., NYS Y., DUNN I.C. (2016): Increasing persistency in lay and stabilizing egg quality in longer laying cycles. What are the challenges? British Poultry Science 57 (3), 330-338.

BOUVAREL I., NYS Y., LESCOAT P. (2011): Hen nutrition for sustained egg quality. In: Nys Y., Bain M., Vanimmerseel F., editors. Improving the Safety and Quality of Eggs and Egg Products, Vol 1: Egg Chemistry, Production and Consumption. Cambridge: Woodhead Publishing Ltd; 261–290.

FAOSTAT (2013): http://www.fao.org/docrep/018/i3107e/i3107e00.htm

HARTEL H. (1990): Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hen. British Poultry Science 1990; 31(3):473-494.

KESHAVARZ K., NAKAJIMA S. (1993): Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poultry Science, 72:144–153.

PAVLOVSKI Z., VITOROVIC D., LUKIC M., SPASOJEVIC I. (2003): Improving eggshell quality by replacement of pulverized limestone by granular limestone in the hen diet. Acta Veterinaria, 53, (1), 35-40.

PELICIA K., GARCIA E.A., FAITARONE A.B.G., SILVA A.P., BERTO D.A., MOLINO A.B., VERCESE F. (2009): Calcium and Available Phosphorus Levels for Laying Hens in Second Production Cycle. Brazilian Journal of Poultry Science, Jan - Mar 2009 / v.11 / n.1 / 39 – 49.

SEFER D., SINOVEC Z. (2008): Opšta ishrana. Faculty of Veterinary Medicine, University of Belgrade, pp 225.

SULTANA F., ISLAM M.S., HOWLIDER M.A.R. (2007): Effect of dietary Calcium Sources and Levels on Egg Production and Egg Shell Quality of Japanese Quail. International Journal of Poultry Science 6 (2): 131-136.

TUNC A.E., CUFADAR Y. (2015): Effect of Calcium Sources and Particle Size on Performance and Eggshell Quality in Laying Hens. Turkish Journal of Agriculture - Food Science and Technology, 3(4): 205-209.

VITOROVIC D., PAVLOVSKI Z., SKRBIC Z., LUKIC M., ADAMOVIC I. (2004): Čvrstoća ljuske jajeta. Biotechnology in Animal Husbandry, 20, 3-4, 107-111.

ZHANG B., CALDAS J.V., COON C.N. (2017): Effect of Dietary Calcium Intake and Limestone Solubility on Egg Shell Quality and Bone Parameters for Aged Laying Hens. International Journal of Poultry Science, 16 (4): 132-138.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EFFECT OF PROTEASE ON PRODUCTION PERFORMANCE, WEIGHTS AND PROPORTIONS OF PRIMAL CUTS AND WEIGHTS OF EDIBLE BY-PRODUCTS OF BROILERS

Vladimir Dosković[1], Snežana Bogosavljević-Bošković[1], Zdenka Škrbić[2], Milun D. Petrović[1], Veselin Petričević[2], Simeon Rakonjac[1]

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

Abstract: This study analyses the effect of different levels of dietary protease supplementation on the weights and proportions of primal cuts (breast, drumsticks, thighs, wings, back, pelvis) and weights of edible by-products (liver, heart, gizzard) in two strains of broilers (fast-growing Cobb 500 and medium-growing Master Gris) in a 49-day experiment. Broilers were allocated to 3 dietary treatment groups: group C (standard diet, 0% protease), group E-I (0.2% protease, crude protein level reduced by 4% compared to C) and group E-II (0.3% protease, 6% reduction in crude protein compared to C). The analysis of production performance showed lower body weights at the end of the fattening experiment, lower feed consumption and poorer feed conversion ratios in Master Gris broilers than in Cobb 500 chickens ($P<0.05$). As determined from data obtained at slaughter, the two broiler strains differed significantly in the weights and proportions of all primal cuts, except the proportion of back, as well as in the weights of all edible by-products, except gizzard. Dietary treatments had a significant effect ($P<0.05$) only on body weight and liver weight in Cobb 500 broilers belonging to E-II compared to C chickens.

Key words: broilers, protease, primal cuts, edible by-products

Introduction

Over the last twenty years, research in poultry farming has been increasingly focused on new production systems for broiler chickens as an alternative to intensive rearing of fast-growing broiler strains, short fattening periods, high stocking densities, etc.

Poultry meat obtained in these novel systems is noted and recognised for its productive traits and quality. In contrast, intensive production has undermined animal welfare, and causes increasing environmental pollution.

Moreover, previous studies have shown great variations in growth and development characteristics among broiler strains, which exhibit specific rates of growth and protein (meat) synthesis and, hence, have specific dietary requirements for protein and other nutrients. A major reason underlying variations in broiler growth and development is nutrition i.e. genotype x nutrition interaction. In addition, broiler nutrition is an important factor influencing meat quality, with the dietary protein level having the greatest effect.

Attention has been given to the production of moderate growth genotypes (*Škrbić et al., 2007, Meluzzi et al., 2009, Blagojević, 2011*), the prolongation of fattening periods (*Souza et al. 2011*), the modification of broiler diet formulations, the use of synthetic amino acids, enzymes, mycotoxin adsorbents, organic acids, probiotics (*Rahman et al., 2009, Dosković et al., 2016*), the use of free range (*Bogosavljević-Bošković et al., 2011*), etc.

The objective of this study was to compare carcass quality of two strains – fast-growing Cobb 500 and medium-growing Master Gris broilers receiving different feed formulations – standard feeds for intensive rearing and feeds containing reduced crude protein levels and supplemented with protease.

Material and methods

Two broiler strains i.e. fast-growing Cobb 500 and medium-growing Master Gris were used in the experiment, which involved 300 day-old chickens of each strain allocated to 3 experimental groups – dietary treatments.

Dietary treatments

Dietary treatments included control C diet containing standard levels of nutrients, E-I diet containing crude protein reduced by 4% relative to the control and 0.2% supplemental protease, and E-II diet containing crude protein reduced by 6% relative to the control and 0.3% supplemental protease (Table 1.). The experiment lasted 49 days. Standard fattening technology was used (10 broilers/m^2, 24-h photo schedule, ad libitum access to feed and water, starter, grower and finisher stages).

The supplementation of the protease enzyme (Ronozyme ProAct, DSM, The Netherlands) was provided through a vitamin and mineral premix.

Table 1. Nutrient composition of diets for broilers across dietary treatments

Ingredient, %	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 49 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
ME, MJ/kg	12.89	12.97	13.02	13.21	13.28	13.32	13.31	13.38	13.42
Crude proteins, %	22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09
Lysine, %	1.33	1.27	1.24	1.15	1.10	1.08	1.05	1.00	0.98
Methionine+cystine, %	0.92	0.90	0.89	0.91	0.89	0.88	0.86	0.84	0.83
Threonine, %	0,90	0,87	0,85	0,75	0,72	0,70	0,67	0,64	0,63
Tryptophan, %	0,30	0,29	0,28	0,23	0,22	0,21	0,20	0,19	0,18

Data collection

At slaughter, 20 broilers (10 females and 10 males) from each group were slaughtered and weight measurements were taken for ready-to-grill carcass, primal cuts (breast, drumsticks, thighs, back, pelvis, wings) and by-products (liver, heart, gizzard).

Statistical analysis

The data obtained were statistically analysed using *ANOVA, Microsoft STATISTICA Ver. 5.0, StatSoft Inc., 1995,* analysis of variance, F-test and Tukey's test (P<0.05).

Results and discussion

Table 2 presents productive traits of two strains of broilers at 49 days of age.

Table 2. Production performance of tested genotypes

Treatment		Body weight, g	Feed consumption, g	Feed conversion ratio, kg/kg prirasta
Hybrid	Protease	49d	1 to 49day	1 to49 day
Cobb 500	No	3001.7^{a}	5931.4^{a}	2.006^{b}
	0.2%	2960.7^{ab}	$5750.\ 1^{a}$	1.971^{b}
	0.3%	2880.1^{b}	5667.2^{a}	1.998^{b}
Master Gris	No	2397.0^{c}	5570.1^{a}	2.365^{a}
	0.2%	2345.9^{c}	5477.0^{a}	2.379^{a}
	0.3%	2383.9^{c}	5350.3^{b}	2.286^{a}
p-value				
Source of variation				
Hybrid		0.001	0.002	0.001
Protease		0.051	0.103	0.379
Hybrid x Protease		0.063	0.918	0.188

$^{a\text{-}c}$ Means within a column with different superscripts differ significantly (P<0.05)

As shown in Table 2, Cobb 500 broilers had greater body weights, higher feed consumption and a lower feed intake per kg of live weight gain compared to Master Gris chickens (P<0.05).

Café et al. (2002) reported an average body weight of 2.705 – 2.748 kg and feed conversion of 2.122 – 2.134 kg feed/kg gain for Cobb 500 broilers at 49 days, and the respective values determined by *Ahmadi and Karimov (2010)* in the same strain of broilers at the same age were 2,580 – 2,698 g and 1.818 – 1.870 kg feed/kg gain. Greater body weights in the present study were primarily due to longer starter (0 to 21 days) and grower (22 to 42 days) stages compared to the abovementioned authors (0-14 days and 14-35 days, respectively), which most likely affected the feed conversion ratio. The body weights of Master Gris broilers in this research were about 400 gr higher and the amount of feed intaken per kg gain was greater than the manufacturer's data (weight 1,963gr, feed conversion 1.96-2.03kg feed/kg gain, Master Gris, 2004). Similarly, *Van Horne et al. (2004)* found a higher feed intake per unit of weight gain in slow-growing broilers than in fast-growing chickens.

These results were supported by similar findings of *Yu et al. (2007)*, *Favero et al. (2009)*, reporting no differences in feed intake per unit of gain in dietary treatments with 0.2% supplemental protease and reduced crude protein levels. However, significance in body weight occurred at 49 days between C and E-II (0.3% protease) broilers.

The weights of edible by-products of both strains of broilers are given in Table 3.

Table 3. Weights of edible by-products of broilers

Treatment		Liver, g	Heart, g	Gizzard, g
Hybrid	Protease			
Cobb 500	No	55.79^{a}	13.65^{a}	45.43
	0.2%	50.99^{ab}	13.78^{a}	46.10
	0.3%	49.87^{b}	12.95^{ab}	44.45
Master Gris	No	48.48^{bc}	11.77^{ab}	47.68
	0.2%	43.91^{c}	10.82^{b}	47.03
	0.3%	45.83^{bc}	11.84^{ab}	46.15
p-value				
Source of variation				
Hybrid		0.001	0.001	0.185
Protease		0.002	0.744	0.624
Hybrid x protease		0.443	0.247	0.907

$^{a-c}$ Means within a column with different superscripts differ significantly (P<0.05)

The data on the weights and proportions of edible by-products (heart, liver and gizzard) presented in Table 3 indicate greater heart and liver weights and lower gizzard weights in Cobb 500 broilers than in Master Gris chickens, but the effect of genotype was non-significant (P>0.05). Dietary treatments had a significant effect only on liver weight in Cobb 500 broilers belonging to group E-II compared to C chickens (P<0.05).

The weights of primal cuts (breast, drumsticks, thighs, wings, back, pelvis) are shown in Table 4.

Table 4. Weights of primal cuts of broilers

Treatment		Breast, g	Drumsticks, g	Thighs, g	Wings, g	Back, g	Pelvis, g
Hybrid	Protease						
Cobb 500	No	798.68[a]	326.45[a]	376.97[a]	261.88[a]	283.53[a]	246.21[a]
	0.2%	791.36[a]	312.57[a]	372.29[a]	252.15[a]	274.78[a]	244.96[a]
	0.3%	764.78[a]	307.96[a]	361.19[a]	253.83[a]	279.95[a]	252.22[a]
Master Gris	No	524.51[b]	277.48[b]	303.78[b]	221.74[b]	222.64[b]	211.47[b]
	0.2%	509.90[b]	264.66[b]	293.25[b]	219.16[b]	209.10[b]	200.89[b]
	0.3%	515.70[b]	265.04[b]	297.08[b]	219.18[b]	216.55[b]	205.55[b]
p-value							
Source of variation							
Hybrid		0.001	0.001	0.001	0.001	0.001	0.001
Protease		0.394	0.113	0.347	0.491	0.332	0.552
Hybrid x protease		0.554	0.921	0.633	0.799	0.950	0.608

[a-b] Means within a column with different superscripts differ significantly (P<0.05)

In line with their greater body weights, fast-growing broilers had greater weights of all primal cuts (breast, drumsticks, thighs, wings, back, pelvis) compared to medium growing broilers (P<0.05). Regardless of feed formulation, the weights of primal cuts were similar in broilers of the same strain; accordingly, no significance was exhibited by dietary crude protein reduction and protease supplementation in either strain (P>0.05).

Based on the weights of primal cuts and ready-to-grill carcass, the proportions of primal cuts were determined (Table 5).

Table 5. Proportions of primal cuts of broilers

Treatment		Breast, %	Drumsticks, %	Thighs, %	Wings, %	Back, %	Pelvis, %
Hybrid	Protease						
Cobb 500	No	33.60[a]	13.69[b]	15.83[bc]	11.02[b]	11.92	10.34[b]
	0.2%	33.93[a]	13.38[b]	15.96[abc]	10.79[b]	11.68	10.47[b]
	0.3%	33.24[a]	13.34[b]	15.47[c]	11.02[b]	12.15	10.91[ab]
Master Gris	No	28.62[b]	15.10[a]	16.53[ab]	12.08[a]	12.11	11.54[a]
	0.2%	28.90[b]	14.97[a]	16.62[a]	12.42[a]	11.86	11.40[a]
	0.3%	28.78[b]	14.77[a]	16.58[ab]	12.22[a]	12.08	11.47[a]
p-value							
Source of variation							
Hybrid		0.001	0.001	0.001	0.001	0.549	0.001
Protease		0.659	0.148	0.388	0.819	0.228	0.195
Hybrid x protease		0.785	0.845	0.431	0.051	0.782	0.134

[a-c] Means within a column with different superscripts differ significantly (P<0.05)

The proportion of breast in Cobb 500 broilers in this study was much higher than in *Café et al. (2002)* for the same strain and age, whereas the proportions of drumsticks, thighs and wings were somewhat lower. Similar data on the proportions of breast, drumsticks and thighs were found by *Ahmadi and Karimov (2010),* as opposed to *Si et al. (2004)* who determined lower breast and higher wing proportions.

The comparison between the two broiler strains suggests that slow-growing broilers had lower breast, but higher drumstick, thigh, wing and pelvis proportions (P<0.05), whereas the proportion of back was similar in both strains (P>0.05). Similarly, *Havenstein et al. (1994)* reported higher drumstick and lower breast proportions in slow-growing broilers than in fast-growing ones, and *Quentin et al. (2003)* found higher drumstick and thigh yields in slow-growing chickens, indicating that carcass composition was generally not affected by dietary treatment (different energy and protein concentrations), which was confirmed by the finding of the present study that crude protein reduction and protease supplementation had no effect on the weights and proportions of primal cuts in both strains of broilers (P>0.05).

Conclusion

The analysis of production performance showed that Master Gris broilers had lower body weights at the end of the fattening experiment – 49 days, lower feed consumption and poorer feed conversion ratios compared to Cobb 500 broilers (P<0.05). As determined from data obtained at slaughter, significant differences were found between the two strains of broilers (P<0.05) in the weights of all primal cuts, proportions of primal cuts, except breast, and the weights of all by-products, except gizzard. The effect of feed formulation was significant (P<0.05) only on the body weight and liver weight of Cobb 500 broilers belonging to E-II compared to C chickens.

Uticaj enzima proteaze na proizvodne rezultate, masu i udeo osnovnih delova trupa i masu jestivih pratećih proizvoda klanja pilića

Vladimir Dosković, Snežana Bogosavljević-Bošković, Zdenka Škrbić,
Milun D. Petrović, Veselin Petričević, Simeon Rakonjac

Rezime

U radu je analiziran uticaj različite koncentracije enzima proteaze, dodate u hranu, na masu i udeo osnovnih delova trupa (grudi, bataci, karabataci, krila, leđa, karlica) i jestivih pratećih proizvoda klanja (jetra, srce, mišićni želudac) kod dva tovna hibrida pilića. U ogledu su korišćeni brzorastući hibrid Cobb 500 i medium-growing linijski hibrid Master Gris. Ogled je trajao 49 dana, a pilići oba hibrida bili su podeljeni u 3 ishranbene grupe: C group (standardni obrok, 0% enzima proteaze), E-I group (0,2% enzyme protease, uz snižen nivo sirovih proteina za 4% u odnosu na C group) and E-II group (0,3% enzyme protease, uz manji nivo sirovih proteina za 6% u odnosu na C group).

Analiza proizvodnih rezultata pokazala je da su pilići hibrida Master Gris imali manju telesnu masu na kraju tova, manju konzumaciju hrane i lošiju konverziju hrane u odnosu na piliće hibrida Cobb 500 (P<0.05). Na osnovu podataka sa linije klanja, utvrđeno je da su između ispitivanih hibrida postojale značajne razlike u masi i udelu svih osnovnih delova trupa, izuzev udela leđa, kao i masi svih pratećih proizvoda klanja, izuzev u masi želuca. Istovremeno, uticaj različitih formulacija obroka ispoljio je signifikantnan efekat (P<0.05) samo kod hibrida Cobb 500, između C i E-II grupe i to na telesnu masu pilića i masu jetre.

Ključne reči: pilići, enzim proteaza, osnovni delovi trupa, jestivi prateći proizvodi klanja

Acknowledgements

This study is part of Project No. 31033 titled “Sustainable Conventional and Revitalised Traditional Production of Value-Added Poultry Meat and Eggs“ financially supported by the Ministry of Science and Technological Development of the Republic of Serbia.

References

AHMADI K., KARIMOV T. (2010): A study on wheat middling’s usage on broilers performances. Australian Journal of Basic and Applied Sciences, 4, 11, 5642-5648.

BLAGOJEVIĆ M. (2011): Uticaj genotipa na intenzitet porasta i kvalitet trupa i mesa brojlerskih pilića u ekstenzivnom sistemu gajenja. Doktorska disertacija. Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad, 1-116.

BOGOSAVLJEVIĆ-BOŠKOVIĆ SNEŽANA, MITROVIĆ S., DOSKOVIĆ V., RAKONJAC S., PETROVIĆ D.M. (2011): Broiler meat quality: the effect of rearing systems and length of fattening period. 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production", 5-7 October 2011, Belgrade, Biotechnology in Animal Husbandry, 7 (4), 1635-1642.

CAFÉ M. B., BORGES C.A., FRITTS C.A., WALDROUP P.W. (2002): Avizyme Improves Performance of Broilers Fed Corn-Soybean Meal-Based Diets. Journal of Applied Poultry Research 11, 29-33.

DOSKOVIĆ VLADIMIR, BOGOSAVLJEVIĆ-BOŠKOVIĆ SNEŽANA, LUKIĆ MILOŠ, ŠKRBIĆ ZDENKA, RAKONJAC SIMEON, PETRIČEVIĆ VESELIN (2016): Effect of dietary protease supplementation and sex on dressing percentage and body conformation in broilers. Biotechnology in Animal Husbandry, 32(2), 185-193.

HAVENSTEIN, G. B., P. R. FERKET, S. E. SCHEIDELER, AND D. V. RIVES. (1994): Carcass composition and yield of 1991 vs 1957 broilers when fed "typical" 1957 and 1991 diets. Poult. Sci, 73, 1795–1804.

FAVERO A., MAIORKA A., ROCHA C., APPELT M.D., SORBARA J.O.B. (2009): Effect of protease enzyme on performance and ileal digestibility of broilers grown to 42 days of age in foor pens. International Poultry Scientific Forum, Atlanta, Georgia, Abstracts of Papers M28, p. 9.

MASTER GRIS M (2004): http://www.hubbardbreeders.com/

MELUZZI A., SIRRI F., CASTELLINI C., RONCARATI A., MELOTTI P., FRANCHINI A. (2009): Infuence of genotype and feeding on chemical composition of organic chicken meat. Italian Journal of Animal Science, 8 (Suppl. 2), 766-768.

QUENTIN M., BOUVAREL I., BERRI C., LE BIHAN-DUVAL E., BAÉZA E., JÉGO Y., PICARD M. (2003): Growth, carcass composition and meat quality response to dietary concentrations in fast-, medium-and slow-growing commercial broilers. Animal Research, 52 (1), 65-77.

RAHMAN A., AHMED S., KHAN D., HUSSAIN M., AHMAD I., SHAH Z., SOHAIL S.M., IKRAM-UL-HAQ, HAFEEZ A., ZIA-UR-REHMAN (2009): Nutritional Importance Of Exogenous Enzymes In Broiler Ration At Finisher Phase. Sarhad Journal of Agricultural, 25 (3), 475-478.

SI JIANLIN, KERSEY J.H., FRITTS C.A., WALDOUP P.W. (2004): An Evaluation of the Interaction of Lysine and Methionine in Diets for Growing Broilers. International Journal of Poultry Science, 3(1), 51-60.

STATSOFT INC. STATISTICA FOR WINDOWS (1995): Version 6.0, Computer program manual. Tulsa: StatSoft Jnc.

ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M. (2007): The effect of fattening duration in different rearing systems on slaughter traits of broiler chickens of genotype Redbro. Biotechnology in Animal Husbandry 23, 3-4, 67-74.

VAN HORNE P.L.M., VAN HARN J.J., RODENBURG T.B. (2004): Slow growing broilers: performance, mortality and welfare. XXI World's Poultry Congress, Instambul-Turkey, 8-13 June 2004, Book of abstracts, p. 326.

YU B., WU S.T., LIU C.C., GAUTHIER ROBERT, CHIOU PETER W.S. (2007): Effects of enzyme inclusion in a maize-soybean diet on broiler performance. Animal Feed Science and Technology, 134, 283-294.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

MEAT QUALITY OF REDBRO CHICKENS REARED EXTENSIVE INDOORS AFFECTED BY DURATION OF FATTENING PERIOD

Zdenka Škrbić[1], Miloš Lukić[1], Veselin Petričević[1], Snežana Bogosavljević-Bošković[2], Vladimir Dosković[2], Simeon Rakonjac[2]

[1]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak
Corresponding author: zdskrbic@gmail.com
Original scientific paper

Abstract: In the development of alternative production systems in the production of poultry meat, the set goals are related to market demands, product quality and production efficiency. In addition to pure and autochthonous breeds of poultry, production in these systems can also be based on more productive hybrids of moderate growth in order to realize higher yields of meat. For the quality of meat from alternative production, the key is the slaughter age of chickens that approaches full maturity. However, slaughtering of older chickens leads to some unfavorable changes in meat quality. The aim of this study was to determine the importance of the duration of fattening period for the quality of chickens of moderate growth of Redbro provenance, grown indoors, based on the chemical composition and individual physical characteristics of the meat. A total of 300 one day old chickens were grown indoors, 12 birds/m^2. The chickens of the first group were reared to the age of 42 days and the chickens of the second group, up to 84 days. Meat quality parameters were studied on samples of white (pectoral muscles) and dark meat (thigh and drumstick muscles) of 12 broilers per treatment, with equal sex representation. The basic chemical composition and parameters of the technological quality of meat were determined (cooking loss, grilling loss, water holding capacity, objective tenderness). Meat of 84 days old Redbro chickens had poorer water holding capacity compared to 42 days old chickens, which is also associated with determined greater cooking loos or grilling loos on the 84th day. The effect of the duration of fattening period on the water holding capacity did not adversely affect the tenderness of the meat, since the meat cutting force was at the same level on the 42nd and 84th day. Chickens aged 84 days, as expected, had higher fat content in meat. A positive aspect of the established higher fat content, especially in white meat, is the possible improvement in the sensory quality of the meat.

Key words: meat quality, Redbro chickens, extensive indoor, slaughtering age

Introduction

Market demands for quality and safe food have initiated the development of alternative breeding systems in poultry production. Commission Regulation 543/2008 sets standards for the production of chicken meat from the system of free range and extensive indoors in the EU countries. In France, the production system and the market for chickens from unconventional systems have been fully developed and standardized, thus providing a standardized quality standard. In our conditions, when the development of market-oriented alternative systems of rearing is initially present in all its forms, it is important that market requirements, product quality and production efficiency are included as criteria for selection. In addition to pure and autochthonous breeds of poultry, adapted to the rearing conditions in these systems, production can also be based on more productive hybrids of moderate growth in order to realize higher body weight and higher meat yield. One of them is the Redbro provenance of chickens, which in previous studies showed good adaptation to systems of production of different intensity. Depending on the nutritional conditions in free range systems, Redbro chickens can reach body weight of 1759 g at the age of 91 days (*Blagojević et al., 2009*), to 3382 g at the age of 84 days (*Škrbić et al., 2013*). In accordance with the above-mentioned body weights are also the relative carcass yields „ready to grill“, which in these studies amounted to 66.43%, and 73.93%, respectively, while the share of abdominal fat ranged from 2.71 to 2.55%. Data on meat quality of Redbro chickens from various unconventional production systems are scarce. The duration of fattening period, in addition to genotypes, breeding systems, nutrition *(Fanatico et al., 2007; Ristić et al., 2007)*, is also important for the meat quality properties. Intensive growth and development of breast muscles of fast growing hybrids is associated with an increase in the size of muscle fibers and reduced deposition of fat, which negatively affects the quality of meat (*Duclos et al., 2007*). According to *Fanatico et al. (2007)*, for the quality of meat from alternative production, of crucial importance is the age of slow-growing slaughter chickens approaching full maturity. Some authors report of a different character of meat quality changes with slaughtering age, depending on the genetic predisposition of broilers for intensity of growth (*Owens et al., 2006; Fanatico et al., 2006).*

The aim of this study was to determine the importance of the duration of fattening period for the meat quality of chickens of moderate growth, of Redbro provenance, reared extensive indoors, based on the chemical composition and individual physical characteristics of the meat.

Material and methods

A total of 300 one day old chickens were grown indoors, 12 birds/m^2. The first group was reared to the age of 42 days and the second group, up to 84 days. The chickens were reared in pens on a deep straw litter, with three repetitions per each treatment. During the first seven days of age, the light program was continuous with 23 hours of light. Subsequently, a lighting program was implemented in accordance with legal norms, which included six hours of darkness in blocks of 4 and 2 hours. Nutrition to day 42 was with three mixtures based on maize/soybean: starter 22% CP, 12.7 MJ ME; grower 19% CP, 13.0 MJ ME; finisher 17% SP, 13.3 MJ ME. The chickens of the second group, after 42 days, until the end of the fattening period, were fed with a finisher mixture (17% CP; 13.3 MJ ME). The availability of food and water was *ad libitum*. Meat quality parameters were studied on samples of white (breast meat) and dark meat (thigh and drumstick muscles) of 12 broilers per treatment, with equal sex representation. The basic chemical composition of meat was determined by reference methods for moisture, fat, protein and ash content according to AOAC (*Association of Official Analytical Chemists, 2011*). Cooking and grilling losses were determined based on the difference in the mass of white and/or dark meat before and after thermal treatment. Thermal processing by boiling was for 10 minutes in boiling water and thermal processing by baking for 25 minutes at 250°C. Water holding capacity (WHC) was tested using the compression method according to *Grau and Hamm (1952).* The resistance to cutting or tenderness of meat was determined on meat subsamples (1×0.5 cm) after thermal treatment, on warm meat, by the action of the blade (Warner-Bratzler Shear) perpendicular to the muscle fibers and by multiplying the obtained value by a coefficient of 0.25.

Statistical data processing was done by analyzing variance with the One-way ANOVA using the statistical software package STATISTICA, version 8, StatSoft, Inc. (www.statsoft.com).

Results and Discussion

The chemical composition of white meat (breast meat) and dark meat (thigh and drumstick meat) of the average mixed-sex sample of Redbro slaughter chickens of 42 and 84 days of age is shown in Table 1. Identified changes in the chemical composition of meat of Redbro chickens as an effect of slaughter age, refer to the mineral component, the content of fat and water. Ash content was significantly lower in meat of chickens aged 84 days compared to chicken meat at the age of 42 days. Determined changes were the same in samples of white and dark meat. The content of intramuscular fat was significantly increased in white meat of broilers at the age of 84 days, and in dark meat, the water content and fat content were under the significant influence of chicken age. Expectedly, the water content decreased and the fat content increased with the age of Redbro slaughter chickens.

Contrary to this, with respect to the same indoors rearing system, the greater slaughter age of Ross broilers did not lead to significant changes in the chemical composition of white or dark meat in the study of *Castellini et al. (2002).* However, by comparing the chemical composition of white meat of Ross broilers in the same research, grown indoors for up to 56 days and in the system with the possibility of using the range to the slaughter age of 83 days, greater fat content in the group of broilers grown for 56 days in the facility is established. These results indicate that, in conditions of the same genotype, the rearing system may have a more important role for the fat content of the meat than the slaughter age of chickens. The absence of differences in the content of water, protein and fat in white meat of the same slow-growing hybrid reared in two systems, at the same slaughter age, is determined by *Wang et al. (2009).*

Analysis of the chemical composition of white meat of Redbro broilers reared in the facility up to 67 days of slaughter, *Fanatico et al. (2005)* determined dry matter content of 27.83%, fat content expressed in the percentage of DM 4.70% and ash 3.93%, which are significantly higher values compared to our results. The explanation is probably in the effect of nutrition. The differences in the chemical composition of white meat between slow, moderate and fast-growing broilers reared in the object, at different slaughter ages (81, 67 or 53 days, respectively), in the same study, were not determined.

Table 1. Chemical composition of the breast meat and legs (drumstick+thigh) meat Redbro broilers

Chemical composition, %	Age, day				p-value
	42		84		
Breast meat	Mean	SD	Mean	SD	
Moisture	74.20	0.76	73.85	0.69	0.263
Protein	24.21	1.04	24.45	0.99	0.565
Lipids	0.59	0.27	0.83	0.25	0.029
Ash	1.21	0.04	1.04	0.06	<0.001
Legs meat					
Moisture	75.86	0.84	74.37	1.03	<0.01
Protein	20.20	0.63	20.60	0.59	0.120
Lipids	2.79	0.63	4.04	0.85	<0.001
Ash	1.14	0.05	1.03	0.05	<0.001

Table 2. Physical characteristics of the breast meat and legs (drumstick+thigh) meat Redbro broilers

Physical characteristics	Age, day				p-value
	42		84		
Breast meat	Mean	SD	Mean	SD	
Cooking loos, %	18.46	1.21	15.92	1.82	<0.01
Grilling loos, %	27.80	1.20	26.75	2.05	0.139
Tenderness	2.22	0.70	2.18	0.41	0.861
Water holding capacity (WHC), %	11.24	0.56	10.19	0.59	<0.01
Legs meat					
Cooking loos, %	25.01	2.06	26.40	2.02	0.109
Grilling loos, %	35.59	3.13	32.07	1.64	<0.01
Tenderness	2.01	1.01	1.98	0.19	0.92
Water holding capacity (WHC), %	13.09	0.52	11.56	0.86	<0.001

Physical characteristics of white and dark meat of Redbro broilers, shown in Table 2, indicate significant differences in losses in thermal processing of meat, where differences in cooking loos were found in white meat (18.46% vs 15.92%), and differences in grilling loos in dark meat (35.59% vs. 32.07%). Both parameters are related to the established significant reduction in water holding capacity with the extension of the fattening period. The difference in the water holding capacity in the meat between the Redbro broilers reared 42 and 84 days, was more pronounced in dark meat samples compared to breast meat, which can be related to the size of muscle fibers and glycogen reserves (*Duclos et al., 2007).* The increased locomotor activity of chickens in conditions of extensive indoor rearing increases the glycogen content in muscle fibers and, consequently, the anaerobic glycolytic potential in muscles, especially in the thigh, which results in lower pH post mortem (*Lawrie and Ledward, 2006*) and leads to denaturation and loss of functionality of numerous proteins responsible for binding of water.

The effect of the duration of fattening period on the water holding capacity did not adversely affect the tenderness of the meat, since the cutting force of the meat remained at the same level, despite the partial negative correlation between the ability to hold water and the meat tenderness (*Lee et al., 2008*). The obtained results are in concordance with the findings that the slaughter age is not the decisive for tenderness of meat, as indicated by previous research *(Castellini et al., 2008). Horsted et al. (2010)* point out the significant role of the genotype, that is, the breed of chickens for the character of changes in the tenderness of meat with the slaughter age. In concordance are the results of the study by *Wang et al. (2009)* showing no differences in the objective texture of white meat of slow-growing broilers reared in the facility and on free range. The opposite effects of slaughter age on meat tenderness are found in fast growing hybrids in relation to slow growing hybrids. Meat of fast-growing hybrids has the tendency of reducing tenderness with extension of the fattening period and weight gain, while in slow-growing races the opposite is determined (*Owens et al., 2006*). The explanation is in the muscle mass and differences in the proteolytic potential associated with the tenderness of the meat. In accordance with the above is a higher degree of tenderness of the Redbro broilers reared until the age of 67 days compared to Cobb broilers reared to the age of 53 days, in the study of *Fanatico et al. (2006).* Extensive rearing systems, especially with the access to range, due to the higher degree of activity of chickens, can result in hard meat in relation to the rearing systems indoors (*Castellini et al., 2002*), while in the study of *Owens et al. (2006)* this effect is related to the slow-growing chicken genotype.

Conclusion

Extensive rearing of Redbro chickens in the facility up to the age of 42 and 84 days resulted in changes in the chemical composition and physical characteristics of the meat. Slaughtering at the age of 84 days reduces the technological quality of meat due to poor water holding capacity, but on the other hand, the acceptability of meat for consumers, based on the determined target tenderness, has remained unchanged. Redbro chickens aged 84 days, as expected, have higher fat content in meat.

A positive aspect of the established higher fat content, especially in white meat, is the possible improvement in the sensory quality of the meat.

Efekat dužine tovnog perioda na kvalitet mesa Redbro pilića gajenih ekstenzivno u objektu

Zdenka Škrbić, Miloš Lukić, Veselin Petričević, Snežana Bogosavljević-Bošković, Vladimir Dosković, Simeon Rakonjac

Rezime

U razvoju alternativnih sistema gajenja u proizvodnji pilećeg mesa postavljeni ciljevi vezani su za zahteve tržišta, kvalitet proizvoda i efikasnost proizvodnje. Pored čistih i autohtonih rasa živine, proizvodnja u ovim sistemima se može zasnivati i na produktivnijim hibridima umerenog porasta u cilju dostizanja većih prinosa mesa. Za kvalitet mesa iz alternativne proizvodnje je ključna starost pilića za klanje koja se približava polnoj zrelosti. Međutim, klanje u starijem uzrastu pilića vodi i određenim nepovoljnim promenama kvaliteta mesa. Cilj ovog istraživanja je bio da se utvrdi značaj dužine tovnog perioda za kvalitet mesa pilića umerenog porasta, Redbro provenijence, gajenih extensive indoor, na osnovu hemijskog sastava i pojedinih fizičkih karakteristika mesa. Ukupno 300 Redbro jednodnevnih pilića je gajeno extensive indoor, 12 birds/m^2. Prva grupa je gajena do uzrasta 42 dana a druga, do 84 dana. Parametri kvaliteta mesa su ispitani na uzorcima belog (pectoralni mišići) i tamnog mesa (mišići bataka i karabataka) od 12 brojlera po tretmanu, sa jednakim učešćem polova. Utvrđen je osnovni hemijski sastav i parametri tehnološkog kvaliteta mesa (kalo kuvanja, kalo pečenja, water holding capacity, objective tenderness). Meso Redbro pilića starosti 84 dana je imalo lošiji water holding capacity u odnosu na uzrast 42 dana, koji je povezan i sa utvrđenim većim cooking loos or grilling loos 84. dana. Efekat dužine tovnog perioda na water holding capacity se nije negativno odrazio na tenderness mesa, s obzirom da je sila sečenja mesa bila na istom nivou 42. i 84. dana. Pilići starosti 84 dana su, očekivano, imali veći sadržaj masti u mesu. Razlike između 42 i 84 dana tovnog perioda u sadržaju masti su bile na nižem nivou signifikantnosti za belo meso, što može dovesti i do izvesnih poboljšanja u proceni senzornog kvalitet belog mesa.

Ključne reči: kvalitet mesa, Redbro pilići, ekstenzivan sistem u objektu, starost za klanje

Acknowledgment

This research is part of the Project EVB: TR-31033 financiallysupported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

References

AOAC. (2011): Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC.
BLAGOJEVIĆ M., PAVLOVSKI Z., ŠKRBIĆ Z., LUKIĆ M., MILOŠEVIĆ N., PERIĆ L. (2009): The effect of genotype of broiler chickens on carcass quality in extensive rearing system. Acta Veterinaria, 59, 1, 91-97.

CASTELLINI C., BERRI C., LE BIHAN-DUVAL E., MARTINO G. (2008): Qualitative attributes and consumer perception of organic and free-range poultry meat. World's Poultry Science Journal, 64, 500-512.

CASTELLINI C., MUGNAI C., DAL BOSCO A. (2002): Effect of organic production system on broiler carcass and meat quality. Meat Science, 60, 219–225.

DUCLOS M. J., BERRI C., LE BIHAN-DUVAL E. (2007): Muscle Growth and Meat Quality. Journal of Applied Poultry Research, 16:107–112.

FANATICO A. C., CAVITT L. C., PILLAI P. B., EMMERT J. L., OWENS C. M. (2005): Evaluation of Slower-Growing Broiler Genotypes Grown with and Without Outdoor Access: Meat Quality. Poultry Science 84:1785–1790.

FANATICO A. C., PILLAI P. B., CAVITT L. C., EMMERT J. L., MEULLENET J. F., OWENS C. M. (2006): Evaluation of Slower-Growing Broiler Genotypes Grown with and Without Outdoor Access: Sensory Attributes. Poultry Science 85:337–343.

FANATICO A. C., PILLAI P. B., EMMERT J. L., GBUR E. E., MEULLENET J. F., OWENS C. M. (2007): Sensory Attributes of Slow- and Fast-Growing Chicken Genotypes Raised Indoors or with Outdoor Access. Poultry Science 86:2441–2449.

GRAU R., HAMM R. (1952): Eine einfache Methode zur Bestimmung der Wasserbildung im Fleisch. Die Fleischwirtschaft, 4, 295-297.

HORSTED K., ALLESEN-HOLM B.H., HERMANSEN J.E. (2010): The effect of breed and feed-type on the sensory profile of breast meat in male broilers reared in an organic free-range system. British Poultry Science, 51: 515–524.

LAWRIE R.A., LEDWARD D.A. (2006): Lawrie`s Meat Science, 7th ed. Cambridge, England: Woodhead Publishing Limited.

LEE Y.S., XIONG R., SAHA A., OWENS C.M., MEULLENET J.F. (2008): Changes in broiler breast fillet tenderness, water-holding capacity, and color attributes during long-term freezing. Journal of Food Science, 73: E162-E168.

OWENS C., FANATICO A., PILLAI P., MEULLENET J., J. EMMERT (2006): Evaluation of alternative genotypes and production systems for natural and organic poultry markets in the U.S. Proceedings XII European Poultry Conference, Verona 10730.

RISTIĆ M., FREUDENREICH P., WERNER R., SCHÜSSLER G., KÖSTNER U., EHRHARDT S. (2007): Hemijski sastav mesa brojlera u zavisnosti od porekla i godine proizvodnje. Tehnologija mesa, 48, 5-6, 203-207.

Statistica-Stat Soft, Inc. version 8.0 (2008), www.statsoft.com

ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M., PETRIČEVIĆ V. (2013): Production performance and carcass quality of coloured broilers differentiated genetic potencial for growth. Biotechnology in Animal Husbandry, 29 (4), 615-624.

WANG K. H., SHI S. R., DOU T. C., SUN H. J.(2009): *)*. Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. Poultry Science, 88: 2219–2223.

THE EFFECT OF STORAGE TIME ON THE EGG QUALITY CHARACTERISTICS IN OLD LAYING HENS

Mirjana Đukić Stojčić, Lidija Perić, Siniša Bjedov

University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Srbija
Corresponding author: djukic@polj.uns.ac.rs
Original scientific paper

Abstract: The objective of this study was to evaluate the effects of storage time on the quality characteristics of eggs from old laying hens at the end of production. Eggs from 72-week old Bovans Brown laying hens were sampled immediately after being laid, and stored inside a refrigerator (5°C) for 1, 2, 3, and 4 weeks. After each period of storing the following most important parameters were evaluated: egg weight, shell breaking force, albumen height, Haugh units (HU), and yolk color. The egg weight, albumen height, Haugh unit, and yolk color were greatly influenced by the storage time. Statistically significant effect of storage time was found on egg weight ($P<0.05$), albumen height ($P<0.01$), HU ($P<0.01$) and yolk color ($P<0.05$). The results of the present study suggest that the most important parameters of egg quality, monitored in this study (egg weight, albumen height, Haugh unit), decrease with the storage period in old laying hens.

Key words: laying hens, egg, quality, storage

Introduction

Table eggs are universal and easily accessible food for all categories of people. The advantage of eggs in comparison with other foods is, among other things, that it can be stored in a refrigerator for a certain period of time. According to Council Regulation (EC) No 589/2008, table eggs should stay fresh if marketed for not longer than 28 days after laying. However, after a longer storage in the refrigerator, table eggs are losing their quality. As soon as a hen lays egg, its quality characteristics begin to fall.

This can be particularly noted for the internal quality of eggs, which depends on several factors, such as: the duration of the storage period (*Roberts, 2004; Jin et al., 2011*), the age of laying hens, genetic factors (*Silversides et al., 2001; Samli et al., 2005*), and nutrition (*Franchini et al., 2002*). One of the most important factors that affect egg quality during storage (among other such as humidity, the presence of CO_2…) is the temperature (*Morais et al., 1997; Menezes* *et al., 2009*). Albumen quality is an important indicator for freshness of egg, but and also for egg breaking industry (*Ahn et al., 1997).* According to *Samli et al*. (2005), storage time and temperature are the most crucial factors affecting albumen quality. It is well known that the albumen quality, egg weight, and albumen height directly affect HU (Haugh unit). Some authors evaluated the effect of storage temperature within different ages. *Santos (2005)* found that, at any age, the internal egg quality of the eggs is better when they are kept under refrigeration. The albumen height decreases with birds age (*Ramos et al., 2008*; *Menezes et al., 2012*), and therefore the increased age of birds decreases HU (*Silversides et al., 1993; Ramos et al., 2008*). *Menezes et al. (2012)* found statistically significant differences of the Haugh unit of eggs from hens between the 35, 40, 45, and 50 weeks of age. The highest quality for the eggs in refrigeration was found in birds at 35 weeks of age, and the lowest in birds at 50 weeks of age. These results can be attributed to physical and chemical reactions that lead to protein degradation.

The objective of this research was to determine the quality of table eggs from 72-week old Bovans Brown laying hens under different storage period (7, 14, 21, and 28 days) in the refrigeration under 5 ºC temperature.

Materials and methods

72-week old Bovans Brown laying hens were housed in battery cages on a commercial farm. Feed and water were offered *ad libitum*. The laying hens were fed a mashed diet, and all nutrient levels met the nutrient requirements suggested in the Bovans Brown Management Guide. The photoperiod was 16 hours light and 8 hours dark. Eggs from laying hens were sampled immediately after being laid (initial sampling period - 0 days), and stored inside a refrigerator (5°C) for 1, 2, 3, and 4 weeks. A random of 30 egg sample was selected from each replicate for analysis. Laboratory tests were performed on the Faculty of Agriculture in Novi Sad, Department of Animal Science, in the laboratory for determining poultry meat and egg quality. After each period of storing, the following most important parameters were evaluated: egg weight, shell breaking force, albumen height, Haugh units (HU), and yolk color.

The egg weight was measured with an electronic balance to the nearest 0.01 g. Shell breaking force was determined by instrument Egg Force Reader (Orka Food Technology Ltd, Israel). Albumen height was measured with a tripod micrometer. Haugh units score was calculated using the egg weight and albumen height (*Haugh, 1937*). The Haugh unit values were calculated for individual eggs using the following formula: $HU = 100*\log(H + 7.6 - 1.7W^{0.37})$, H = Observed height of the albumen in mm; W = Weight of egg in grams. Egg yolk colour was determined according to Roche yolk colour fan.

Statistical analyses were done in program STATISTICA 13 (*Stat Soft, 2016*) using ANOVA utilizing the GLM analysis. The model included the main effect of the storage times. When the effect of the main factor was significant, the means were separated using Duncan's test. Probabilities of less than 0.05 were considered significant for all analyses.

Results and discussion

The effects of different storage times on egg quality characteristics are presented in the Table 1. Egg weight constantly decreased from the first day of storage in refrigerator until the 28th day. Statistically significant difference ($P < 0.05$) in egg weight was found in third week, and after that the quality further deteriorated. In investigation from *Samli* (*2005),* egg weight was not significantly decreased by storage in refrigerator from 0 until 10 days.

The weight loss was in range between 1.03 and 1.50 grams, but no statistically significant difference was determined between four weeks of storage. Authors *Scott and Silverside (2000)*, also did not found the egg weight loss in the eggs stored for 10 days. Authors *Jin et al. (2011)* determined that egg weight was not significantly decreased by storage for 0 to 10 days at 5°C. However, these authors considered that a percentage of weight loss was significantly decreased with increasing storage time. This is opposite to the results of our research.

In the paper published by *Aykerek and Okur (2009); Tabidi (2011)*; *Chung and Lee (2014)*, the duration of the storage period had a significant influence on the egg weight loss. Increase in storage duration generally increased egg weight loss.

By measuring the shell breaking force it was observed that the shell strength ranged from 3.30 to 4.71 (average 3.87) in four weeks of storage. There was no statistically significant difference ($P > 0.05$) between shell strength in the different weeks of storage. In investigation from *Jones and Musgrove (2005*), there were also no differences observed in shell strength during storage. On the other hand, *Perić et al. (2017)*, pointed out that old hens (59-weeks) had significantly lower shell strength (3.63 kg) compared to the eggs from younger hens (4.31 kg). Authors *Navarro et al. (2002)* stated that eggshells from aged hens had a lower breaking strength compared to the eggs laid by young hens.

The albumen height significantly decreased with extended storage ($P < 0.01$), from first to the fourth week of storage. Albumen height was approximately 3 mm higher in initial sampling than at the end of experiment. These results are in agreement with those of *Scott and Silversides (2000)*; *Samli (2005),* which found significant decrease in albumen height due to storage time.

Table1. Effects of storage time on egg quality characteristics at 72 weeks of hen's age

Storage length, weeks	Egg weight (g)	Loss, (g)	Shell breaking force, (kg)	Albumen height, (mm)	Haugh units, (HU)	Yolk color
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
0	67.15 ± 3.98^{a}	-	4.71 ± 0.95	7.70 ± 1.25^{a}	85.14 ± 7.54^{a}	11.77 ± 1.39^{b}
1	66.79 ± 4.34^{ab}	1.29 ± 2.68	3.57± 0.67	6.37 ± 1.15^{a}	75.77 ± 8.89^{a}	12.54 ± 0.99^{a}
2	65.24 ± 4.48^{ab}	1.03 ± 0.91	3.30 ± 0.78	5.07 ± 0.82^{b}	66.17 ± 8.11^{b}	12.88 ± 0.82^{a}
3	65.71 ± 4.17^{b}	1.17 ± 0.66	3.42 ± 0.71	4.85 ± 0.96^{b}	63.54 ± 9.53^{b}	12.46 ± 0.95^{a}
4	63.69 ± 4.74^{b}	1.50 ± 0.58	4.36 ± 5.33	4.52 ± 0.98^{b}	61.17 ± 9.59^{b}	12.69 ± 1.05^{a}

[a-b] Different letters indicate significant differences between the means in each column ($P < 0.05$); ($\bar{x}$) - Mean, SD - Standard Deviation

Since HU is depending on the albumen height, it decreased in eggs from first until fourth week of storage (75.77 vs. 61.17). Significant ($P < 0,01$) negative effect on HU was determined after two weeks of storage. After the third week and in the fourth week the quality further deteriorated. *Silversides* and *Villeneuve (1994)* found that at 65 weeks of age, the value of HU was 77.40. Opposite to those investigations, *Aykerek and Okur (2009)* reported that HU decreased in 50 weeks of age from 81.53 to 32.55. *Barbosa et al. (2004); Menezes et al. (2012),* reported that the storage time affects the quality of eggs, exerting a negative influence on the Haugh unit. However, authors *Jin et al. (2011)* did not find a reduction in HU by keeping eggs in the fridge at 5°C for 0 to 10 d.

On the first day of measurements, the yolk color was 11.77, while in the next four weeks the yolk color significantly increased (12.54, 12.88, 12.46, 12.69 respectively). Results from *Jin et al. (2011)* show a significant darker yolk color after only two days of storage at 4^{o}C, similar to our study. In contrast to our study, *Perić et al. (2017)*, reported a reduction of yolk color during storage in older hens. *Maria Elena et al. (2006)* point out that under lower temperatures (4^{o}C) no changes in yolk color occurred, even after 30 days of storage.

Conclusions

The results of the present study suggest that the most important parameters of egg quality, monitored in this study (egg weight, albumen height, Haugh unit), decrease with the storage period in laying hens at the end of their production cycle. Generally it can be concluded that eggs from old laying hens have reductions of internal quality during storage period, but can be used four weeks, if kept under refrigeration.

Uticaj vremena skladištenja na karakteristike kvaliteta jaja starijih kokoši nosilja

Mirjana Đukić Stojčić, Lidija Perić, Siniša Bjedov

Rezime

Cilj ovog rada je bio da se proceni efekat vremena skladištenja na kvalitet konzumih jaja kod starih kokošaka, na kraju proizvodnog ciklusa. Jaja od 72-nedelja starih kokoška, hibrida Bovans Brauna su sakupnjena na komercionalnoj farmi i istog dana uzorkovana (uzorak od 30 jaja) i ostatak jaja čuvan u frižideru na temperature od 5 ° C, tokom četiri naredne nedelje. Nakon svakog perioda čuvanja od nedelju dana, mereni su sledeći najvažniji parametri kvaliteta jaja: masa jajeta, gubitak mase skladištenjem, sila loma, visina belanca, Hogove jedinice (HU) i boja žumanceta. Na masu jajeta, visinu belanca, Hogove jedinice i boju žumanceta uticalo je vreme skladištenja. Statistički značajan efekat skladištenja je utvrđen na masu jajeta ($P < 0{,}05$), visinu belanca ($P < 0{,}01$), HU ($P < 0{,}01$) i boju žumanca ($P < 0{,}05$).

Rezultati ovog istraživanja sugerišu da najvažniji parametri kvaliteta konzumnih jaja, koji se prate u ovom istraživanju (masa jajeta, visina belanca, Hogove jedinice), smanjuju se sa periodom skladištenja, kod koka nosilja na kraju proizvodnog ciklusa.

Ključne reči: kokoši nosilje, jaje, kvalitet, skladištenje

Acknowledgements

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31033.

References

AHN D. U., KIM S. K., SHU H. (1997): Effect of egg size and strain and age of hen on the solids content of chicken eggs. Poult. Sci. 76, 914–919.

AKYUREK H., OKUR A.A. (2009): Effect of storage time, temperature and hen age on egg quality in free range layer hens. Journal of Animal and Veterinary Advances 8, 1953-1958.

BARBOSA N.A.A., FREITAS E.R., SAKOMURA N.K. et al. (2004): Efeito da temperatura e do tempo de armazenamento na qualidade interna de ovos de poedeiras comerciais. Brazilian Journal Poultry Science 6, 60-65.

CHUNG H. S. H., LEE K.W. (2014): Effect of Hen Age, Storage Duration and Temperature on Egg Quality in Laying. International Journal of Poultry Science 13,11, 634-636.

FRANCHINI A., SIRRI F., TALLARICO N., MINELLI G., IAFFALDANO N., MELUZZI A. (2002): Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. Poultry Science, 81, 1744-1750.

HAUGH R. R. (1937): The Haugh unit for measuring egg quality U.S. Egg Poultry Mag. 43, 552-555, 572-573.

JIN Y. H., LEE K. T., LEE W. I., HAN Y. K. (2011): Effects of Storage Temperature and Time on the Quality of Eggs from Laying Hens at Peak Production. Asian-Aust. J. Anim. Sci. 24, 2, 279 – 284.

JONES D. R., MUSGROVE M. T. (2005): Effects of Extended Storage on Egg Quality Factors Poultry Science 84,1774–1777.

MARIA ELENA C. J., LEONOR S. G., EDUARDO M. B., SILVIA C. D., AVILA A. G., BENJAMIN F. M., MIRIAM R. P., FERNANDO P. G. R. (2006).: Shrimp head meal in laying hen rations and its effects on fresh and stored egg quality. INCL.

MENEZES P.C., CAVALCANTI V.F.T., LIMA E.R., NETO J.E. (2009): Aspectos produtivos e econômicos de poedeiras comerciais submetidas a diferentes densidades de alojamento. Revista Brasileira de Zootecnia 38, 11, 2224-2229.

MENEZES P. C., LIMA E. R., MEDEIROS J. P., OLIVEIRA W. N. K., EVÊNCIO-NETO J. (2012): Egg quality of laying hens in different conditions of storage, ages and housing densities. R. Bras. Zootec. 41, 9, 2064-2069.

MORAIS C.F.A., CAMPOS, E.J., SILVA, T.J.P. (1997): Qualidade interna de ovos comercializados em diferentes supermercados na cidade de Uberlândia. Arquivo Brasileiro de MedicinaVeterinária e Zootecnia 49, 365-373.

PERIĆ L., ĐUKIĆ STOJČIĆ M., BJEDOV S. (2017): The effect of storage and age of hens on the quality of table eggs. Animal Science and Biotechnologies 50, 1.

RAMOS K.C.B.T., FLOR H.R., CAMARGO A.M. (2008): Aspectos qualitativos de ovos comerciais armazenados em diferentes embalagens. In: Encontro latino americano de pós graduação, 8., , São Carlos, SP. Anais... São Carlos, SP, 1-4.

ROBERTS J.R. (2004): Factors affecting internal quality and egg shell quality in laying hens, Journal of Poultry Science, 41, 1661-177.

SAMLI H.E., AGMA A., SENKOYLU N. (2005): Effects of storage time and temperature on egg quality in old laying hens. Journal of Applied Poultry Research 14, 548-553.

SANTOS M.S.V. (2005): Avaliação do desempenho e qualidade dos ovos de poedeiras comerciais, submetidas às dietas suplementadas com diferentes óleos vegetais. Fortaleza, 174f. Tese (Doutorado em Zootecnia) - Universidade Federal do Ceará, Fortaleza.

SCOTT T. A., SILVERSIDES F. G. (2000): The effect of storage and strain of hen on egg quality. Poultry Science 79,1725–1729.

SILVERSIDES F. G., SCOTT T. A. (2001): Effect of storage and layer age on quality of eggs from two lines of hens. Poultry Science 80, 1240-1245.

SILVERSIDES F.G., TWIZEYIMANA F., VILLENEUVE P. (1993): Research note: a study relating to the validity of the Haugh unit correction for egg weight in fresh eggs. Poultry Science 72, 760-764.

SILVERSIDES F.G., VILLENEUVE, P. (1994): Is the Haugh unit correction for egg weight valid for eggs stored at room temperature? Poultry Science 73, 50-55.

TABIDI M. H. (2011): Impact of Storage Period and Quality on Composition of Table Egg, Advances in Environmental Biology 5, 5, 856-861.

POSTER SECTION III

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

COMPARATIVE STUDY OF FATTENING AND SLAUGHTER PROPERTIES OF FEMALE SIMMENTAL CATTLE AND CROSSES OF SIMMENTAL AND CHAROLAIS

Maja Petričević, Aleksandar Stanojković, Dušica Ostojić-Andrić, Dragan Nikšić, Veselin Petričević, Marija Gogić, Violeta Mandić

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia
Corresponding author: majanovakovic@live.com
Original scientific paper

Abstract: In this paper, the slaughtering properties, conformation score and fat tissue cover of carcass are presented, as well as the composition of female carcass sides of two genotypic groups: domestic Simmental breed (A) and its crosses with Charolais breed (B). The sample included a total of 20 heads, 10 in each group. The animals were slaughtered at pre-slaughter weight of about 480 kg. After slaughtering, individual measurements of warm carcass sides with and without kidney fat were performed. After the cooling, the left carcass side was cut into the basic parts according to the Rulebook ("Official Gazette SFRY", No. 34/74, 26/75, 13/78 - Rulebook, 1/81 - Rulebook and 2/85 - Rule book). The obtained results of the study show that the bovine groups (A) achieved statistically significantly ($p<0.01$) higher warm carcass yield with and without fat in comparison with group B. Statistically significant difference was not established in the share of the accompanying slaughter products, the conformation of carcasses and share of main carcass parts.

Key words: slaughter properties, composition of carcass sides, carcass conformation

Introduction

Weight gain, nutrition and quality of the carcass are of great economic importance for beef producers. Changes occurring in these properties are attributed to differences in genotype, nutrition and gender (*Bureš and Bartoň, 2012*). Fattening and slaughtering characteristics are the basic characteristics of each breed which have impact on the quantity and quality of the final products. On the basis of data on body development, the quantity of bones in the carcass, the bone : meat ratio, and the total amount of meat can be predicted with more or less accuracy

(*Ostojić-Andrić et al., 2007*). The conformation and coverage of the carcass with fatty tissue, colour and texture of the meat are characteristics that are responsible for the quality of the carcass (*Reddy et al., 2015*).

The conformation and the fatty cover of the carcass are important criteria in modern systems of quality assessment of the carcass quality (classification of EUROP). In this way, the corresponding result is realized in accordance with the requirements of the market. Consumer behavior is influenced by information on the origin, appearance, nutritional value, health effects and sensory characteristics of the meat.

A fast and economical way to improve the fattening and slaughtering properties of cattle is achieved by crossing the Simmental breed as the basis with specialized fattening/meat breeds (*Bogdanović et al., 2005)*. French fattening breeds are characterized by favourable fattening and slaughtering qualities as well as good quality of meat with a small fraction of fat tissue in the carcasses (*Petričević et al., 2015*).

Materials and Methods

The trial was performed at the experimental farm of the Institute for Animal Husbandry (Belgrade, Serbia). Two groups of female calves were formed: group A (n = 10) Simmental breed and group B (n = 10) F1 generation crosses of this breed with Charolais. Both groups of cattle were fed ad libitum with a combined diet consisting of a mixture of the whole maize silage according to the nutrition table depending on the weight group. The final pre-slaughter weights were about 470 kg. One day before slaughter, the cattle did not receive food, but they had free access to water. Slaughter and primary processing were performed in the experimental slaughterhouse of the Institute for Animal Husbandry. Animals were measured before slaughter. After primary processing, the weight of the warm carcass was measured, the weight of the intestines (heart, lung, liver, kidneys, spleen), tongue, head, tail and kidney fat. After primary treatment, the carcasses were cooled to 4^0C for the next 24 hours. After chilling, the carcasses were measured and split along the vertebral column in two halves, and the left side was used for all measurements. The left side of each carcass was divided into twelve anatomical regions: round, beef steak, loin, shoulder, back, neck, chest, blade, ribs, flank, foreshank and shin, using a standard technique. Evaluation of the carcass side was determined on the basis of two systems: JUS (carcass conformation and carcase covering with fatty tissue - shown in grades 1 to 5, where 1 is very low 2-low, 3-average, 4-high or 5-very high) and EUROP (carcass conformation and carcass cover with fat tissue are shown in grades 1 to 5, where 5- (E) -excellent, 4-(U) -very good, 3- (R) -good, 2- (O) -moderate and 1- (P) - poor).

The obtained data were processed by using the analysis of variance in one-way ANOVA program SPSS Statistics 20. The statistical significance of the differences between mean values was determined by t-test.

Results and Discussion

The average values of the slaughtering results are shown in Table 1. The results of the study show that statistically significantly ($p<0.01$) higher yield of warm-carcass with and without kidney fat was achieved by the cattle in group (A), by 3.22% and 1.39% in relation to the cattle group (B).

Table 1. Average values of slaughter properties of cattle

Indicators	Cattle group		Singificance of differences
	A	B	t-test
Pre-slaughter weight (kg)	477.25 ± 7.27	467.33 ± 8.10	ns
Daily gain (g)	910.25 ± 123.14	1213.33 ± 127.27	ns
Warm carcass weight (kg)	280.18 ± 8.71	259.07 ± 11.67	ns
Warm carcass yield (%)	58.70 ± 1.16	55.48 ± 0.78	**
Warm carcass weight without fat (kg)	268.90 ± 6.02	248.20 ± 3.66	ns
Warm carcass yield without fat (%)	56.35 ± 0.73	54.96 ± 1.89	**
Kidney fat	2.34 ± 0.54	1.73 ± 0.46	ns

ns – not significant
** significant at the level of ($p<0,01$)

Bureš and Bartoň (2012), on the basis of their research, state that the weight of warm carcasses of the Simmental cattle breed is 299.6 kg, while *Petrićević et al. (2015)* indicate the value of 292.21 kg for the same property. In the research of *Petrićević et al. (2015),* the values of the warm carcass yield of 57.92% and the share of kidney fat of 1.97 kg are determined for the female cattle of the Simmental breed. *Pečiulaitienė et al. (2015)* report that the female cattle of the Simmental breed weighing pre-slaughter 456.19 kg have achieved a yield of 51.34%.

The share of the accompanying slaughtering products did not differ significantly between the groups of cattle (Table 2). The share of liver (1.29%) and skin (7.46%) was higher in group (B), but there was no statistical significance between identified differences. From Table 2 it can be seen that the average share of internal organs was approximately the same in both groups of studied cattle. *Aleksić et al. (2002),* in their study, report the value of the liver (1.16%), spleen (0.37%), kidney (0.15%) and heart (0.37%) for the young bulls of the Simmental breed, which is similar to the results obtained in our research. *Petričević et al. (2011)* report following values of the share of internal organs in Simmental bulls: liver (1.12%), lung (0.76%), spleen (0.19%), kidney (0.18%), tongue (0.35%) and heart (0.37%), which is also in line with the results of our research.

Table 2. The share of the accompanying slaughtering products (%)

Name	Cattle group		Significance of differences
	A	B	t-test
Liver	1.20 ± 0.09	1.29 ± 0.21	ns
Lungs	0.54 ± 0.06	0.58 ± 0.10	ns
Spleen	0.21 ± 0.05	0.18 ± 0.04	ns
Kidneys	0.21 ± 0.01	0.20 ± 0.02	ns
Tongue	0.27 ± 0.05	0.24 ± 0.04	ns
Heart	0.41 ± 0.05	0.39 ± 0.01	ns
Head	2.52 ± 0.26	2.30 ± 0.06	ns
Tail	0.19 ± 0.05	0.25 ± 0.04	ns
Skin	7.09 ± 0.53	7.46 ± 0.59	ns

ns – not significant

Differences in the carcass conformation score of female cattle showed no statistical significance between groups (Table 3). Evaluation of the conformation and coverage of the carcass with fat tissue is of great importance in modern systems of quality assessment of carcasses *(Petričević et al., 2015).* Crosses of Charolais with domestic Simmental breed had carcass conformation score of 3.94 and covering of round with fat tissue of 3.77 *(Ostojić-Andrić et al., 2011). Karolyi et al. (2006)* have established favorable carcass conformation in heifers of the Simmental breed, of which about 1/3 of the carcasses are rated the highest E class (classification according to the EUROP system). The covering of carcass by fat tissue in heifers was less favourable and classified as highly fatty (class O) and therefore less valuable.

Table 3. Carcass conformation score using two systems (JUS and EUROP)

Trait	Cattle group		Significance of differences
	A	B	t-test
	JUS		
Conformation	4.00 ± 0.01	3,83 ± 0.29	ns
Fat cover	4.00 ± 0.02	4.17 ± 0.29	ns
Meat colour	4.38 ± 0.29	4.67 ± 0.28	ns
	EUROP		
Conformation	4.00 ± 0.01	3,83 ± 0.27	ns
Fat cover	4.00 ± 0.02	4.00 ± 0,01	ns
Meat colour	4.62 ± 0.48	4.67 ± 0,58	ns
Fat tissue colour	4.00 ± 0.20	4.07 ± 0.19	ns

ns – not significant

Pečiulaitienė et al. (2015) state that the muscle tissue content in the carcass increases with the increase in pre-slaughter weight. By increasing pre-slaughter weight of 500 kg by 50 kg, a moderate increase in muscle tissue of 0.9% in the carcasses of Simmental breed heifers of class U was obtained. The same authors also report a moderate increase in muscle tissue of 16.5% in carcasses of R-class heifers. All analyzed groups of animals (bulls, heifers and cows) according to the assessment of conformation are mainly classified in O class. They have registered a trend that with the increase of weight and increase of animals' pre-slaughter age, the meat yield is improved and the muscularity of the carcasses increases. *Ostojić-Andrić et al. (2011)* have found in their researches that the crosses of Charolais with the domestic Simmental breed have had the carcass conformation score of 3.94 and the covering of carcass with fat tissue 3.77, which is approximate to the results of our investigation.

The share of main carcass side parts of the females did not statistically significantly differ between groups (Table 4). The share of round (29.71%) was higher in group B, while the other parts of the carcass side (beefsteak, back-loin part, shoulder) had lower values than group A. Similar results are reported by *Karolyi et al. (2008)* in their research. The authors state that the share of the category I carcass (round) was 30.62%, the share of the back 8.81% and the shoulder 13.84%, which is similar to the results achieved in our research.

Table 4. The share of main carcass side parts of the cattle

Carcass side parts (%)	Cattle group		Significance of differences
	A	B	t-test
Beefsteak	1.10 ± 0.08	1.01 ± 0.04	ns
Round	28.65 ± 1.24	29.71 ± 0.12	ns
Shin	3.66 ± 0.23	3.91 ± 0.51	ns
Back-loin part	10.72 ± 1.21	10.15 ± 0.16	ns
Shoulder	12.88 ± 3.16	11.00 ± 0.80	ns
Shank	2.98 ± 0.64	2.86 ± 0.47	ns

ns – not significant

Conclusion

Based on the results of the study, it can be concluded that there is no statistically significant influence of the genotype on slaughter properties, the share of individual parts of the carcass side and the accompanying slaughter products. Based on the results presented in this paper, it can be concluded that the cattle groups (A) and (B) did not differ statistically significantly in the carcass conformation evaluation. A statistically significant difference was found for the yield of warm carcass side with kidney fat, which amounted to 58.70% and of the warm carcass side without kidney fat 56.35%, in the case of the bulls of group (A). The share of the main parts of the carcass side did not differ statistically significantly among the groups of studied cattle.

Uporedno ispitivanje tovnih i klaničnih osobina junadi ženskog pola simentalske rase i meleza šarolea sa simentalskom rasom

Maja Petričević, Aleksandar Stanojković, Dušica Ostojić-Andrić, Dragan Nikšić, Veselin Petričević, Marija Gogić, Violeta Mandić

Rezime

U radu su prikazane klanične osobine, ocena konformacije i prekrivenosti trupova masnim tkivom i sastav polutki junadi ženskog pola dve genotipske grupe:

domaće simentalske rase (A) i njenih melaza sa šarole rasom (B). Uzorkom je obuhvaćeno ukupno 20 grla, po 10 u svakoj grupi. Grla su zaklana pri dostizanju težine oko 480 kg. Nakon klanja obavljeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Posle hlađenja leva polutka je rasecana u osnovne delove prema pravilniku ("Sl. list SFRJ", br. 34/74, 26/75, 13/78 – dr. pravilnik, 1/81 – dr. pravilnik i 2/85 – dr. Pravilnik). Dobijeni rezultati istraživanja pokazuju da su junad grupe (A) ostvarila statisticki značajno (p<0,01) veći randman toplog trupa sa i bez loja u poređenju sa grupom B. Statistički značajna razlika nije utvrđena kod udela pratećih proizvoda klanja, ocene konformacije trupova i udela osnovnih delova trupa.

Ključne reči: klanične osobine, sastav polutki, konformacija trupa

Acknowledgements

This research was financed by the Ministry education and Technological Development, Republic of Serbia, project TR 31053.

References

ALEKSIĆ S., MIŠČEVIĆ B., PETROVIĆ M.M., PAVLOVSKI Z., JOSIPOVIĆ S., TOMAŠEVIĆ D. (2002): Ispitivanje faktora značajnih za rezultate vrednosti radmana klanja muške tovne junadi domaće simentalske rase i meleza domaće simentalske rase sa limuzinom.Biotehnology in Animal Husbandry 18 (3-4): 9–15.

BOGDANOVIĆ V., ĐEDOVIĆ R., PERIŠIĆ P., PETROVIĆ M.M. (2005): Cattle breeding goals and programmes in Serbia. Biotechnology in Animal Husbandry, 21, 1, (5-6): 15–21.

BUREŠ D., BARTOŇ L. (2012): Growth performance, carcass traits and meat quality of bulls and heifers slaughtered at different ages. Czech J. Anim. Sci., 57 (1): 34–43.

KAROLYI D., ĐIKIĆ M., SALAJPA K., ČUBRIĆ ČURIK V., JURIĆ I. (2006): Carcass traits of baby beef Simmental cattle. Meso, 7 (6): 346–350.

KAROLYI D., ĐIKIĆ M., SALAJPA K., ČUBRIĆ ČURIK V., JURIĆ I. (2008): Composition of carcass cuts in young Simmental bulls and heifers. Acta agriculturae Slovenica, (2): 65–70.

OSTOJIĆ-ANDRIĆ D., ALEKSIĆ S., PETROVIĆ M.M., HRISTOV S., PANTELIĆ V., NOVAKOVIĆ Ž., NIKŠIĆ D. (2011): The effect of crossing of domestic Simmental breed and french fattening breeds on conformation and fat cover of beef carcasses. Biotechnology in Animal Husbandry 27 (2): 137–145.

OSTOJIĆ-ANDRIĆ D., BOGDANOVIĆ V., ALEKSIĆ S., PETROVIĆ M. M., MIŠČEVIĆ B., PANTELIĆ V., JOSIPOVIĆ S. (2007): Uticaj genotipa na tovne osobine i telesnu razvijenost junadi. Biotechnology in Animal Husbandry 23 (3-4): 31–39.

PEČIULAITIENĖ N., JUKNA V., MEŠKINYTĖ-KAUŠILIENĖ E., KERZIENĖ S., MOLEIKAITIENĖ S. (2015): Efects of weight and age on carcass yield and conformation of cattle. Biotechnology in Animal Husbandry 31 (1): 73–84.

PETRIČEVIĆ M., ALEKSIĆ S., PETROVIĆ M. M., PANTELIĆ V., OSTOJIĆ-ANDRIĆ D., STANIŠIĆ N., NIKŠIĆ D. (2015): Comparative study of fattening and slaughter traits of male simmental breed and crosses with charolais breed. Biotechnology in Animal Husbandry 31(2): 265–272.

PETRIČEVIĆ M., ALEKSIĆ S., STANIŠIĆ N., NIKŠIĆ D., STANOJKOVIĆ A., PETRIČEVIĆ V., GOGIĆ M., MANDIĆ V. (2015): Comparative testing of slaughter traits and meat quality of male and female Ssmmental cattle. Biotechnology in Animal Husbandry 31(3): 375–383.

PETRIČEVIĆ M., STANIŠIĆ N., SRETENOVIĆ LJ., PETROVIĆ M. M., STAJIĆ S., NIKŠIĆ D. (2011): Properties and composition of carcass of domestic spotted young cattle of two preslaughter weights. Biotechnology in Animal Husbandry 27 (4): 1443–1450.

REDDY V. B., SIVAKUMAR S. A., JEONG W. D., WOO Y. B., PARK S. J., LEE S. Y., BYUN J. Y., KIM C. H., CHO S. H. , HWANG I. (2015): Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. Animal Science Journal, 86: 1–16.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

SLAUGHTERHOUSES, POSSIBLE SOURCE OF BACTERIA

Snežana Ivanović, Ksenija Nešić, Boris Pisinov, Ivan Pavlović

Institute of Veterinary Medicine of Serbia, Autoput 3, 11070 Belgrade, Serbia
Corresponding author: snezaivanovic57@gmail.com
Review paper

Abstract: Slaughterhouses are objects in which animals are slaughtered and meat products are produced respecting the principles of hygiene and technology and under the veterinary-sanitary control. The main purpose of the slaughterhouse is the production of meat for human consumption. Technological operations, such as skinning and evisceration, are critical points where bacteria contaminate carcases, but are also transferred to equipment, walls, floors, aprons and boots of workers. If complete cleaning, washing and disinfection are not performed, microorganisms survive in slaughterhouses, reproduce and become ready for the subsequent contamination of meat and meat products. In addition to this requirement, microorganisms have the ability to develop a defence mechanism to disinfection and thus survive. The aim of this paper is to present the microbial resistance to disinfection and their survival in the slaughterhouse where goats are slaughtered.

Key words: slaughterhouses, bacteria, disinfectant, biofilm

Introduction

Slaughterhouses are objects in which, while respecting the principles of hygiene and technology and under the veterinary-sanitary control, animals are slaughtered and meat products are produced. The main purpose of the slaughterhouse is the production of meat for human consumption.

As with all other animals, goats and sheep at the slaughter could be a major source of the bacteria which are distributed on the skin of an animal and in the digestive tract. These are mainly pathogens belonging to enterobacteria (*Salmonella spp.*, *Escherichia coli*, *Proteus spp.*). Besides the enterobacteria some Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus species*, *Listeria monocytogenes*, *Lactobacillus spp.*, *Bacillus spp.*) could be found during the process. Within Enterobacteria greatest importance is given to the genus

Salmonella spp. (*Humphrey, 2000, Botteldoorn et al., 2003, Ivanovic et al., 2009, 2014*).

Technological operations, such as skinning and evisceration, are critical points where bacteria contaminate carcases, but are also transferred to equipment, walls, floors, aprons and boots of workers. Equipment in the slaughterhouse is numerous, various and includes conveyors, tanks, equipment for cooling and freezing, skinning, accessories for cutting. All such equipment is complex, with narrow openings, accessible for dirt, but inaccessible for washing, cleaning and disinfection. So it is not surprising if we find bacteria on equipment that is washed and disinfected.

Using these obstacles with the help of its own defence mechanisms, microorganisms survive in slaughterhouses, reproduce and are ready for the subsequent contamination of meat and meat products. In this way they circulate. The aim of this paper is to present the microbial resistance to disinfection and their survival in the slaughterhouse where goats and sheep are slaughtered.

Susceptibility of bacteria to disinfectants

According to the Codex Alimentarius disinfection is use of chemical agents and/or physical methods for reduction of the number of microorganisms in the environment to the level that does not threaten food safety and usability. Different types of microorganisms differ in susceptibility to disinfectants due to differences in their cell structure, chemical composition and physiological processes (*Vučinić et al., 2004*).

Bacteria are susceptible to standard disinfectants. Based on the chemical origin and mechanisms of action, disinfectants are divided into: acids, bases, salts, heavy metals, chlorine preparations, cresols and phenols, alcohols, iodophores, surfactants and gaseous disinfectants (*Hrgović, 1986, Ivanović et al., 2012*).

Susceptibility

When considering the mechanisms of susceptibility of microorganisms to disinfectants, it is necessary to point out that there are two types of resistance of microorganisms in relation to chemical agents used for their inactivation. These are the natural resistance (tolerance) and acquired resistance (resistance). It is accepted view that resistance is a form of acquired resistance resulting from changes in the genetic material, i.e. microbial genome (a nucleic acid). However, the resistance implied and acquired forms of bacterial resistance which is result of phenotypic adaptation to the conditions of life, as is the case with the biofilm. Mechanisms of resistance of microorganisms are:

- natural microbial resistance to disinfectants (tolerance) and acquired resistance of microorganisms to disinfectants (resistance);
- resistance of microorganisms to sublethal doses of disinfectants;
- the formation of biofilms and
- creating pseudobiofilm and interaction among remedies for sanitary cleaning and disinfection.

Sublethal/subinhibiting doses of disinfectant as a cause of resistance

In a population of microorganisms, there are different subpopulations of various sensitivity to disinfectants. When some of the chemicals affect these populations, they inactivate microorganisms sensitive to the used remedy. There remain two more subpopulations: the subpopulations of microorganisms on which a disinfectant achieved a germistatic effect, i.e. only stopped their growth and a subpopulation of microorganisms that disinfectant does not affected at all. For the population to which the disinfectant acted germistaticly it takes some time to recover, after which they will freely reproduce. So, these last two subpopulations are able to continue to grow and reproduce. At a certain area where the disinfectant was used, they will multiply and create a resistant population. The same happens if the disinfectant is used in sublethal/subinhibiting concentrations, and if more water is left behind on surfaces that have been subjected to sanitary washing as the preparations for disinfection. If a disinfectant does not inactivate bacterial cell, then it activates its mechanisms of defence, recovery and repair damage, such as:

- production of neutralizing enzymes that break down disinfectants;
- repair damage to nucleic acids;
- reduced permeability of bacterial lipopolysaccharide layer of cells wall for disinfectants;
- reduced permeability of pores for disinfectants and
- enhanced efflux of disinfectants in bacterial cells.

It is believed that all these mechanisms of defence have the genetic-molecular basis and it is not just about changing the genetic code towards the synthesis of neutralizing enzymes, but also the synthesis of new structural and functional proteins; the change in conformation of the protein molecules in the structure of the membrane and the wall of the bacterial cell, as well as the change in fatty acids in the bacterial wall.

The consequence of production of the neutralizing enzymes (peroxidase, catalase, glutathione reductase) is a phenomenon of resistance to the peroxides. As a further consequence of the bacterial resistance to peroxides is cross-resistance to heat, ethanol and hypochlorous compounds. This type of resistance has been described in *Escherichia*, *Salmonella* species, *Campylobacter, Haemophilus* and *Bacillus* species (*Mcdonnell and Russell, 1999, Vučinić et al., 2004*). The resistance of microorganisms to sublethal/subinhibiting dose of disinfectant is a form of acquired resistance.

The formation of biofilms and pseudobiofilms

The formation of biofilms is one of the mechanisms of resistance of microorganisms to disinfectants. The biofilm formation (Figure 1) is regulated by different genetic and environmental factors. Genetic studies have shown that bacterial mobility, cell membrane proteins, extracellular polysaccharides and signalling molecules play significant roles in biofilm formation (*Watnick and Kolter, 2000*).

Bacterial mobility is enabled by two types of protein growths on the cell surface, flagella and fimbriae. Flagella are long, spiral growths that enable bacteria to float in liquid medium, and fimbriae are short, straight growths that enable limited, twitching movements of bacteria on substrate surface (*Maric and Vranes, 2007*).

It is a mixture of microorganisms, food components and exopolysaccharides (EPS), substances produced by microorganisms that are bound to the solid surface. They are by nature very sticky and are attached to the surface along with the bacteria, and are particularly well adhered and stay long on the metal surfaces. The formation of biofilms, in fact, presents the ability of bacteria to linger on wet surfaces and grow into microcolonies in areas where flow water, organisms and firm surface exist (*Boulange-Petermann, 1996; Janković et al., 2009; Kokare et al.,2009*). Bacteria in the biofilm not need to be pathogenic, but they easily adhere.

Biofilms are complex structures composed of associated bacterial populations attached to surface. They can be made up from populations that have evolved from one bacterial species or communities of several types and can be formed in a number of substrates. In nature, biofilm is forming by rod-shaped bacteria, then to them is joined stem and filamentous and silicate algae. The biofilm forming process consists of several stages (Figure 1).

First, the bacteria attached temporarily to an appropriate carrier (step 1). After some time, they remain attached to surface and begin forming microcolonies (step 2). Then, bacteria are growing, reproducing itself and mutually connecting (step 3 and 4). Afterwards, biofilm matures, some other bacteria, algae, fungi can join it. At the end, cells separate from biofilm, and biofilm separate from surface (step 5).

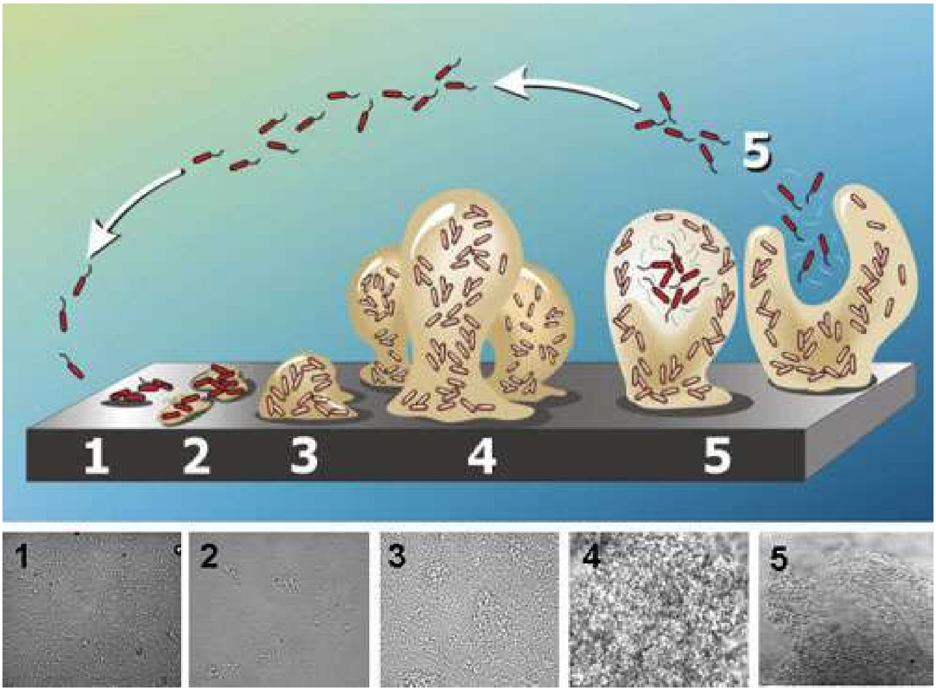


Figure 1. The biofilm forming process

In biofilm, bacteria communicate to each other via a mechanism called *quorum sensing* and in this way regulate the expression of genes. The expression of genes is process by which the information of a gene is used for synthesis of functional gene product. Often, these products are proteins. The bacteria involved in *quorum sensing* communication create specific chemical signalling molecules (autoinducers or pheromones). Biofilm provides affordable micro-habitat for bacteria. Communication in cells and exchange of genetic material is more common in biofilm. Also, it protects the bacteria from toxic substances, physical force, macrophages.

The biofilm formation is of particular importance to the meat industry. It is difficult to remove it from surfaces on which it was created, and is resistant to the detergents, i.e. to almost all substances used for sanitary washing (*Neting, 2001; Vučinić et al., 2004*). Bacterial resistance to disinfectants resulting from formation of biofilms is a form of physiological phenotypic adaptation. Biofilm consists of viable and nonviable microorganisms coated by polyanion extracellular substances attached to the surface.

Extracellular polysaccharide matrix (EPS) has a significant role in biofilm formation. Research respective authors showed that the bacteria lose ability to form biofilm if the genes responsible for synthesis of EPS matrix are inactivated. Interactive communication via signalling molecules enables bacteria to organize into a community so that the biofilm functions as a multicellular organism.

Studies on *Listeria monocytogenes* biofilm formation showed that a too low or too high level of phosphates in the environment reduces biofilm formation, while the presence of carbohydrates mannose and trehalose stimulates biofilm formation (*Kim and Frank,1995*). Biofilm formation in *E. coli* is regulated by the presence of oxygen. In case of insufficient oxygen supply biofilm does not form, since bacteria cannot adhere to substrate surface (*Landini and Zehnder, 2002*). Studies on the influence of temperature on *L. monocytogenes* showed that biofilm did not form if temperature was high, because the process of connecting bacteria to substrate surface was inhibited (*Gorski et al., 2003*). Environmental pH is also important for biofilm formation, which was shown by studies carried out on *V. choleare*. Optimal pH for multi-plication of *V. cholerae* is 8.2, and if pH value is less than 7, that is if the solution is acid, the ability of these bacteria to form a biofilm is reduced due to the fact that bacterial cells lose their mobility (*Hommais et al., 2002*). Unlike *V. cholerae,* bacteria *S. epidermidis* and *E. coli* do not need alkaline environment to multiply, so they can form a biofilm on urethral catheters where urine pH is acidic.

Extracellular polymeric substances (EPS) include polysaccharides, proteins, phospholipids, teichonic and nucleic acids, long polymeric substances and water in an amount of 85-95 %. Extracellular polymeric substances protect the microorganisms in the biofilm from disinfectants, metal ions, toxins and drying and they concentrate the nutrients needed by bacteria (*Lindberg et al., 2001; Vućinić et al., 2004*). Biofilm on accessories, equipment and work areas in a slaughterhouse, contains large amounts of organic and mineral nutrients originating from the raw materials themselves, but also from the water used in the production process.

The biofilm is an organized collection of microorganisms within the exopolymer polysaccharides. In relation to the type of microorganisms, biofilm may be composed of a monoculture, several different types or different phenotypes (strains) of the same bacterial species (Figure 2). The biofilm may be of a high level of organization, to contain one or more species of microorganisms, to be of the single- or three-dimensional shape, as is the case for the creation of aggregates (flocks and granules). A greater number of microorganisms in the biofilm are bonded to the surface - sessile organisms, although there are present free organisms that make biofilm plankton.

In addition to these factors, the formation of biofilms is affected by the composition of the substrate, the chemical composition of the substrate on which is biofilm formed, surface topography (smooth surface, scratched and rough surfaces, etc.) and water flow. Besides the working surfaces, in the food industry a biofilm is often created in waterducts, where it can be a source of interference in the flow of water and also cause corrosion of metal surfaces (*Lindberg et al., 2001; Vućinić et al., 2004*). In the meat industry the formation of biofilms on the working surfaces is one of the causes of foodborne infections and intoxications.

In different parts of the biofilm, bacteria have disparate access to nutrients so that their physiological characteristics are changed. In the inner part of the biofilm, due to the limited amount of nutrients, the growth of bacteria is slowed down. For sessile microorganisms also, because of their immobility, access to nutrients is relatively limited. That is why they are different from planktonic bacteria that float freely in the superficial parts of the biofilm. The presence of *Listeria monocytogenes* (*Ivanović et al., 2009*), *Pseudomonas spp*., *Bacillus spp*. and *Salmonella spp*. has been confirmed in the biofilm. There are several reasons for reduced susceptibility to disinfectants in biofilm:

- reduced penetration of disinfectants to bacteria within the biofilm, which may be affected by the type of disinfectant, disinfectant binding capacity for biofilm glycocalyx and by speed of the growth of colonies of microorganisms in relation to the degree of diffusion of disinfectants;
- chemical interaction between biofilm and disinfectant;
- changes in the microenvironment that are associated with nutrient limitation and growth rate of bacteria;
- production of enzymes that break down antiseptics and disinfectants or chemicals that perform the neutralization of disinfectants and
- genetic changes in the bacteria within the biofilm formed by exchanging plasmids.

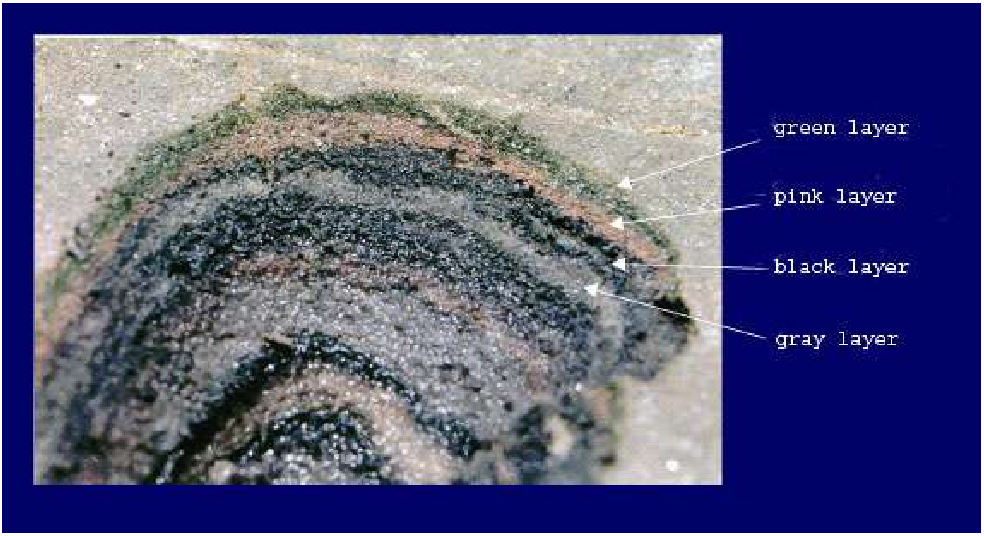


Figure 2. The biofilm structure organization

The bacteria which are isolated from the biofilm and cultured in nutrient media become sensitive to disinfectants again, as well as planktonic bacterial cells in the laboratory cultures. The removal of organic matter and moisture and good maintenance of working areas besides the use of appropriate water temperature for cleaning of work surfaces and equipment are of crucial importance in preventing the formation of biofilms in the slaughterhouse. It should be noted that it is necessary to properly combine cleaning and washing, because some components together may be incompatible and some inefficient alone.

The formation of biofilms on the worn and damaged equipment and work surfaces is a common problem. However, it is often formed in the channels and on the walls of the slaughterhouse. Aerosols which arise during or after the sanitary washing are one of the methods of dispersion of bacteria from the biofilm through the plant. The aerosol is formed during washing and spraying of fluids to work surfaces, but also during the drying. These are two moments that should be carefully considered in the process of preventing the formation of biofilms.

Pseudobiofilm is a similar phenomenon that has been associated with bacterial resistance to disinfectants (*Mcdonnell and Russell, 1999; Vucinic et al., 2004*). Cases of creating pseudobiofilm are described in medicine and related to diagnostic, surgical and additional medical equipment which is not properly cleaned before the sterilization by glutaraldehyde.

Interactions of remedies for sanitary washing and disinfection

Many remedies for sanitary washing are uniogen surfactants (emulsifiers and detergents), anionic surfactants or a mixture of both types of surfactants. Uniogen surfactants are neutral in the solution, while anionic surfactants and negatively charged. When detergents are applied to dirty vertical surfaces, in 15 to 20 minutes all liquid slips down. However, a small amount of detergent is still maintained at the surface, which is mostly anionic surfactant. If the surface is not thoroughly rinsed after the action of detergents, and quaternary ammonium compounds were applied as disinfectants, which are cationic surfactants, it may lead to complete inactivation of the disinfectant.

In the interaction between quaternary ammonium compounds and anionic surfactants, residues are formed of these two compounds or the film which do not have a germicidal activity. Complex disinfectant-detergent, without germicidal activity, also contains nutrients suitable for microbial growth and helps their proliferation if left undetected (*Mcdonnell and Russell, 1999; Vucinic et al., 2004*).

Conclusions

Disinfection is reduction by chemical agents and/or physical methods, number of microorganisms in the environment at a level that does not endanger the safety of food or its usability.

Different types of microorganisms differ in sensitivity to disinfectants precisely because of differences in cellular structure, chemical composition and physiological processes that take place within their organizational units. If disinfectant not inactivated bacterial cell, then it activates its defences mechanisms, recovery and repair damage.

It is believed that the resistance is form of acquired resistance resulting due to changes in the hereditary material, i.e. microorganisms genome (nucleic acid). However, under the resistance are include and acquired forms of bacterial resistance resulting phenotypic adaptation to living conditions, as is the case with the biofilm.

The biofilm represents a specific life form of microorganisms which provides not only efficient protection from negative outside influence, but also physically and chemically suitable microenvironment necessary for growth and survival.

Creating a biofilm on the worn and mechanically damaged working areas and equipment regularly presents a problem.

However, it frequently generated in the channels and on the walls of slaughterhouses. Aerosols that occur during and after sanitary washing is one of the ways of bacterial dispersion from the biofilm through facility. Aerosol is formed during washing and spraying fluids on working areas, as well as during surface drying. Exactly, these two moments should be observed in the process of preventing the creation of biofilm.

Klanice, mogući izvor bakterija

Snezana Ivanovic, Ksenija Nesic, Boris Pisinov, Ivan Pavlovic

Rezime

Klanice su mesta na kojima se vrši klanje životinja i obrada njihovog mesa po higijenskim principima, propisanim tehnološkim uslovima i pod veterinarsko-sanitarnom kontrolom. Osnovna uloga klanica je proizvodnja mesa za ljudsku upotrebu. Tehnološki postupci kao skidanje kože i evisceracija predstavljaju kritične tačke za bekterijsku kontaminaciju polutki, ali se isto tako bakterije prenose i na opremu, zidove, podove, kecelje i čizme radnika. Ako se ne sprovodi kompletno pranje, čišćenje i dezinfekcija, mikroorganizmi preživljavaju u klanicama, umnožavaju se i spremni su da kontaminiraju meso i mesne prerađevine. Pored toga, mikroorganizmi su sposobni da razviju različite oblike zaštite od dezificijenasa kako bi preživeli. Cilj ovog rada je da prikaže rezistenciju mikroorganizama prema dezicinficijensima i njihovo preživljavanje u klanicama gde se vrši klanje životinja.

Ključne reči: klanice, bakterije, dezificijensi, biofilm

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053 and III 46009.

References

BOTTELDOORN N., HEYNDRICKX M., RIJPENS N., GRIJSPEERDT K., HERMAN L. (2003): Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of Apply Microbiology 95, 891-903.

BOULANGE-PETERMANN L. (1996): Processes of bioadhesion on stainless steel surfaces and cleanability: a review with special reference to the food industry. Biofouling 10 (4): 275-300.
CODEX ALIMENTARIUS COMMISSION (2003): Recommendet international Code of Practice: General Principles of Food Hygiene. CAC/RCP 1-1969, Rev. 4.
GORSKI L., PALUMBO J.D., MADRELL R.E. (2003): Attachment of Listeria monocytogenes to radish tissue is dependent upon temperature and flagellar motility. Apply and Environent Microbiology 69, 258-266.
HOMMAIS F., LAURENT-WINTER C., LABAS V., KRIN E., TEN-DENG C., SOUTOURINA O., DANCHIN A., BERTIN P. (2002): Effect of mild acid pH on the functioning of bacterial membranes in Vibrio cholerae. Proteomics 2, 571-579.
HRGOVIC N. (1986) : Zoohigijena. Belgrade: Faculty of Veterinary Medicine, Serbia.
HUMPHREY T. (2000): Public-health Aspects of Salmonella Infection. In: C. Wray, A. Wray (Eds.), Salmonella in Domestic Animals. CAB International, 245-263
IVANOVIC S., ZUTIC M., RADANOVIC O. (2009): Kontaminacija klaničnih površina i opreme sa Listeria monocytogenes. Arhiv of Veterinary Medicine 2 (2), 39-48.
IVANOVIC S., TEODOROVIC V., BALTIC Z.M. (2012): Kvalitet mesa – Biološke i hemijske opasnosti. Naučni institut za veterinarstvo Srbije, Beograd, pp. 356
IVANOVIC S., BALTIC Z.M., NESIC K., ZUJOVI M., IVANOVIC J., VUKOVIC S. (2014): Superficial Bacterial Contamination of Goats Carcasses. Journal of Pure and Apply Microbiol**o**gy 8 (1), 101-108.
JANKOVIC LJ., RADENKOVIC-DAMNJANOVIC B., VUCINIC M., DJORDJEVIC M., TEODOROVIC R. (2009): Biofilm opasnost u proizvodnji hrane Zbornik radova XX Savetovanja dezinfekcija, dezinsekcija i deratizacija u zaštiti zdravlja životinja i ljudi sa međunarodnim učešćem, May 28-31, Divcibare, 127-131.
KIM K.Y., FRANK J.F. (1995): Effect of nutrients on biofilm formation by Listeria monocytogenes on stainless steel. Journal of Food Protection 58, 24-8.
KOKARE C.R., CHAKRABORTY S., KHOPADE A.N., MAHADIK K.R. (2009): Biofilm: Importance and applications. Indian Journal of Biotechnology 8,159-168.
LANDINI P., ZEHNDER A.J.B. (2002): The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown Escherichia coli by modulating expression of flagellar genes and lipopolysaccharide production. Journal of Bacteriology 184, 1522–1529.

LINDBERG L.E., HOLMBOM R.B., VAISANEN M.O., WEBER A., SALKINOJA L.M. (2001): Sugar copmosition of biofilm produced by paper mill bacteria. Apply Microbiology and Biotechnology 55 (5), 638-643.

MARIC S., VRANES J. (2007) : Characteristics and significance of microbial biofilm. Periodica Biologica 109 (2), 115-121.

MCDONNELL G., RUSSELL D. (1999): Antiseptic and Disinfectants: Activity, Action and Resistance. Clinical Microbiology Review 12 (1), 147-179.

NETTING J. (2001): Sticky situation. Science News 160, 28-30.

Importance and applications. Indian Journal of Biotechnology 8, 159-168.

VUCINIC M., RADENKOVIC-DAMNJANOVIC B., TODOROVIC R. (2004). Bakterijska rezistencija na biocide. Zbornik radova XV savetovanja dezinfekcija, dezinsekcija i deratizacija u zaštiti životne sredine sa međunarodnim učešćem, May 20-23, Tara, Tara, 17-23.

VUCINIC M., RADENKOVIC-DAMNJANOVIC B. (2004): Biofilm i pseudobiofilm kao oblik bakterijske otpornosti prema dezificijensima. Zbornik radova XV savetovanja dezinfekcija, dezinsekcija i deratizacija u zaštiti životne sredine sa međunarodnim učešćem, May 20-23, Tara, 60-67.

WATNICK K., KOLTER R. (2000): Biofilm, city of microbes. J. Bacteriol. 182*:* 2675-79.

web.bf.uni-lj.si/mikro//…/mikrobnaBIODIVERZITETA.pdf

www.binghamton.edu/biology/faculty/davies/research.htm

SEASONAL CHANGES OF COMPOSITION AND SOMATIC CELL COUNT OF BUCKET MILK FROM JERSEY CROSSBRED COWS IN NORTHERN TURKEY

Metin Davut, Savaş Atasever

Ondokuz Mayis University, Faculty of Agriculture, Department of Animal Husbandry, 55139, Samsun, Turkey.
Corresponding author: Savaş Atasever, satasev@omu.edu.tr
Original scientific paper

Abstract: The objectives of this study were to determine the composition and somatic cell count (SCC) of bucket milk of Jersey crossbred cows and to reveal the seasonal factors affecting these properties. In total, 189 bucket milk samples those collected from local milk selling points in the Black Sea region of Turkey were analyzed by somatic cell count (SCC), fat, protein, lactose and density in four seasons. While SCC was highest ($P<0.01$), other components were lowest ($P<0.001$) in the summer. Negative or positive correlation coefficients ($P<0.01$ or $P<0.001$) were revealed among the parameters except for fat and protein or lactose. Calculated SCC mean ($483x10^3$ cells/ml) was found to be close to the highest limit that declared by Turkish Food Codex. As the result, taking additional precautions at hot months is suggested to boost bovine raw milk quality.

Key words: cow, season; raw milk; somatic cell count; milk composition.

Introduction

Animal production is not only important to ensure food materials, but also has been reached to main labor section in many countries. For instance, it was reported that 90 percent of total milk production of Turkey has been ensured from cattle in 2016. At this point, cattle husbandry might be referred as dominant occupation in the Turkish dairy industry. According to some studies those conducted to determine bovine raw milk composition (*Skrzypek et al., 2004; Pavel and Gavan, 2011; Atasever and Stadnik, 2015a*) many environmental factors such as test day, year, farm, calving season, parity, stage of lactation, body condition score (BCS) affect the milk properties. The studies have clearly pointed out that milk quality could be elevated in terms of decreasing the effect of non-genetic factors those directly affecting raw milk components. Actually, the principal target of enterprises dominating dairy sector is both boosting raw milk quality and quantity. In this context, achieving relatively lower microbial load and somatic cell count (SCC) including milk may be seen as a major item by herd owners.

Somatic cells are known as body origin and they pass from blood to milk. These cells may find as ephitel, erythrocyte, lenfocyte or lococyte forms and significantly increase during any trauma or infection in the udder. In addition to the health conditions of the cows, husbandry practices, breed, environment, season, milking management, age or stage of lactation may be closely related to SCC (*Atasever et al., 2012; Atasever and Stadnik, 2015b; Mikone Jonas et al., 2016*). While the highest threshold for SCC in EU countries has been declared as $400x10^3$ cells/ml, this limit has been reported to be $500x10^3$ cells/ml by Turkish Food Codex. An earlier study that carried out to estimate the milk production losses caused by SCC (*Erdem et al., 2010*), daily milk yield (dMY) and 305 dMY losses were informed to be about 15% and 14%, respectively. In another investigation conducted in Lithuanian conditions (*Juozaitiene et al., 2008*), the milk yield loss was calculated to be 14.4% when SCC elevated from $200x10^3$ cells/ml to $800x10^3$ cells/ml.

Water, fat, protein, ash, lactose and minerals may be grouped as the main parameters of cow's raw milk and highly wide correlations between some components and lactation persistence have been reported (*Mikone Jonas et al., 2016*). Correlation of the components with each other and SCC has been investigated in first parity Holsteins (*Atasever and Stadnik, 2015a*). However, according to the literature, detailed studies those investigating raw milk quality in bucket milk samples, especially collected from Jersey or crossbreds are still rare. Besides, many reports focused either cow or tank milk bases. That's why, revealing the milk compounds of bucket milk samples of Jersey crossbred cows throughout the production period may be seen an approach to remove an important omission for dairy science.

The aim of this study was to evaluate the milk composition and SCC by season in bucket milk samples collected from Jersey crossbreds.

Materials and Methods

The bucket milk samples were collected from milk selling points of Çarşamba and Bafra districts and Samsun province (41° 17′ arid and 36° 20′E) those located in the Black Sea region of Turkey. Throughout a year period (from April 2014 to January 2015), approximately 50 ml milk was ensured for each farm in four seasons. All farms had Jersey crossbreds (Jersey x Turkish native) and similar feeding-managemant conditions during the study. Collected raw milk samples were immediately transferred to the laboratory in a bag with ice-boxes. Fat (F), protein (P), lactose (L) and density were used as composition markers and analyzed with Funke Gerber Lactostar device. SCC analysis was performed by Somatic Cell Counter DCC (DeLaval Group, Swiss). Due to obtained SCC data showed a wide variation, values were transferred to log 10 base to ensure homogeneity of variance.

In the statistical assessment, the following mathematical formula was used:

$$Y_{ij}=\mu+S_i+ e_{ij}$$

where; Y_{ij}: observation value, μ: mean of the population, a_i: effect of sampling season (i= 1,2,3,4), e_{ij}: random error term.

Differences among the groups were compared with Duncan's multiple comparison test, and the relations among the parameters were estimated using Pearson's phenotypic correlation coefficients. All statistical analyses of the present study were performed by SPSS 17.0 for Windows computer program at 0.05 significance level.

Results and Discussion

Table 1. Seasonal changes of parameters

Season	n	logSCC**	Fat***	Protein***	Lactose***	Density***
Spring	57	5.44 ± 0.62^{ab}	5.16 ± 3.77^{a}	2.85 ± 0.58^{ab}	3.88 ± 0.79^{a}	1.022 ± 0.006^{a}
Summer	50	5.59 ± 0.44^{a}	2.70 ± 1.75^{b}	2.62 ± 0.47^{a}	3.54 ± 0.64^{b}	1.021 ± 0.004^{a}
Autumn	34	5.45 ± 0.45^{ab}	3.28 ± 2.01^{b}	2.85 ± 0.24^{ab}	3.90 ± 0.33^{a}	1.023 ± 0.004^{ab}
Winter	48	5.31 ± 0.28^{b}	2.77 ± 0.87^{b}	3.02 ± 0.23^{b}	4.11 ± 0.28^{a}	1.025 ± 0.002^{b}
General	189	5.45±0.48	3.57±2.66	2.83±0.45	3.85±0.61	1.023±0.004

(**P<0.01; ***: P<0.001; logSCC: somatic cell count in log 10 base)

Changes of milk components according to season are given in Table 1. As seen, season significantly (P<0.01 or P<0.001) affected all parameters. Really, milk samples had higher logSCC values in the summer when compared to the winter samples. This finding might be evaluated as an expected case because SCC tended to increase with physiologic stress due to high temperature and humidity in the summer seasons. *Zucali et al*. (*2011*) emphasized that SCC closely related to hygiene scores of cows and milk samples collected in the summer had higher microbial load and SCC. In our study, relatively lower SCC in the winter might alse be explained as keeping herds in the closed barns and sharing more time for milking cows in this period. *Sandrucci et al. (2004*), who founded similar results in an earlier study, certainly advised the premilking teat disinfection to reduce SCC.

As seen from the Table 1, season significantly (P<0.001) affected milk fat. Obtained finding was founded as different from some study results that informed higher fat in the autumn (*Pavel and Gavan, 2011; Marcondes et al., 2014*) or in the winter (*Jez et al., 2012*). In the present investigation, calculated highest fat mean (5.16%) in the spring may be referred to abundant fresh- green pasture and roughage in this period. At this period, readjustments on feeding strategies should be suggested to herd owners to prevent sudden droppings in milk fat percentage. Besides, calculated overall mean for fat (3.57%) in this study was lower than some earlier study findings (*Andrew, 2000; Kucjaz, 2001; Dehinenet et al., 2013*), but similar to mean of a study that conducted by *Jez et al. (2012)*. Differences in the feeding programs and breeds of the farms could be the main reasons for these variations in the investigations.

While the protein mean for the summer was lowest and for winter was highest, these means differed (P<0.001) from each other. This case might be caused by keeping cows in the barns in the cold weather with applying concentrate feeding. This finding was harmonic with the study results of *Pavel and Gavan (2011)* and *Wangdi et al. (2014)* those obtained the highest protein percentages in the spring and autumn, respectively. Different management and feeding programs of the farms or different genetic structures of the cows might be the main reasons of reached case by protein percentage.

The season also affected the lactose content of milk in this study (Table 1). As seen, lactose percentage was lower in the summer when compared to the other seasons (P<0.001). Really, lactose percentage of this season was 0.31% lower than the overall mean of the investigation. This case might be explained by the adverse effect of hot climate on pasture and also lactose level. Besides, calculated overall lactose mean (3.85%) was found as lower than the result of *Jez et al. (2012)* who conducted a similar study in Serbia conditions. As understood, examined raw milk samples had important quality problems especially in the summer months.

In this study, the season was an effective factor on density of milk. Such that, density of milk collected in the winter was different (P<0.001) from spring and summer (Table 1). This case could be associated with the effect of serving more concentrates to the cows in the closed barns in the cold months as explained earlier.

Pearson's correlation coefficients among the evaluated parameters are given in Table 2. According to these estimations, SCC negatively correlated with protein, lactose and density (P<0.01). While density negatively correlated with fat, this parameter positively correlated (P<0.01) with protein and lactose. Besides, significant correlations were estimated between protein and lactose (P<0.01). Finally, estimated relationships among the milk components indicated that multi markers should be regarded during the evaluation of quality of bovine raw milk to obtain more accurate results.

Table 2. Pearson's phenotypic correlation coeefficients among the components

Parameter	Protein	Lactose	Density	logSCC
Fat	0.141	0.125	-0.387**	0.048
Protein		0.979**	0.780**	-0.300**
Lactose			0.811**	-0.285**
Density				-0.290**

(*P<0.05; **: P<0.01, logSCC: somatic cell count in log 10 base)

However, calculated SCC mean of the present study (483x10^3 cells/ml) was higher than the results of *Kucjaz (2001), Skrzypek et al. (2004)* and *Yarabbi et al. (2014),* but lower than the means of *Atasever et al. (2012).* Also, this value was near to the highest limit that reported by Turkish Food Codex. At this point, preventing bacterial load and controlling SCC by means of showing more attention to hygienic conditions (*Yamane, 2006; Nagahata, 2007*) can be suggested.

Conclusion

Finally, it was revealed that bucket milk samples collected from Jersey crossbred cows had marked quality problems. Especially, summer season had an adverse effect on milk components. Estimated correlations among the parameters clearly pointed out that multi components should be evaluated together to decide quality degree of raw milk. Besides, additional precautions are needed on the farms in the hot months to prevent an elevation of SCC and decline of other traits.

Sezonske promene sadržaja i broja somatskih ćelija namuženog mleka meleza džersej rase goveda u severnoj Turskoj

Metin Davut, Savaş Atasever

Rezime

Ciljevi ove studije bili su da se odrede sastav i broj somatskih ćelija (SCC) namuženog mleka džersej rase goveda i da otkrije sezonske faktore koji utiču na te osobine. Ukupno 189 uzoraka namuženog mleka prikupljeno od lokalnih tačaka za prodaju mlijeka u regionu Crnomorske Turske analizirani su na broj somatskih ćelija (SCC), mast, protein, laktozu i gustinu u četiri sezone. Dok je SCC bio najveći (P<0,01), ostale komponente su bile najniže (P<0,001) tokom leta. Negativni ili pozitivni koeficijenti korelacije (P<0,01 ili P<0,001) su utvrđeni između parametara, osim za mast i proteina ili laktozu. Utvrđeno je da je izračunata srednja vrednost za SCC (483x10^3 ćelije/ml) blizu gornje granice koju određuje turski Kodeks za hranu. Kao rezultat toga, uzimanje dodatnih mera predostrožnosti u letnjim mesecima predlaže se za povećanje kvaliteta sirovog mleka od goveda.

Ključne reči: Krava, sezona, sirovo mleko, broj somatskih ćelija, sastav mleka

References

ANDREW S.M. (2000): Effect of fat and protein content of milk from individual cows on the specificity rates of antibiotic residue screening tests. Journal of Dairy Science, 83, 2992–2997.

ATASEVER S., ERDEM H., DEMIRYUREK K. (2012): Association of some milking parameters with milk quality of smallholder dairy farms in Samsun region, Turkey. Journal of Environmental Biology, 33, 126-129.

ATASEVER S., STADNIK L. (2015a): Factors affecting daily milk yield, fat and protein percentage, and somatic cell count in primiparous Holstein cows. Indian Journal of Animal Research, 49,3, 313-316.

ATASEVER S., STADNIK L. (2015b): Assessing milk yield, somatic cell count and some milk components in Czech Holstein cows. IDF World Summit, Sep. 20-24, Vilnius, Lithuania.

DEHINENET G. MEKONNEN H., ASHENAFI M., EMMANUELLE G. (2013): Determinants of raw milk quality under a smallholder production system in selected areas of Amhara and Oromia National Regional States, Ethiopia. Agriculture and Biology Journal of North America, 4,1, 84-90.

ERDEM H., ATASEVER S., KUL E. (2010): Determination of milk production characteristics and milk losses related to somatic cell count in Jersey cows raised in the Black Sea Region of Turkey. Asian Journal of Animal and Veterinary Advances, 5,3, 217-222.

JEZ G., PANDUREVIC T., CVETANOVIC D., JANKOVIC J. (2012): Phenotype variability of basic components of cow's milk in conventional conditions of production. Third International Scientific Symposium, November 15-17, (Proceedings Book: 554-558 p), Jahorina, Serbia.

JUOZAITIENE V., JUOZAITIS A., MICIKEVICIENE R. (2006): Relationship between somatic cell count and milk production or morphological traits of udder in Black-and-White cows. Turkish Journal of Veterinary and Animal Science, 30, 47-51.

KUCJAZ M. (2001). Interrelations between year season and raw milk hygienic quality indices. Electronic Journal of Polish Agricultural Universities, 4,1.

MARCONDES M.I., JACOME D.C., DA SILVA A.L., RENNO L.N., DOS SANTOS PIRES A.C. (2014): Evaluation of raw milk quality in different production systems and periods of the year. *Revista* Brasileira *de* Zootecnia, 43,12, 670-676.

MIKONE JONAS E., ATASEVER S., GRAFF M., ERDEM H. (2016): Non-genetic factors affecting milk yield, composition and somatic cell count in Hungarian Holstein cows. Journal of Faculty of Veterinary Medicine, Kafkas University, 22, 3, 361-366.

NAGAHATA H., ITO H., MARUTA H., NISHIKAWA Y., SUSUKİNO H., MATSUKI S., HIGUCHI H, OKUHIRA T., ANRI A. (2007): Controlling highly prevalent *Staphylococcus aureus* mastitis from the dairy farm. The Journal of Veteinary Medical Science, 69, 9, 893-898.

PAVEL E.R., GAVAN C. (2011): Seasonal changes in bulk tank milk composition of dairy cows. Animal Science and Biotechnologies, 44, 2, 444-449.

SANDRUCCI A., BAVA L., ZUCALI M., TAMBURINI A. (2014): Management factors and cow traits influencing milk somatic cell counts and teat hyperkeratosis during different seasons. Revista Brasileira de Zootechnia, 43,9, 505-511.

SKRZYPEK R., WOJTOWSKI J., FAHR, R.D. (2004): Factors affecting somatic cell count in cow bulk tank milk: A case study from Poland. Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine, 51, 3, 127-131.

WANGDI J., DEMA T., KARMA M., BHUJEL P. (2014): The compositional quality of cows milk in Bhutan. Issues in Biological Sciences and Pharmaceutical Research, 2 ,7, 62-68.

Yamane I (2006): Management practices associated with somatic cell counts in bulk milk. Journal of the Japanese Veterinary Medical Association, 59 (10): 674-678.

YARABBI H., MORTAZAVI A., MEHRABAN M., SEPEHRI N. (2014): Effect of somatic cells on the physic-chemical and microbial properties of raw milk in different seasons. International Journal of Plant, Animal and Environmental Sciences, 4, 3, 289-298.

ZUCALI M., BAVA L., TAMBURINI A., BRASCA M., VANONI L., SANDRUCCI A. (2011): Effects of season, milking routine and cow cleanliness on bacterial and somatic cell counts of bulk tank milk. Journal of Dairy Research, 78,436-441.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EXAMINATION OF THE TIME OF EXCRETION OF PENICILLIN RESIDUES IN COWS MILK BY FORMING INTERVAL SERIES AND ABSOLUTE FREQUENCYES

Vujadin Vuković[1], Nada Plavša[2], Jasna Kureljušić[1]

[1]Institute of Veterinary Medicine of Serbia, 11070 Belgrade, Auto put 3. Srebia
[2] Faculty of Agriculture, University of Novi Sad, Trg D. Obradovica 8
Corresponding author: vujadin.vukovic@gmail.com
Original scientific paper

Abstract: A total of 16 dairy cows intrauterine received a dose of 800,000 IU per penicillin. The excretion of antibiotics residues in milk was monitored using a modified method Resasurine test (*Vuković, 1999*): 12, 24, 36, 48, 60, 72 and 84 hours after application. Based on the study findings raw formed 8 series interval of 10 hours. Next, the column is formed of the absolute frequency (f) with the number of frequencies for each interval series. In this way, we achieved a basic table for calculating statistical parameters: mean value, positional averages absolute parameter variability and the coefficient of variation.

Key words: penicillin, residues, milk, interval series, frequency, statistics

Introduction

Statistical methods and procedures are applicable in all scientific fields and disciplines, however, they need to be properly selected, adapted to the study in order to obtain a clear and credible way to display the results. This scientific approach has been used in our present tests and in the study of the presence of antibiotic residues in cows' milk.

In addition to the usual procedures of planning, setting up and carrying out of experiments/trials, sampling and laboratory testing, within the method of statistical analysis, statistical processing of data is included, which, for reasons of clarity, most often expressed in tables and/or graphs.

The main goal of this study is the simplification and application of statistical methods and procedures in the processing of the data obtained in these studies. In doing so, the results are presented using appropriate statistical parameters: arithmetic mean, positional mean values, absolute parameters of variability and the coefficient of variation.

Material and Methods

A total of 16 cows of East Friesian breed received penicillin intrauterine in a dose of 800,000 I.U./cow. Samples of milk from treated cows were taken manualy, milking each udder quarter into one composite sample and stored in sterilized bottles in the fridge until the beginning of the test. Milk sampling times were: 12, 24, 36, 48, 60, 72 and 84 hours after treatment, or until two consecutive negative findings. Detection of penicillin residues in milk was performed in the laboratory with the test method Resasurine with *Str. thermophilus*, the level of sensitivity of 0.01 I.U./ml (positive result), and 0.008 and 0.009 I.U./ml (suspicious findings) (*Vuković, 1991; Vuković, 2001; Vuković, 2005).*

Based on the obtained retention time of penicillin residues in milk, a table was formed with intermittent series of approximately 10 hours (9.99 h) and absolute frequencies in the normal distribution. Interval series are shown also as mean values (X_{sr}) of the interval *(Benčić and Šuvak, 2013; Joksimović, 2006; Žižić et al., 2002).* The aim of the paper was to present also the statistical processing of data, by using the interval series from which all planned statistical parameters were calculated based on the statistical formula.

Results and Discussion

Total of 16 milking cows, which have received penicillin intrauterine at a dose of 800,000 I.U./cow, excreted residues of antibiotics into milk in the following time intervals: 60, 24, 60, 48, 84, 36, 60, 72, 72, 72, 48 , 72, 36, 60, 60 and 84 hours post-treatment.

Based on these data a table is formed that included interval series of approximately 10 hours (9.99 h), which are presented also as the mean value of the interval (X_{sr}) (Table 1).

Table 1. Interval series of retention time of penicillin residues in cows' milk 16 after dose of 800,000 I.U. (in h).

Interval series (X) (in h.)	X_{sr} (in h.)	Absolute frequency (f)	(f)x(X_{sr}) (h.)
10-19	15	0	0
20-29	25	1	25
30-39	35	2	70
40-49	45	2	90
60-69	65	5	325
70-79	75	4	300
80-89	85	2	170
		$\sum$ =16	$\sum$= 980

Also, 16 absolute frequencies are presented (f), of which 5, or the highest frequencies were for interval series of 60 to 69 hours of extraction. After multiplying the number of frequencies with the corresponding mean values of interval series, a total score of 980 hours of excretion was obtained.

Subsequent to administration of intrauterine dose of 800,000 I.U./cow (*Kasalica and Vuković, 2007*), same as in our trials, residues of antibiotics have been excreted in the milk of treated cows 30 to 72 (average 53.8) hours. In our experiments, the arithmetic mean was 61.25 hours and all 16 cows have excreted the antibiotic. On the other hand, our findings are not consistent with the data provided by *Mc Clary (1984).* Namely, subsequent to administration of intrauterine dose of 1,000,000 I.U. of penicillin/cow, in a 12 hour trial, the author proved residues in milk from 10% of cows. In our trial, the residues were detected in all cows in hours 12 and 24 of the trial. Our findings are partially in agreement with the results given by *Haaland et al. (1984)*, who have detected penicillin residues in milk for 60 to 84 hours, subsequent to i.u. dose of 1,500,000 I.U. of penicillin.

Calculation of statistical parameters

1. The parameter mean values

1.1. The calculation of the arithmetic mean value ($\overline{X}$)

The arithmetic mean represents the central value in respect of which the sum of all deviations must be zero. The arithmetic mean is calculated by dividing the total sum of hours of excretion by the sum of the number of frequencies.

$$\overline{X} = \frac{\sum (f)x(Xsr)}{\sum f}$$

Data presented in Table 1 show that there were in total 16 cows in the experiment, and 16 frequencies, and the total sum of 980 hours of excretion.

$\sum (f)$ x(X_{sr}) =980 hours; $\sum f$ =16

$$\overline{X} = \frac{\sum (f)x(Xsr)}{\sum f} = \frac{980}{16} = 61.25 \text{ hours}$$

The arithmetic mean for the duration of excretion of penicillin residues in milk from treated cows is 61.25 hours.

2. The positional mean values
2.1. The calculation of the mode (Mo)

The mode is the value in the series, which is the most common, or whose frequency is the highest. This value in interval series is calculated using the following formula:

$$Mo = L_{Mo} + \frac{f_{Mo} - f_{Mo-1}}{(f_{Mo} - f_{Mo-1}) + (f_{Mo} - f_{Mo+1})} x\Delta$$

L_{Mo} – onset of the modal interval = 60 hours
f_{Mo} - frequency of the modal interval = 5
f_{Mo}-1- 1 frequency pre - modal = 2
f_{Mo}+1 - 1 frequency post - modal = 4
Δ - interval width = 10 hours

The data presented in Table 1 show that the number 5 is the highest frequency that occurs. The variation interval of 60 to 69.99 hours corresponds to this value.

$$Mo = 60 + \frac{5-2}{5-2+5-4} x10 = 67.50 \text{ hours}$$

2.2. The calculation of the median (Me)

The median is the middle value of a series that is obtained based on the total number of frequencies. The median in interval series is calculated using the following formula:

$$Me = L_{Me} + \frac{\frac{\sum f}{2} - f(sumofallabove)}{f_{Me}} x\Delta$$

$$\frac{\sum f}{2} = \frac{16}{2} = 8$$

L_{Me} – onset of the interval for the median class, i.e. position 8 = 60 hours
f (sum of all above) - the sum of all frequencies before the median = 5
f_{Me} – frequency of the median class = 5
Δ - interval width - 10 hours

Me=60+ $\frac{8-5}{5} x10$ = Me = 66.0 hours

3. The absolute variability parameters
3.1. The interval of variation - (Iv)

The variation interval is a range that contains all values of interval series, i.e. the difference between the maximum and minimum values of interval series, and it is calculated using the following formula:

Iv = X_{max} - $X_{min,}$ where:
X_{max} - maximum value of the interval series (in hours) = 89 hours
X_{min} - minimum value of the interval series (in hours) = 10 hours
Iv = 89 - 10 = 79 hours

3.2. The variance –mean square deviation (σ^2)

The variance represents the mean square deviation which is obtained/calculated by squaring the sum of all individual deviations. The variance can be relative to the arithmetic mean, mode and median.

Table 2. The variance from the arithmetic mean, median and mode for the retaining time of penicillin residues in milk of 16 cows subsequent to administration of i.u. dose of 800,000 I.U. (in hours)

X_{sr} (in hours)	Absolute frequency (f)	The deviation from the arithmetic mean (61.25 hours)		The deviation from the mode (67.50 hours)		The deviation from the median (66.0 hours)	
		X_{sr}- $\bar{X}$ (in hours)	fx(X_{sr}- $\bar{X}$)^2 (u čas.)	X_{sr}-Mo (in hours)	fx(X_{sr}-Mo)^2 (in hours)	X_{sr}-Me (in hours)	(f)x(X_{sr}-Me)^2 (in hours)
15	0	-46.25	0x2,139.06	-52.50	0x2,756.25	-51.0	0x2601
25	1	-36.25	1x1,314.06	-42.50	1x1,806.25	-41.0	1x1681
35	2	-26.25	2x689.06	-32.50	2x1,056.25	-31.0	2x961
45	2	-16.25	2x264.06	-22.50	2x506.25	-21.0	2x441
65	5	3.75	5x14.06	-2.50	5x6.25	-1.0	5x1
75	4	13.75	4x189.06	7.50	4x56.25	9	4x81
85	2	23.75	2x564.06	17.50	2x306.25	19	2x361
	$\sum$ =16		$\sum$= 5,174.96		$\sum$= 5,899.75		$\sum$= 5,536

3.2.1. The variance of the arithmetic mean

The variance (mean square deviation) from the arithmetic mean is calculated using the following formula:

$$\sigma^2 = \frac{\sum(f)x(X_{sr} - \bar{X})^2}{\sum f}$$

The arithmetic mean ($\bar{X}$) for retaining of penicillin residues in milk of 16 cows after i.u. dose of 800,000 I.U. (in hours) is 61.25 hours, a sum of square deviation from the mean is 5,174.96 hours.

$$\sigma^2 = \frac{\sum(f)x(X_{sr} - \bar{X})^2}{\sum f}$$

$\sum(f)$ x $(X_{sr} - \bar{X})^2$ = 5,174.96 hours; $\sum$ f = 16

$\sigma^2 = \frac{5.174,96}{16}$ = 323.43 hours

3.2.2. The variance of the mode

The variance (mean square deviation) of the mode is calculated using the formula:

$$\sigma^2 = \frac{\sum(f)x(X_{sr} - Mo)^2}{\sum f}$$

The mode (Mo) for retaining of penicillin residues in milk of 16 cows after i.u. dose of 800,000 I.U. (in hours) is 67.50 hours, a sum of square deviation from the mode is 5,899.75 hours.

$$\sigma^2 = \frac{\sum(f)x(X_{sr} - Mo)^2}{\sum f}$$

$\sum(f)$ x $(X_{sr} - Mo)^2$ = 5.899,75 hours; $\sum(f)$ = 16

$\sigma^2 = \frac{5.899,75}{16}$ = 368.73 hours

3.2.3. The variance of the median

The variance (mean square deviation) of the median is calculated using the formula:

$$\sigma^2 = \frac{(\sum f)x(X_{sr} - Me)^2}{\sum f}$$

The median (Me) for retaining penicillin residues in milk of 16 cows after i.u. dose of 800,000 I.U. (in hours) is 66.0 hours, and the sum of square deviations from the median is 5,536.0 hours.

$\sum(f)$ x $(X_{sr}$ - Me$)^2$ = 5,536.0 hours; $\sum$ f = 16

$$\sigma^2 = \frac{5.536{,}0}{16} = 346.0 \text{ hours}$$

3.3. The standard deviation (Sd)

The standard deviation is the second root of the variance (mean square deviation) The standard deviation can be relative to the arithmetic mean, mode and median and it is calculated according to the formula: Sd = $\sqrt{\sigma^2}$

3.3.1. The standard deviation from the mean

$\text{Sd}_{(\overline{X})} = \sqrt{\sigma^2}$

σ^2 - the variance from the mean = 323.43 hours

$\text{Sd}\,\overline{X} = \sqrt{323{,}43}$ = 17.98

3.3.2. The standard deviation from the mode

$\text{Sd}(_{Mo}) = \sqrt{\sigma^2}$

σ^2 - the variance from the mode = 368.73 hours

$\text{Sd}(_{Mo}) = \sqrt{368{,}73}$ = 19.20

3.3.3. The standard deviation from the median

$\text{Sd}(_{Me}) = \sqrt{\sigma^2}$

σ^2 - the variance from the median = 346.0 hours

$\text{Sd}(_{Me}) = \sqrt{346{,}0}$ = 18.60

4. Relative parameters

4.1. The coefficient of variation (Cv)

The coefficient of variation indicates the variability between two or more series or the homogeneity of mass. Mass is homogeneous if the coefficient of variation does not exceed 30%. The coefficient of variation is determined using the following formula:

$$Cv = \frac{Sd}{\bar{X}, MoorMe} \text{x}100$$

4.1. The coefficient of variation of the mean

$$Cv = \frac{Sd}{\bar{X}} \text{x}100$$

Sd= 17.98; $\bar{X}$ = 61.25 hours

$$Cv = \frac{17.98}{61.25} \text{x}100 = 29.35\%$$

4.2. The coefficient of variation of the mode

$$Cv = \frac{Sd}{Mo} \text{x}100$$

Sd= 19.20; Mo = 67.50 hours

$$Cv = \frac{19.20}{67.50} \text{x}100 = 28.44\%$$

4.2. The coefficient of variation of the median

$$Cv = \frac{Sd}{Me} \text{x}100$$

Sd= 18.60; Me = 66.0 hours

$$Cv = \frac{18.60}{66.0} \text{x}100 = 28.18\%$$

Conclusion

Subsequent to intrauterine (i.u.) dose of 800,000 I.U./cow, penicillin residues were excreted for 24 to 84 hours after application. In this case, the arithmetic mean ($\overline{X}$) was 61.25 hours, with mode (Mo) of 67.50 hours and the median (Me) of 66.0 hours. As for the variation, the interval of variation (Iv) was 79 hours, within which the variance (σ^2) of the arithmetic mean, mode and median had an approximate value (from 323.43 to 368.73), and hence the standard deviation (Sd) of 17.98 to 19.20. Also, the coefficient of variation (Cv) of the arithmetic mean, mode and median ranged from 28.18 to 29.35%, which shows that the statistical sample is a homogeneous mass.

Ispitivanje vremena izlučivanja rezidua penicilina mlekom krava korišćenjem intervalnih serija i apsolutnih frekvencija

Vujadin Vuković, Nada Plavša, Jasna Kureljušić

Rezime

Ukupno 16 muznih krava intrauterino dobilo je dozu od po 800.000 i.j. penicilina. Izlučivanje rezidua antibiotika mlekom praćeno je korišćenjem modifikovane metode Rezazurin testa (*Vuković, 1999*): 12, 24, 36, 48, 60, 72 i 84 čava nakon aplikacije. Na osnovu dobijenih sirovih nalaza formirane su intervalne serije od po 10 časova (9,99 čas.). Dalje, formirana je kolona za apsolutnu frekvenciju (f) sa brojem frekvencija za svaku intervalnu seriju. Na taj način dobijena je osnovna tabela za izračunavanje statističkih parametara: aritmetičke sredine, pozicionih srednjih vrednosti, apsolutnih parametara varijabiliteta i koeficijenta varijacije.

Ključne reči: penicilin, rezidue, mleko, intervalne serije, frekvencija, statistika

Acknowledgment

Project MNTR Government of Serbia no. 46009.

References

BENČIĆ M., ŠUVAK N. (2013): Primjenjena statistika. Izdavač Sveučilište J.J. Štrosmajera, Osijek.

HAALAND, M.A. et all (1984): Antibiotics residues in milk after i.u. infusion. Vet. Med. and small Animal Clinical, 79 (3), 382-386.

JOKSIMOVIĆ D. (2006): Poslovna statistika. Univerzitet Megatrend, Beograd.

MC CLARY, D.G. (1984): B. stearothermophilus disc assay detection of penicillin in milk of dairy cows after postestrual i.u. infusion. Am.J. of Vet. Resear. 45, 3, 416-419.

VUKOVIĆ, V. (1991): Residue resorption and secretion of penicillin, streptomycin and oxytetracycline in milk after intrauterine administration. Doctoral thesis, Faculty of Veterinary Medecine, Belgrade.

VUKOVIĆ, V. (1999): Determination of penicillin residues in milk by using the modified resasurine methode. 2. Slovenian Congres, with International Participation «Milk and dairy products», Portorož, Slovenija, Book of abstracts, 65.

VUKOVIĆ V. (2001): Investigations of sensitivity level of Delvo and Resasurine test for presence of penicillin and oxytetracycline in milk. Vet. glasnik, 55, 1-2, 55-59.

VUKOVIĆ V. (2005): Rezidue antibiotika u mleku krava posle intrauterine aplikacije. Izdavač „Naučni institut za veterinarstvo Srbije, Beograd.

VUKOVIĆ V.**,** KASALICA A. (2007): Residues of penicillin in the milk after intrauterine treatment of lactating cows. Biotechnology in animal husbandry, 2 nd International Congress on animal husbandry new perspectives and challenges of sustainable livestock farming, Belgrade- Zemun- Serbia, vol. 23 (5-6), book 2, 77-85; 3-5. oktobar.

ŽIŽIĆ M., LOVRIĆ M., PAVLIČIĆ D. (2002): Metodi statističke analize. Ekonomski fakultet Subotica.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EXAMINATION OF THERAPEUTIC EFFECT TIAMULIN IN THE TREATMENT OF BLOODY DIARRHEA PIGLETS

Vujadin Vuković, Mihajlo Vićentijević, Milan Radović

Institute of Veterinary Medicine of Serbia, 11070 Belgrade, Auto put 3. Srebia
Corresponding author: vujadin.vukovic@gmail.com
Original scientific paper

Abstract: The trials involved a total of 28 piglets with clinical signs of bloody diarrhoea. The diagnosis was based on clinical picture, clinical symptoms and bacteriological findings. Affected animals were divided into experimental group, which was treated with a drug Tiamulin, and a control group that received the drug Hemutin S. Drugs were administered intramuscularly, once in 5 days, at a dose of 1 ml/12.5 kg of body weight. We assessed The effects of the experimental compared to the control drug were monitored and assessed, as well as the incidence of death, termination of symptoms of illness, full recovery and recurrence.

Key words: tiamulin, piglets, bloody diarrhoea, treatment, diagnosis

Introduction

Bloody diarrhoea is a disease which affects all breeds and all ages of pigs. In modern livestock production, disease is particularly widespread, globally and in our country (*Stamatović and Šamanc, 2001*), with significant mortality (*Lončarević, 1997*). The disease, in addition to causing serious health problems, leads to economic losses that are reflected in mortality, an increase in the number of stillborn piglets, reduced percentage of fertilization, and reduced number of fattening pigs produced per sow, lower growth and feed conversion rates, treatment expenses, etc. (*Gagrčin and Kovčin, 1999; 2002)*. The etiology of disease involves different types of microorganisms (enterovirus, bacteria, spirochetes ...), and can also be the case of poly-etiology, where the etiology is always associated with particular factors which influence and induce the development of the disease. This primarily applies to the problems with nutrition, including food quality (*Baumann and Bilkei, 2002*), whereby, for example, diet with content below 6.1% of easily digestible fibres reduces the risk of manifestation of clinical symptoms of endemic dysentery and reduces mortality of pigs (*Bilić and Bilkei G., 2003; Mirko and*

Bilkei, 2005). Furthermore, the low immunity, poor water quality, particularly or directly, including the infected water, poor housing conditions, stressful factors (transportation stress, changes in food, changing accommodation, bad weather ...) contribute to the development of the disease. The causes of bloody diarrhoea are present in the intestines, in faeces, the material/bedding of housing boxes, the equipment, they can be found in food, water ... (*Šamanc, 2009*).

When *Serpulina (Borrelia) hyodysenteriae* is the cause of bloody diarrhoea, the disease causes health, reproduction and production problems (*Gagrčin et al., 2001; Gagrčin and Stančić, 2002*). The disease usually occurs in the acute clinical form.

In our experiments, *Serpulina (Borrelia) hyodysenteriae*, gram negative spirochete, was isolated as the cause of bloody diarrhoea. The disease has manifested by persistent bloody diarrhoea, along with the emergence of dehydration. The studied drug was Tiamulin ad us. vet. It was decided that the efficacy of the investigational drug will be measured/rated by the success in the treatment of bloody diarrhoea and by comparing the results achieved by the control drug (Hemutin S). Tiamulin, as active substance, is a bacteriostatic antibiotic which inhibits protein synthesis by binding to the 50-S ribosomal subunit. It is well absorbed from the digestive tract (over 90%), and the maximum serum concentration was reached after 4 hours. Its action and effect of the Serpulina hyodysenteriae as a cause of dysentery of piglets and pigs is also very important, and the dose is 10 mg/kg body weight (*Jezdimirović, 2000*).

Materials and Methods

The experiments were conducted on a total of 28 piglets suffering from bloody diarrhoea, of domestic white breed, both sexes, aged about 2 months, average body weight 20 kg. Piglets were housed in the appropriate boxes (isolated from healthy piglets), farm type of housing. The disease began as an ordinary diarrhoea, which has turned into slimy, bloody diarrhoea, with faecal matter of chocolaty appearance. The situation with the piglets' appetite ranged from slight loss of it to complete absence of it, in addition to the reduction in body weight, with a slight increase in body temperature. The piglets' hair was cowed. Signs of dehydration ranged from the elementary to expressed, and the general condition was not damaged. The clinical diagnosis of the disease was the bloody diarrhoea, and the objective diagnosis was made based on clinical symptoms and examination of rectal swabs. In the laboratory conditions, *Serpulina hyodysenteriae* was isolated.

Previously, in the laboratory conditions, complete laboratory trials of the drug Tiamulin ad us. vet., planned to be used in the trial, were carried out. The drug contains 100 mg of tiamulin base in 1 ml of injectable solution. A total of 14 piglets were the experimental group that received study drug, and the remaining 14 pigs were given the drug Hemutin S, as a control drug, and comprised the control group. In both groups of pigs, drugs were administered once intramuscularly in a dose of 1 ml/12.5 kg of body weight. The treatment lasted 5 days. The animals have endured well the drug application and no side effects have been observed.

The obtained results are presented descriptively, expressed in percentages, also as the interval of variation (Iv), as the difference between the maximum and minimum values, and as modal values of (Mo), i.e. as a value with the highest frequency.

Results and Discussion

In the study of the therapeutic effect of tiamulin as active substance in the experimental drug (Tiamulin ad us. Vet.), and in the control drug (HEMUTIN S ad us. Vet.), in the treatment of bloody diarrhoea in piglets, the following parameters were observed: termination of diarrhoea, normalization of the appetite, normalization of the body temperature, the number of cured animals and the potential mortality and the potential incidence of recurrence of disease (Table 1).

Table 1. Testing of the therapeutic efficacy of drugs Tiamulin ad. us.vet. and Hemutin S ad. us.vet., in the treatment of bloody diarrhoea in piglets

Studied parameter	Trial group (Tiamulin)	%	Control (Hemutin S)	%
Number of piglets at the beginning of the trial	14	100%	14	100%
Duration of treatment	5 days		5 days	
Termination of diarrhoea	day 1	0	day 1	3 piglets (21.43%)
	day 2	4 piglets (28.57%)	day 2	11 piglets (78.57%)
	day 3	9 piglets (64.28%)		
	day 4	1 piglet (7.14%)		
Normalisation of the appetite	day 2	10 piglets (71.43%)	day 2	9 piglets (64.28%)
	day 3	4 piglets (28.57%)	day 3	5 piglets (35.71%)
Normalisation of the body temperature	day 1	7 piglets (50.0%)	day 1	9 piglets (64.28%)
	day 2	7 piglets (50.0%)	day 2	5 piglets (35.71%)
Full recovery	day 3	13 piglets (92.86%)	day 3	14 piglets (100%)
	day 4	1 piglet (7.14%)		
Number of piglets at the end of trial	14	100%	14	100%
The number of relapses	0		0	

Data presented in the Table 1 show that no piglets died in the experimental and control group. Also, it can be seen that in both groups all the pigs were healed and there was no recurrence.

As far as symptoms of diarrhoea, in the trial group, the termination of diarrhoea occurred from day 2 to 4 of the trial with an interval of variation (Iv) of 8 (57.14%) piglets (Iv_{max} = 9 or 64.28% of piglets, Iv_{min} = 1 or 7.14% of piglets). In addition, on day 3 of the trial, the highest frequency was obtained (modus - Mo = 9 (64.28% of piglets). In the control group, the termination of diarrhoea occurred on day 1 and 2 of the trial, however with the completely same result for the interval of variation as in experimental group (Iv_{max} = 11 or 78.57% of piglets, Iv_{min} = 3 or 21.43% of pigs). The modal value (Mo) was 2 days with 11 (78.57% of piglets) frequencies (Table 1).

The following parameter - the normalization of appetite, in both groups, occurred within the days 2 and 3 of the trial, with an interval of variation (Iv) of 6 piglets or 42.86% (Iv_{max} = 10 or 71.43%, Iv_{min} = 4 or 28.57%) piglets. In the control group, the value of the interval of variation (Iv) was 4 piglets or 28.57% (Iv_{max} = 9 or 64.28%, Iv_{min} = 5 or 35.71%) piglets. The modal value in both groups was the second day of the trial with 10 (71.43%) frequencies in the experimental group and 9 (64.28%) frequency in the control group (Table 1).

The table 1 shows that, in both groups, the body temperature has normalized during the first two days of the experiment, with the interval of variation (Iv) of 0 piglets for experimental group, and Iv of 4 piglets, or 28.57% piglets (Iv_{max} = 9 or 64.28%, and Iv_{min} = 5 or 35.71%). In the experimental group, the number of frequencies was the same, but in the control group the modal value (Mo) was the first day with 9 frequencies (64.28% of piglets).

The healing of piglets in the experimental group took place on days 3 and 4 of the trial, with Iv 12 or 85.71% (Iv_{max} = 13 or 92.86%, Iv_{min} = 1 or 7.14%), while in the experimental group all animals were healed on day 3 of the experiment (Table 1).

Gagrčin et al. (2004) provide data about the high level of mortality in all categories of pigs suffering from bloody diarrhoea. Piglets planned for our trial (prior to treatment) had no observed mortality, nor was there mortality post - treatment. *Jezdimirović (2000)* points out that tiamulin base can be successfully used in the treatment of *Treponema hyodysenteriae*, which was confirmed in our experiment in the struggle against pathogen *Serpulina hyodysenteriae*. Also, *Popovic and Gagrčin (1990),* provide information about the importance of timely and appropriate treatment in order to improve chances for cure and a significant mortality rate. In our experiment, due to the success of the therapy, it is obvious that the treatment was appropriate and timely. In the study of the drug Rodotium ad. us. vet., in the treatment of swine dysentery, animals of about 4 months of age and with the control study which included the drug Hemutin S, same as in our experiment, all 18 pigs (9 + 9) has been successfully healed. In fact, the symptoms of the diarrhoea disappeared within 3-4 days in the experimental, and 2-4 days in the control group. The highest frequency was on day 3 in both groups, i.e. 66.66% of pigs in the experimental group, and 55.50% in the control group. These results are close to our findings, in the experimental group with the highest frequency of 9 piglets (64.28%) on day 3 of the experiment, and in the control group, the highest frequency of 11 (78.57%) piglets on day 2 of the experiment. Also, the parameter - normalization of appetite, in trials with Rodotium and Hemutin S, took place on days 3 and 4 of the trial, in both groups, with identical highest frequency of 77.77% of pigs on day 3. These results are similar to our findings, on days 2 to 3 in both groups, with the highest frequency of 10 (71.43%), piglets on day 2 of the trial in the experimental group and 9 piglets (64.28%) piglets in the control group (NIVS, 2003).

Conclusion

Subsequent to i.m. treatment of bloody diarrhoea in piglets using the drug Tiamulin ad us. vet. as an experimental drug, and the drug Hemutin S as a control drug, there was no mortality of piglets, all the animals were healed and no recurrence of disease was observed. Symptoms of diarrhoea terminated from day 2 to 4 in the experimental group, with the highest frequency on day 3, and in the control group, termination of diarrhoea was observed on days 1 and 2, with greater frequency on day 2. In both groups, normalization of appetite occurred in 2 to 3 days, and normalization of body temperature in one to two days. Full recovery occurred on day 3 day of the trial in the experimental group in 92.86% of cases, and in 100% of cases in the control group. It should be noted, along with the success of the treatment, that both the experimental and control drug gave approximately similar results in the treatment of bloody diarrhoea.

Ispitivanje terapijskog efekta tiamulina u lečenju krvavog proliva prasadi

Vujadin Vuković, Mihajlo Vićentijević, Milan Radović

Rezime

U ogledima je učestvovalo ukupno 28 prasadi sa kliničkom slikom krvavog proliva. Dijagnoza je postavljena na osnovu kliničke slike, kliničkih simptoma i bakteriološkog nalaza. Obolele životinje su podeljene u oglednu grupu, koja je tretirana lekom Tiamulin, i kontrolnu grupa koja je dobila lek Hemutin S. Lekovi su davani jednokratno intramuskularno u toku 5 dana, u dozi od 1 ml/12,5 kg t.m. Praćeni su efekti oglednog u odnosu na kontrolni lek, pojava uginuća, prestanak simptoma obolenja, potpuni oporavak i pojava recidiva.

Ključne reči: tiamulin, prasad, proliv, terapija, dijagnoza

Acknowledgment

Project MNTR Government of Serbia no. 46009.

References

BAUMANN D., BILKEI G. (2002): Effect of highly fermentable dietary fiber on the development of swine dysentery and on pig performance in a „Pure – Culture Challenge Model.“, Berl Münchn Tierärztl Wschr, 115:37-42.

BILIC B., BILKEI G. (2003): Effect of highly fermentable dietary fiber on pig performance in a large unit, infected with endemic swine dysentery. Acta Veterinaria Beograd, Vol. 53, 4, 229-238.

GAGRČIN M., KOVČIN S. (1999): Zdravstveni, reproduktivni i proizvodni rezultati na farmama svinja u Vojvodini u 1998. godini. Zbornik radova, I simpozijum iz oblasti veterinarske nauke i prakse, Zlatibor, 124-129.

GAGRČIN M., KOVČIN S. (2002): Problemi i rešenja u svinjarskoj proizvodnji, Revija – agronomska saznanja, XII, 1-2, 65-67.

GAGRČIN M., KOVČIN S., STANČIĆ B. (2001): Zdravstveni i proizvodni rezultati u farmama svinja sa područja Vojvodine za 2000. godinu. Savremena poljoprivreda, 3-4, 251-256.

GAGRČIN M., KOVČIN S., STANČIĆ B. (2002): Zdravstveni i proizvodni rezultati u farmama svinja sa područja Vojvodine za 2001. godinu. Savremena poljoprivreda, 3-4, 265-267.

GAGRČIN M., STANČIĆ B., KOVČIN S., DOŠEN R. (2004): Results of swine production in Serbia from 1998-2003. Veterinarski glasnik, Vol. 58, 3-4, 501-512.

JEZDIMIROVIĆ M. (2000): Veterinarska farmakologija, II prerađeno i dopunjeno izdanje, Fakultet veterinarske medicine, Beograd.

JEZDIMIROVIĆ M. (2009): Osnovi farmakoterapije i gotovi veterinarski lekovi. Fakultet veterinarske medicine, Beograd.

KIRKWOOD R. N., HUANG S. X., Mc FALL M., AHERNE X. (2000): Dietary factors do not influence the clinical expression of swine dysentery. Swine Health and Prod., Vol. 8, 73-76.

LONČAREVIĆ A. (1997): Zdravstvena zaštita svinja u intenzivnom uzgoju. Naučni institut za veterinarstvo Srbije, Beograd.

MILNER J.A, SELLWOOD R. (1994): Chemotactic response to mucin by Serpulina hyodysenteriae and other porcine spirochetes a potential role in intestinal colonization. Infect Immun.Vol. 62, 4095-4099.

MIRKO C.P., BILKEI G. (2005): Risk factors associated with swine disentery in East European pig production units. Vet. glasnik, Vol. 59, 3-4, 349-362.

NIVS (2002): Izveštaj o kliničkom ispitivanju leka Tiamulin ad us.vet. br. 504/1.

NIVS (2003): Izveštaj o kliničkom ispitivanju leka Rodotium ad us.vet. br. 577/1.

POPOVIĆ, M., GAGRČIN M. (1990): Dizenterija svinja kao ekonomski i zdrstveni problem i značajnija diferencijalno dijagnostièka oboljenja. Vet. glasnik, Vol.44, 3-4, 287-298.

STAMATOVIĆ S., ŠAMANC H. (2001): Bolesti svinja. Veterinarska komora Srbije, Beograd.

ŠAMANC H. (2009): Bolesti svinja. Fakultet veterinarske medicine, Beograd.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

EXAMINATION OF THERAPEUTIC EFFECTS GENTAMICIN 5% INJ. ad. us. vet. IN THE TREATMENT OF NEONATAL COLIBACILLOSIS CALVES

Vujadin Vuković, Mihajlo Vićentijević, Milan Radović

Institute of Veterinary Medicine of Serbia, 11070 Belgrade, Auto put 3. Srebia
Corresponding author: vujadin.vukovic@gmail.com
Original scientific paper

Abstract: In trials involving a total of 24 calves suffering from neonatal colibacillosis. The diagnosis was based on clinical picture, clinical symptoms of disease and bacteriological findings. The animals were divided into two groups, experimental and control. Experimental group was treated with the drug Gentamicin 5% inj. ad. us. vet., whereas the control group received the composition Gentamicin 5. Drugs were administered by intramuscular route for 7 days at a dose of 4 ml/50 kg body weight, twice a day. We assessed the effects of the experimental compared to the control drug, the phenomenon of death, cessation of symptoms of illness, full recovery and recurrence.

Key words: gentamicin, calves, colibacillosis, therapy, diagnosis

Introduction

The etiology of neonatal calf diarrhea involves various types of microorganisms: viruses, bacteria, protozoa, fungi ... The pathogenic strains of *E. coli* (ETEC, EIEC, DAEC, EAggEC, EHEC), with different O:H serovars lead in the bacterial etiology, causing colibacillosis depending on the virulence mechanism of action ... *(Samokovlija and Đuričić, 2009). E. coli* is a gram negative rod, of the family *Enterobacteriaceae*. The disease occurs in conjunction with enteropathogenic bacteria and viruses, as well as a series of unfavorable factors (inadequate hygiene, poor housing conditions, untimely drying and nutrition of pregnant cows, poor immunity, etc.) (*Sinovec, 2003; Vujanac et al., 2011*). Furthermore, it is necessary that the calf in the first hours after birth gets colostrum and to continue to consume it as lon as it is excreted (*Jonić et al., 2012*). The greatest risk of developing the disease is until the age of one week, but the risk exists to one month of age, which causes severe losses in cattle production (*Stamatović and Jovanović, 1994*). The mortality of animals, the cost of treatment, poor growth of animals, etc. cause economic losses. Colibacillosis is characterized by: watery diarrhea, pale to pale yellow in colour with frequent effortless defecation, loss of appetite, dehydration of the body, cold extremities, difficulties when getting up, disorder of the general condition of the animal, increased pulse and drop in body temperature. Because of the above mentioned, the preventive action is necessary, however, when the disease occurs, prompt and proper diagnosis and determination of appropriate therapy are crucial, including liquid electrolytes, immunoglobulins ... as well as the application of a series of protective measures (*Jezdimirović, 2000; 2009; Prka 2008*).

Material and Methods

The trials were carried out on 24 calves of Holstein-Friesian breed, both sexes, to 30 days of age and average body weight of about 60 kg. The animals were reared in farm system of housing. The clinical diagnosis of the disease was colibacillosis in acute progress, and objective diagnosis was based on clinical symptoms and bacteriological examination of rectal swabs. The enteropathogenic *E. coli* (ETEC) was isolated in laboratory conditions. The clinical picture presented profuse diarrhea of varying intensity (watery to mucous, pale to pale yellow in colour, and in some cases with blood elements). Also, the dehydration symptoms were in the range from the initial symptoms to the pronounced symptoms. Among other symptoms, the triad was disturbed, thirst increased, appetite decreased, along with the general state of disorder. The position of the animal was mostly lying down, getting up was with difficulties.

A total of 24 calves were divided into two equal groups, experimental and control group, treated with gentamicin, as the active ingredient in finished drugs Gentamicin 5% ad us. vet. and Gentamicin 5 ad us. vet. Gentamicin is an aminoglycoside antibiotic with broad anti-bacterial spectrum (*E. coli, Pseudomonas spp., Proteus, Klebsiella,* streptococci and *staphylococci).* Subsequent to an intra-muscular injection, it reaches maximum concentration in serum in 30 to 60 minutes, which is sustained during 8 to 10 hours. It is also used in cases of infections caused by enterobacteria, especially in young animals (*Jezdimirović, 2000*). In our trials, animals suffered precisely this kind of infections, and the diseased individuals were the mentioned age. The calves were divided into two equal groups of 12 animals (experimental and control). In both groups, the drug was administered intramuscularly to all calves at a dose of 4 ml/50 kg body weight, every 12 hours. The treatment lasted 7 days. All animals endured well the application of the drug and showed no side effects.

Results and Discussion

The examination of the therapeutic effect of gentamicin as an active substance in the experimental and control drug, in the treatment of colibacillosis in calves, was monitored by observing the following: the mortality of calves, the termination of the disease symptoms and complete recovery.

Data presented in Table 1, show the same number of calves at the beginning and end of the trial, in the experimental and the control group, meaning that there were no deaths.

Table 1. Testing of the therapeutic efficacy of drugs Gentamicin 5% inj. ad. us.vet. and Gentamicin 5 ad. us.vet., in the treatment of colibacillosis of neonatal calves

Studied parameter	Trial group (Gentamicin 5%)	%	Control (Gentamicin 5)	%
Number of calves at the beginning of the trial	12	100%	12	100%
Duration of treatment	7 days		7 days	
Termination of diarrhoea	Day 1	9 calves (75.0%)	Day 1	7 calves (58.33%)
	Day 2	3 calves (25.0%)	Day 2	4 calves (33.33%)
			Day 3	1 calf (8.33%)
	Day 2	8 calves (66.66%)	Day 2	6 calves (50.0%)
Normalisation of the triad	Day 3	4 calves (33.33%)	Day 3	6 calves (50.0%)
	Day 5	11 calves (91.66%)	Day 5	12 calves (100%)
Full recovery	Day 6	1 calf (8.33%)		
	Day 1	9 calves (75.0%)	Day 1	7 calves (58.33%)
Number of calves at the end of trial	12	100%	12	100%
The number of relapses	0		0	

Termination of the symptoms of diarrhea in both groups, exclusively and mostly occurred during the first two days of the experiment: in the experimental group in 100% and in the control group in 91.66% calves. At the same time, also in both groups, the highest frequencies of termination of diarrhea were during the first day and amounted to 9 (75.0%) and 7 (58.33%) calves (Table 1).

As for the normalization of the triad, in both groups, the situation normalized in all calves days 2 and 3 of the trial. It is notable that in the experimental group, two thirds of frequencies (8 of 12 calves, or 66.66%) of the normalization of triad occurred on day 2 of the experiment, whereas in the control group this ratio was equal (Table 1).

Data presented in Table 1 show that all calves had complete recovery. Most calves in the experimental group recovered on day 5 of the experiment, i.e. 11 of 12 (91.66%) calves, and in the same period, in the control group, all calves recovered (100%).

Also, the Table 1 shows that there was no recurrence of disease.

In the experimental and the control group all the animals were healed, and experimental and control drug were successful in the treatment of neonatal colibacillosis in calves. However, variations in the obtained results, both within groups and between groups, are the result of the biological differences between individual animals, severity of the disease, etc., and does not represent a response relevant to the comparative – experimental drugs.

Our findings are partially consistent with the scientific indicators presented by *Stamatović and Jovanovic (1994)*, showing calves suffering from colibacillosis, that respond favorably to therapy, recovering normally within 24 to 36 hours. In our experiments, the termination of diarrhea was 24 to 48 hours, in the experimental group, as well as in the control group, in 11 of 12 calves.

The treatment of colibacillosis in calves with a combination of sulphadimidine and trimethoprim managed to stop the diarrhea after 3 days, and complete recovery of calves was after 6 days (*Nivs, 2002*). Adequate therapy enteritis of calves, with isolated *E. coli* for 3 to 5 days, results in the efficacy in healing of 92.0% (*Spoo and Riviere, 1995*), and 95.0% (*Goodman and Gilman (1995*). Also, *Ito et al. (1997),* provide data on successful treatment of neonatal colibacillosis citing the importance of the timely treatment and the proper choice of antibiotics. These data are in agreement with our results, which show 100% efficacy in the experimental and control group.

Conclusion

Subsequent to intra-muscular treatment of neonatal colibacillosis in calves with Gentamicin 5% inj. ad. us.vet., as a experimental drug, and Gentamicin 5, as a control drug, there was no mortality in calves. In both groups of calves, diarrhea has stopped in 2 to 3 days, the triad was normalized within 2 to 4 days, and complete recovery was after 7 days.

It should be noted that the experimental and control drug were successful in treatment and had almost identical results.

Ispitivanje terapijskog efekta gentamicina 5% inj. ad. us. vet. u lečenju neonatalne kolibaciloze teladi

Vujadin Vuković, Mihajlo Vićentijević, Milan Radović

Rezime

U ogledima je učestvovalo ukupno 24 teleta obolelih od neonatalne kolibaciloze. Dijagnoza je postavljena na osnovu kliničke slike, simptoma obolenja i bakteriološkog nalaza. Životinje su podeljene u dve jednake grupe, oglednu i kontrolnu. Ogledna grupa je tretirana lekom Gentamicin 5% inj. ad. us.vet., a kontrolna grupa je dobila preparat Gentamicin 5. Lekovi su davani intramuskularno u toku 7 dana u dozi od 4 ml/50 kg t.m., dva puta na dan. Praćeni su efekti oglednog u odnosu na kontrolni lek, pojava uginuća, prestanak simptoma obolenja, potpuni oporavak i pojava recidiva.

Ključne reči: gentamicin, telad, kolibaciloza, terapija, dijagnoza

Acknowledgment

Project MNTR Government of Serbia no. 46009.

References

BOJKOVIĆ-KOVAČEVIĆ S. JEREMIĆ I., KIROVSKI D., POLOVINA M., VUJANAC I., PRODANOVIĆ R., MILOŠEVIĆ S. (2011): Influence of Peripartum Dietary Energy Supplementation on some Blood Biochemical Parametars in Dairy Cows.Vet. glasnik, Vol. 65,1-2, 3-12.

GOODMAN L.S. and GILMAN A. (1995): The Pharmacological Basis of Therapeutics. The Macmillan Company, London.

ITO T, AKINO E., HIRAMATSU K. (1997): Evaluation of antibiotics used for enterohemorrhagic Esherichia coli O157 enteritis – effects of various antibiotics on extracellular release of verotoxin. Kansenshogaku Zasshi. J Jpn Assoc Infect Dis, 71: 130-137.

JEZDIMIROVIĆ M. (2000): Veterinarska Farmakologija. II prerađeno i dopunjeno izdanje, Fakultet veterinarske medicine, Beograd.

JEZDIMIROVIĆ M. (2009): Osnovi farmakoterapije i gotovi veterinarski lekovi. Fakultet veterinarske medicine, Beograd.

JONIĆ B., DIMITRIJEVIĆ B., MIRILOVIĆ M., OBRENOVIĆ S. i BACIĆ D. (2012): Evaluation of colostrum protection of calves. Vet. glasnik, Vol. 66, 1-2, 13-25.
NIVS (2002): Izveštaj o kliničkom ispitivanju leka Baktapram 30% inj. ad us.vet. br. 511/1.
NIVS (2003): Izveštaj o kliničkom ispitivanju leka Gentamicin br. 19/586.
PRKA I., VUKOVIĆ D. (2008): Oboljenja teladi- prolivi. Vet.inf.br. 30/31, str.111-115.
SAMOKOVLIJA A., ĐURIČIĆ B. (2009): Escherichia coli – stari problem, nova patologija. Vet. glasnik 63 (1-2) 113 - 123.
SINOVEC Z. (2003): Uloga ishrane u etiopatogenezi zdravstvenih poremećaja krava u peripartalnom periodu. Vet. glasnik, Vol. 57, 3-4, 137-148.
STAMATOVIĆ S. i JOVANOVIĆ M. (1994): Bolesti papkara I. Bolesti goveda. Univezitet u Beogradu, Veterinarski fakultet
SPOO J. and RIVIERE J. (1995): Chloramphenicol, Macrolides, Lincosarnides, Fluoroquinolones and Miscellaneous antibiotics. Veterinary pharmacology and therapeutics ed R.H. Iowa State University Press/Ames pp. 820-854. and Ther., 1995).

COMPARATIVE ANALYSIS OF FATTY ACIDS IN THE MEAT OF THE MACEDONIAN AND OHRID TROUT FROM AQUACULTURE PRODUCTION

Aleksandar Saveski[1], Tatjana Kalevska[1], Viktorija Stamatovska[1], Zora Uzunoska[1], Elena Josevska[2]

[1]University St. Kliment Ohridski, Bitola, Faculty of Technology and Technical science, Add. Dimitar Vlahovnn, Veles, R. Macedonia
[2] University St. Kliment Ohridski, Bitola, Faculty of biotechnical science, Add. Partizanska nn Bitola, R. Macedonia
Corresponding author: saveskia@yahoo.com
Original scientific paper

Abstract: The aim of this research is to conduct a comparative analysis and to verify the amount of fatty acids in the meat of the Macedonian trout (*Salmomacedonicus*) and the Ohrid trout (*Salmoletnica*), from aquaculture production, nurtured in a fish farm at controlled conditions. The analyses of the amount of fatty acids have been conducted according to an accredited method AO AC 996.06/2005. It has been confirmed that the amount of fatty acids in the Macedonian trout is divided in: 40.16% monounsaturated, 35.407% unsaturated and 25.554% saturated fatty acids. On the other hand, in the Ohrid trout the entire amount of fatty acids is divided as follows: 39.462% monounsaturated, 35.204% unsaturated and 25.335% saturated fatty acids. The most prevailing saturated fatty acid is the palmitic acid, from the group of monounsaturated acids most common is the oleic acid and the most prevailing unsaturated acids is the linoleic fatty acid. The proved difference of fatty acids in the meat of the Macedonian and Ohrid trout is significant on a level $p>0.05$. The amount of n-3 fatty acids in the Macedonian trout is proved to be 4.904% or 4.917% in the Ohrid trout, whereas the amount of n – 6 fatty acids in the Macedonian trout is 30,503% and 30.287% in the Ohrid trout.

Key words: Macedonian trout (*Salmomacedonicus*), Ohrid trout (*Salmoletnica*), fatty acids, n -3, n -6.

Introduction

The nutrition and health significance of fish is one of the reasons for the constant demand of fish, especially fish from aquaculture *(Burger and Gochfeld, 2009)*. The high nutritional value of fish is manifested through the favorable content and ratio of proteins, fats, carbohydrates, minerals, vitamins, as well as the significant content of unsaturated fatty acids, especially n-3 polyunsaturated fatty acids *(Conor, 2000; Sidhu, 2003)*. In fish meat from 17% up to 21% are present saturated fatty acids and 79% up to 83% unsaturated fatty acids. Of the unsaturated fatty acids, the most important is the content of n-3 polyunsaturated fatty acid *(Conor, 2000; Sidhu, 2003)*. The content of fats and the composition of fatty acids in fish varies within and between species *(Haliloglu et al., 2002)*, and many factors such as temperature, water quality, species, availability of food, seasonality, age, gender, reproductive status, geographical location and individual differences are considered important and additionally contribute to these variations.

The fatty acid composition of the food affects the composition of fatty acids in fish meat. The food rich in n-3 fatty acids, under the same conditions of cultivation, has an effect on increasing the ratio of n-3 / n-6 polyunsaturated fatty acids in fish tissues *(Vranić et al., 2012)*.

Of the unsaturated fatty acids in fish in large quantities are represented: oleic, linolenic, linolenic and arachidonic acid, which are defined as essential fatty acids, which have a role of cofactor in the processes of metabolism and have the function of maintaining the normal state of health of the human organism The total amount of polyunsaturated fatty acids with four, five or six double bonds is lower in freshwater fish (about 70%) compared to sea fish (about 88%) *(Huss, 1988)*.

The unsaturated fatty acids can be divided into two groups: n-3 and n-6 polyunsaturated fatty acids. Fish fats are rich in n-3 polyunsaturated fatty acids *(Mason, 2000)*.

Both groups of unsaturated fatty acids are important for the human health, and they differ in the chemical structure, i.e. in the position of the double bond in the chain. In the n-3 fatty acids, the double bond is located on the third C atom, and in the n-6 fatty acids in the sixth C atom.

A beneficial effect on the human health has the n-3 fatty acids, alpha-linolenic (ALA), eicosapentaic acid (EPA) and the docosahexaenoic acid (DHA). There are food products that are enriched with EPA and DHA, which are naturally contained in fish and seafood *(Bender 2011)*.

The ripe fish contains a larger amount of n-6 polyunsaturated fatty acids, linolenic (18: 2 n-6 fatty acid) and arachidonic (20: 4 n-6 fatty acid), but also significant amounts of n-3 fatty acid EPA (20: 5 n-3 fatty acid) and DHA (22: 6 n-3 fatty acid).

The ratio of essential fatty acids to n-3 / n-6 in river fish, ranges from 1 to 4, and in sea fish, this ratio of the content of n-3 fatty acids ranges between 5 and 10. The composition of fatty acids in the river and sea fish depends on the fat content in their foods.

Open water fish compared to the same type of fish produced in aquaculture production contains less fat, and a higher amount of n-3 fatty acid. The intake of n-3 polyunsaturated fatty acids in the human body is higher when consuming fish that are grown in aquaculture production *(Cahu et al., 2004; Lichtenstein et al., 2006).*

The interest in conducting research on potential positive effects on human health from the use of fish in their diet, appears in the 1950s, when it was found that fish oil positively affected the alleviation of symptoms of atypical eczema and arthritis, as well as the impact of lowering the level of cholesterol in the blood *(Riediger et al., 2009).*

According to *Kris-Etherton et al. (2002)* increasing the consumption of fish meat has great significance for the human health,because it allows normal development and functioning of the organism, as well as reducing the occurrence of cardiovascular diseases.

Materials and methods

The purpose of the research was with scientific and objective methods to determine the fatty acid composition of the meat of the Macedonian (Salmomacedonicus) and the Ohrid trout (Salmoletnica) with a consumption size from 200g to 300g of aquaculture production. Samples of the two species of trout were bred in the same conditions at the fishing farm in the village of Izvor, Republic of Macedonia.

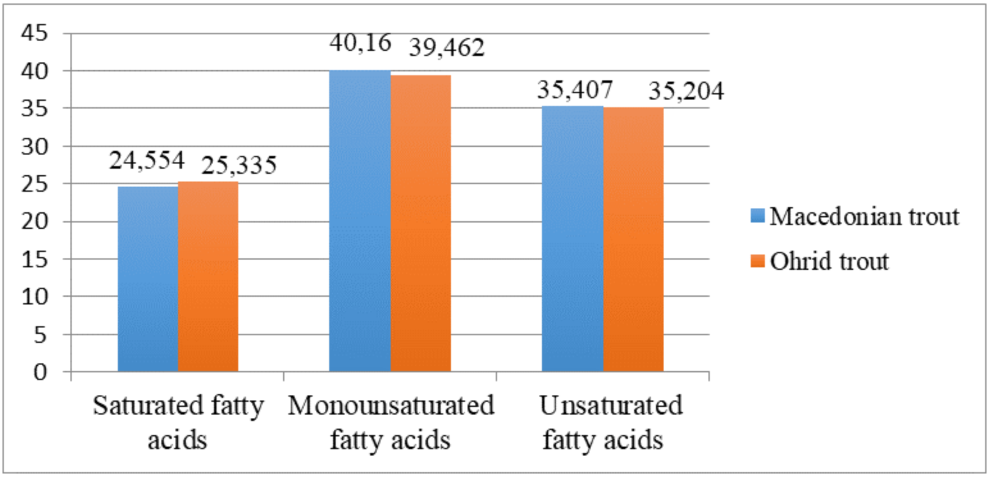
The fish farm uses spring water from the spring of the river Bleshnitsa, in the immediate vicinity of the village of Izvor, in the Porech region and at the same time it represents a repro-center for the Ohrid trout. The water temperature in the fish farm depends on the weather conditions and the season, and has an average temperature of 7-12^{0}C. For feeding of the trout, the same food has been used, which was produced by Nutreco Company Skretting - Italy.

According to the declaration, in the composition of the food there were: wheat, soy beans, wheat flour, processed animal protein from poultry, hydrolyzed food from feathers, fish oil, blood products - pig hemoglobin, extracted sunflower oil, starch, rapeseed oil, Soybean oil, wheat gluten. The presence of constituents in food depends on the type and weight of the fish.

The analysis of fatty acid constituent from trout meat was done according to the method AOAC 996.06/2005 in the accredited laboratory of the "Institute of Food" at the Faculty of Veterinary Medicine in Skopje.

Results and discussion

The average content of total fatty acids in the meat from the Macedonian and Ohrid trout are shown in graph 1.



Graph 1. Comparasion of average content (%) of total saturated, monounsaturated and unsaturated fatty acids in meat from Macedonian and Ohrid trout

According to our results, the average content of total saturated fatty acids in the Macedonian and Ohrid trout was 24.554%, ie 25.335%. According to the results of Ackman's studies (1989), the fish have a low content of total saturated fatty acids (<30%), which is confirmed by the results of our research on the analysis of fatty acids in the meat of Macedonian and Ohrid trout.

The content of monounsaturated fatty acids in the Macedonian trout is 40.16%, while in the Ohrid trout meat it was 39.462%. Our results fully coincide with the results of *Geri et al. (1995)*. Similar results indicate *(Trbović et al., 2012)* and *(De Francesko et al., 2004)*. The polyunsaturated fatty acids in the meat of the Macedonian trout were represented with 35.407%, i.e. 35.204% in the Ohrid trout and were approximate to the value of unsaturated fatty acids in the study of *Trbović et al. (2012)*.

The results obtained from the analysis of the composition of fatty acids in the meat of the Macedonian and Ohrid trout are shown in Table 1.

Table 1. Composition of fatty acids (%) in meat from Macedonian and Ohrid trout

Structure	Macedonian trout $\bar{x} \pm S\bar{x}$	Ohrid trout $\bar{x} \pm S\bar{x}$
C14:0	2.433 ± 0.05	2.688 ± 0.07
C16:0	16.930 ± 0.19	17.505 ± 0.18
C18:0	3.487 ± 0.06	3.570 ± 0.06
C20:0	0.440 ± 0.004	0.270 ± 0.03
C21:0	1.265 ± 0.06	1.302 ± 0.07
C16:1n7	3.835± 0.15	4.050 ± 0.08
C18:1n9	35.865± 0.21	34.937 ± 0.58
C22:1n9	0.460± 0.05	0.475 ± 0.05
C18:2n6	25.237± 0.38	24.515 ± 0.39
C18:3n3	3.412± 0.12	3.427 ± 0.07
C20:3n6	0.383± 0.03	0.452 ± 0.03
C20:5n3	1.492± 0.04	1.490 ± 0.04
C22:6n3	4.883± 0.37	5.320 ± 0.25

From the results it can be concluded that from saturated fatty acids, the most common was the palmitic fatty acid (C16:0) with 16.930% ± 0.19% in the Macedonian trout, or 17.505% ± 0.18% in the Ohrid trout. Similar results to ours of the palmitic acid content in the carp fillets from 16.25% i.e. 19.04% in the trout, were found by *Trbović et al. (2012).*

Stearic fatty acid (C18:0) is represented by 3.487% ± 0.06% in the Macedonian and 3.570% ± 0.06% in the Ohrid trout. The least content in the meat of the Macedonian trout has the arachidonic fatty acid (C20:0) which is 0.440% ± 0.004%, or 0.270% ± 0.03% in the meat of the Ohrid trout. In fatty acids, C16: 0 ($p = 0.040$); C18: 0 ($p = 0.189$) and C20: 0 ($p = 0.004$) in the meat of Macedonian and Ohrid trout were found statistically significant difference at the level of p>

0.05. Of the monosaturated fatty acids, the most common was the oleic (C18: 1n-9) fatty acid with 35.865% ± 0.21% in the Macedonian and 34.937% ± 0.58% in the Ohrid trout. The content ofpalmitoleic fatty acid (C16: 1n-7) is 3.835% ± 0.15% in the Macedonian, i.e. 4.050% ± 0.08% in the Ohrid trout. The least contents in the meat of the Macedonian and Ohrid trout has the erucic fatty acid (C22: 1n-9) of 0.460 %± 0.05% i.e. 0.475% ± 0.05% respectively.

Of the unsaturated fatty acids, the highest content was found in linoleic fatty acid (C18: 2n-6), which in the Macedonian trout is 25.237% ± 0.38% and 24.515% ± 0,39% in the meat of the Ohrid trout. A linolenic acid (C18: 3n-3) is represented by 3.412% ± 0.12% in the Macedonian, i.e. by 3.427% ± 0.07 % in the Ohrid trout. The content of docosahexaenoic acid (DHA) (C22: 6n-3) in the Macedonian is 4.883% ± 0.37%, and somewhat greater content of 5.320 ± 0.25 is found in the Ohrid trout.

Trbović et al. (2012) and *Caballero et al. (2002)* cite results with different content of (C22: 6n3) from our results. The Eicosapentaenoic acid (EPA) (C20: 5 n-3) is represented with 1.492% ± 0.04% , In the Macedonian, i.e. with 1.490% ± 0.04% in the Ohrid trout. A higher content of 2.4% of (C20: 5n-3) was determined by (Caballero et al 2002). Of the polyunsaturated fatty acids, the least content was found in eicosatric acid (C20: 3 n-6) from 0.383% ± 0.03% in the Macedonian, i.e. 0.452% ± 0.03% in the Ohrid trout.

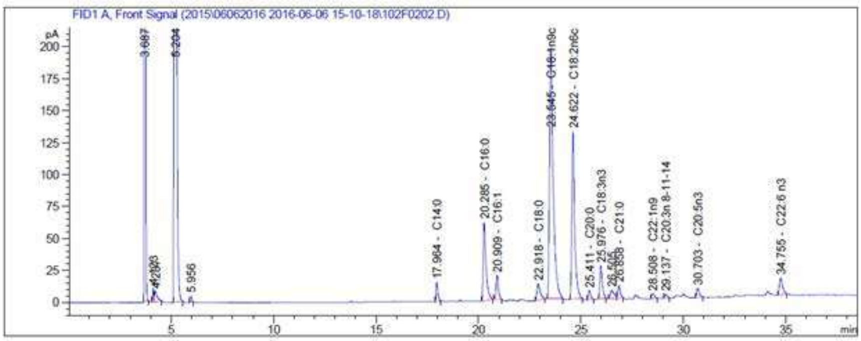


Fig. 1. Chromatogram from analysis of fatty acid composition of meat from Macedonian trout

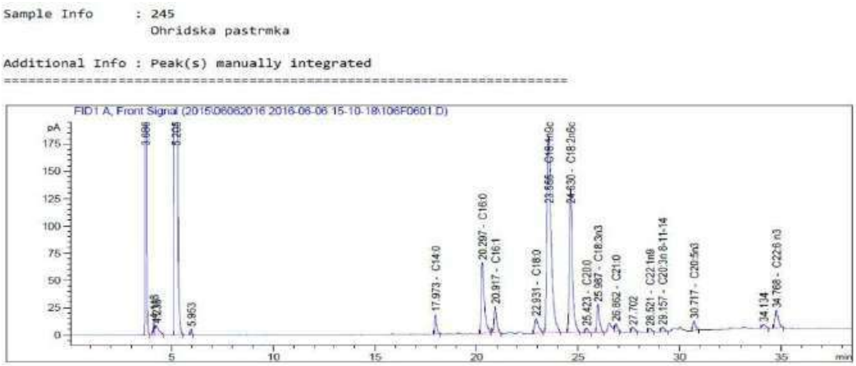
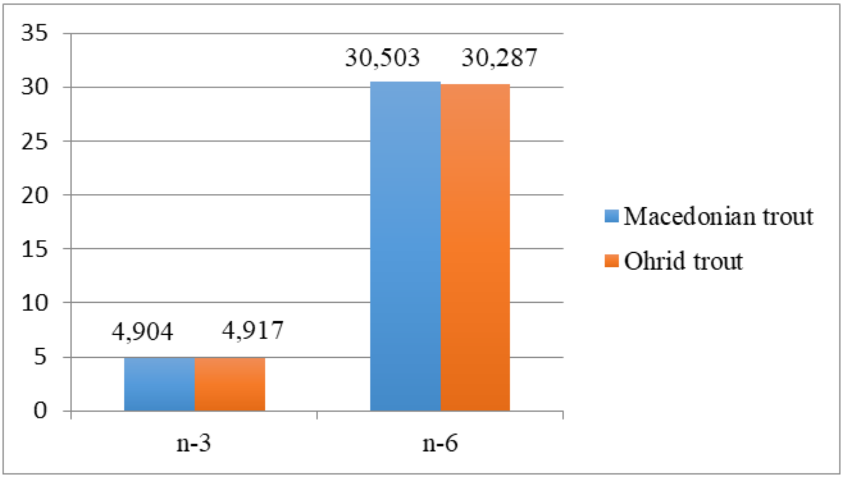


Fig. 2 Chromatogram from analysis of fatty acid composition of meat from Ohrid trout

The average contents of total n – 3 and n - 6 fatty acids (%) in the meat from the Macedonian and Ohrid trout are shown in graph 2.



Graph 2. Comparasion the content of n-3 and n-6 fatty acids (%) in meat from Macedonian and Ohrid trout

The total content of n-3 fatty acids in the meat of the Macedonian trout was 4.904% and 4.917% in the meat of the Ohrid trout. The total content of n-6 fatty acids in the meat of the Macedonian and Ohrid trout was 30.503% and 30.287%, respectively. According to *Valfre et al. (2003)*, the ratio of n-3 and n-6 fatty acids in sea fish is 5: 1 to 10: 1, while in freshwater fish, this ratio is 4: 1 due to the greater prevalence of n-6 fatty acids, primarily linoleic and arachidonic acid.

The fish from aquaculture production have slightly lower content of n-3 fatty acids compared to the open water fish (*Kris-Etherton et al., 2002; Spirić et al., 2009; Trbović et al., 2012; Ljubojević et al., 2013*). This conclusion is confirmed by the results of our research.

Conclusion

According to results of total fatty analysis of the meat from the Macedonian (Salmomacedonicus) and the Ohrid trout (Salmoletnica) from aquaculture production, it can be concluded that the monounsaturated fatty acids had the highest determined content of 40.16% in the Macedonian i.e 39.462% in the meat of the Ohrid trout.

The content of polyunsaturated fatty acids in the meat of Macedonian trout was 35.407%, and in the meat of Ohrid trout 35.204%. In both types of trout, the lowest was the content of saturated fatty acids: 24.554% in the Macedonian, i.e. 25.335% in the Ohrid trout. In both species of trout, from the saturated fatty acids, the most common is C16: 0, from the monounsaturated C18: 1n-9 and from the polyunsaturated fatty acids C18: 2n-6.

The approximate total content of n-3 fatty acids of 4.904% is determined in the meat of Macedonian trout and 4.917% in the meat of the Ohrid trout. The total content of n-6 fatty acids in meat of the Macedonian trout and the Ohrid trout was 30.503% and 30.287% respectively.

As a result of the higher content of monounsaturated and polyunsaturated fatty acids compared to saturated fatty acids, as well as the favorable composition of fatty acids and the ratio of n - 6 and n - 3 fatty acids, the meat of the Macedonian and the Ohrid trout have high nutritional value and act positively on the human health.

Considering the fact that the two species of trout were grown in the same fishing farm, under the same ambient conditions and nutrition, the differences determined by the comparative analysis of the Macedonian and Ohrid trout samples are due to their genetic differences.

Uporedna analiza masnih kiselina u mesu makedonske i ohridske pastrmke iz akvakulture

Aleksandar Saveski, Tatjana Kalevska, Viktorija Stamatovska, Zora Uzunoska, Elena Josevska

Rezime

Cilj ovog istraživanja je da se sprovede komparativna analiza i proveri količina masnih kiselina u mesu makedonske pastrmke (*Salmomacedonicus*) i ohridske pastrmke (*Salmoletnica*), iz akvakulture, gajene u ribnjaku u kontrolisanim uslovima. Analize količine masnih kiselina izvedene su prema akreditovanoj metodi AO AC 996.06/2005. Utvrđeno je da je količina masnih kiselina u makedonskoj pastrmki sledeća: 40,16% mononezasićenih masnih kiselina, 35,407% nezasićenih i 25,554% zasićenih masnih kiselina. S druge strane, u ohridskoj pastrmki, celokupna količina masnih kiselina podeljena je na sljedeći način: 39.462% mononezasićenih masnih kiselina, 35.204% nezasićenih i 25.335% zasićenih masnih kiselina. Najzastupljenija zasićena masna kiselina je palmitinska kiselina, od grupe mononezasićenih kiselina najčešća je oleinska kiselina, a od nezasićenih kiselina - linolna masna kiselina. Utvrđena razlika masnih kiselina u mesu makedonske i ohridske pastrmke je značajna na nivou p> 0,05. Utvrđena je količina n-3 masnih kiselina u makedonskoj pastrmki od 4,904% odnosno 4,917% u ohridskoj pastrmki, dok je količina n-6 masnih kiselina u makedonskoj pastrmki bila 30,503% odnosno 30,287% u ohridskoj pastrmki.

Ključne reči: makedonska pastrmka (*Salmomacedonicus*), ohridska pastrmka (*Salmoletnica*), masne kiseline, n -3, n -6

References

ACKMAN R. G. (1989): Nutritional composition of fats in seafoods. Progress in Food and Nutrition Science 13, 161-241.

BURGER J., GOCHFELD M. (2009): Perceptions of the risks and benefits of fi sh consumption: Individual choices to reducerisk and increase health benefits. Environmental Research, 109, 343–349.

BENDER D.V. (2011): Omega-3 masne kiseline – svojstva I djelovanje, Zavod za gastroenterologiju I hepatologiju, Centar za kliničku prehranu, Klinika za unutrašnje bolesti, KBC Zagreb – Rebro, 234-240.

CONNOR E. W. (2000): Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition, 71, 171S–175S.

CABALLERO M. J., OBACH A., ROSELUND G., MONTERO D., GISVOLD M., IZQUIERDO M. S. (2002): Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchusmykiss. Aquaculture 214, 253-271.

CAHU C., SALEN P., DE LORGERIL M. (2004): Farmed and wild fish in the prevention of cardiovasular diseases: Assessing possible differences in lipid nutritional values. Nutrition Metabolism and Cardiovascular Diseases, 14, 34–41.

DE FRANCESCO M., PARISI G., MEDALE F., LUPI P., KAUSHIK S. J., POLI B. M. (2004): Effect of long-term feeding with a plant protein mixture based of large rainbow trout (Oncorhynchusmykiss). Aquaculture 236, 413-429.

GERI G., POLI B. M., GUALTIERI M., LUPI P., PARISI G. (1995): Body traits and chemical composition of muscle in the common carp (Cyprinuscarpio L.) as influenced by age and rearing environment. Aquaculture 129, 329-333.

HUSS H. H. (1988): Fresh fish quality and quality changes. FAO Fisheries Series, No.29.

HALILO LU H. I., ARAS N. M. (2002): Comparison of muscle fattyacids of three trout species (Salvelinusalpinus, Salmotruttafario, Oncorhynchusmykiss) raised under the sameconditions. Turkish Journal of Veterinary and AnimalSciences, 26, 1097–1102.

KRIS-EHTERTON P. M., HARRIS W. S., APPEL L. J. (2002): Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. Circulation, 106, 2747–2757.

LICHTENSTEIN A. H., APPEL L. J., BRANDS M., CAMETHON M., DANIELS S., FRANCH H. A., FRANKLIN B., KRIS –ETHERTON P., HARRIS W. S., HOWARD B., KARANJA N., LEFEVRE M., RUDEL L., SACKS F., VAN HOM L., WINSTON M., WYLIE –ROSETT J. (2006): Diet and lifestyle recommendations revision 2006: A scientifi c statement from the American Heart Association Nutrition Committee. Circulation, 114, 82–96.

LJUBOJEVIĆ D., ĆIRKOVIĆ M., ĐORĐEVIĆ V., TRBOVIĆ D., VRANIĆ D., NOVAKOV N., MAŠIĆ Z. (2013): Hemijski sastav, sadržaj holesterola I sastav masnih kiselina šarana (Cyprinuscarpio) iz slobodnog izlova, poluintenzivnog I kaveznog sistema gajenja. Tehnologija mesa, 54, 1, 48–56.

MASON P. (2000): Fish oils- an update. The Pharmaceutical Journal, 265, 720–724.

RIEDIGER N. D., OTHMAN R. A., SUH M., MOGHADASIAN M. H. (2009): A Systemic Review of the Roles of n-3 Fatty Acidsin Health and Disease. Journal of the American DieteticAssociation, 109, 668–679.

SIDHU K. S. (2003): Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology, 38 (3), 336–344.

SPIRIĆ A., TRBOVIĆ D., VRANIĆ D., ĐINOVIĆ J., PETRONIJEVIĆ R., MILIJAŠEVIĆ M., JANKOVIĆ S., RADIĆEVIĆ T. (2009): Uticaj masnih kiselina u hrani na sastav masnih kiselina I količinu holesterola kod kalifornijske pastrmke (Oncorhynchusmykiss). Tehnologija mesa, 50, 3–4, 179–188.

TRBOVIĆ D., VRANIĆ D., PETRONIJEVIĆ R., BATLIĆ M., ĆUPIĆ V. (2012): Sastav masnih kiselina I sadrźaj holesterola u pred konzumnoj pastrmci I šaranskoj mlađi, Institut za

higijenu I tehnologiju mesa, Beograd, Fakultet veterinarske medicine, Beograd.
VRANIĆ D., BALTIĆ Ž. M., TRBOVIĆ D., ĐINOVIĆ -STOJANOVIĆ J., MARKOVIĆ R., PETRONIJEVIĆ R., SPIRIĆ A. (2012): Mlađ I konzumna kalifornijska pastrmka (Oncorhynchusmykiss): hemijski sastav, sadržaj holesterola I masnokiselinski sastav fileta. Tehnologija mesa 53, 1, 26–35.
VALFRE F., CAPRINO F., TURCHINI G. M. (2003): The Health Benefit of Seafood. Veterinary Research Communications, 27,1, 507–512.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

CROPPING SYSTEM AND FERTILIZATION REGIME AS FACTORS OF MAIZE GRAIN QUALITY

Vesna Dragičević, Milena Simić, Branka Kresović, Milan Brankov

Maize Research Institute ”Zemun Polje”, Slobodana Bajića 1, 11185 Zemun Polje, Republic of Serbia
Corresponding author: vdragicevic@mrizp.rs
Original scientific paper

Abstract: Maize cropping systems include continuous cropping (monoculture), which is one of the widespread systems in Serbia and in the world, as well as rotations which include rotation of crops of different species, enabling better control of diseases, pests and weeds, as well as better utilization of water and nutrients from soil, having as a consequence increased yields. When leguminous crop is prior to maize in rotations, the maize grain yields are much higher than in rotations when some other crops are prior to maize. The aim of experiment was to evaluate different cropping systems (maize monoculture (MM), maize-winter wheat rotation (M-W), maize-field pea rotation (M-P) and maize-field pea-winter wheat rotation (M-P-W)) and four fertilization regimes (control - without fertilization, $N_{80}P_{60}K_{40}$, $N_{120}P_{90}K_{60}$ and $N_{160}P_{120}K_{80}$) on maize grain yield and grain quality (protein and oil content) during two meteorologically opposite years (2014 and 2015). The growing season and its interaction with cropping system and fertilization regime were the main factors that influenced significant variations in maize grain yield, protein and oil content. A double lower yield, slightly lower oil content and about 1/3 higher protein content were obtained in dry 2015, in relation to 2014, as a season with high precipitation level and lower average temperature. Rotation with higher crop number included (M-P-W rotation) expressed the highest impact on maize yield increase, together with protein and oil content in grain, particularly during stressful 2015. Similar trend was observed in two crop rotation, where legume crop is prior to maize. What is more, increased fertilization level was positively reflected on yielding potential and protein content increase, but it decreased oil content in grain. Poorer growth conditions, present in maize monoculture revealed lower grain yield and protein content, with greater oil content, particularly in treatment without fertilizer application.

Key words: fertilization, grain yield, maize, oil content, protein content, rotation

Introduction

Continuous cropping (monoculture) is one of the widespread systems of maize growing in Serbia and in the world. It has many disadvantages, reflecting through increased infection by diseases and pests, weed spreading, etc. (*Kovačević, 2003)*. On the other hand, cropping systems which include rotation of crops of different species, when they are properly applied, enables better control of diseases, pests and weeds, as well as better utilization of water and nutrients from soil, having as a consequence increased yields *(Kovačević et al., 2008)*.

Combination of grain crops, cereals, forages and legumes in sequences includes different crop densities, application of various tillage practices, fertilizers, herbicides, etc. changing soil physical and chemical properties and microbiota, maintaining fertility to some extent. *Govaerts et al. (2007)* also indicated that cropping systems that include rotation of different crops, zero tillage and crop residue retention contributes to increased soil microorganisms biomass, activity and diversity, when compared to continuous cropping. Combination of these systems meliorates conditions for development of antagonists and predators, thus increasing ecological stability. *Karlen et al. (2006)* depicted that extended rotations that include three year of forage crops in sequences established the highest soil quality index, suggesting that conservation programs which include more diverse and extended crop rotations are connected to increased soil quality, grain yields and so, increased profit. When leguminous crop is prior to maize in rotations which include increased number of different crops, the maize grain yields are much higher than in rotations when some other crops are prior to maize (*Berzsenyi et al., 2000*). This is of particular importance, since legume crop enriches soil with fixed N, increase mineralization and nutrient absorption, leading as a consequence to the yield increase, especially in cases when fertilizers are not applied in maize crop (*Horst and Härdter, 1994*). *Yusuf et al. (2009)* separated effect of N added by fixation of previous legume crop and "other effect of rotation" and calculated that in maize grain yield that increased up to 1.2-1.3 fold, effect of fixed N ranged from 124 to 279 kg ha^{-1} while rotation effects ranged between 193 and 513 kg ha^{-1}.

Some researchers also have underlined importance of increased N uptake of maize included in rotation with other crops, particularly during anthesis, was positively reflected on protein status in maize grain (*Montemurro et al., 2006). Saha et al. (2008)* applied increased NPK levels in maize-wheat rotation system and achieved higher maize grain yield and protein level when compared to maize monoculture, what means that rotation enables better nutrient utilization by maize crop. What is more, when leguminous crop like soybean is preceding crop to sorghum, sorghum grain yield and quality was raised, reflecting through increased nitrogen level and grain hardness *(Mady Kaye et al., 2007).*

The aim of this experiment was to evaluate different cropping systems, which include maize monoculture, maize-winter wheat rotation, maize-field pea rotation and maize-field pea-winter wheat rotation and three fertilization regimes, with different NPK levels on maize grain yield and grain quality (protein and oil content) during two opposite years in meteorological factors.

Material and Methods

Experiment was settled up in Maize Research Institute "Zemun Polje" during 2014 and 2015 growth seasons, with the aim to evaluate effects of different cropping systems (rotations) and fertilization regimes on maize grain yield and grain quality reflected through protein and oil content during two opposite seasons. Experiment included four cropping systems: maize monoculture (MM), two year rotations: maize-winter wheat rotation (M-W), maize-field pea rotation (M-P) and three year rotation: maize-field pea-winter wheat rotation (M-P-W) and four fertilization regimes: control - without fertilization (F1), $N_{80}P_{60}K_{40}$ (F2), $N_{120}P_{90}K_{60}$ (F3) and $N_{160}P_{120}K_{80}$ (F4). The relation between nutrients is 1:0.75:0.50 for maize fertilization and winter wheat, while field pea was fertilized with half of the N dose applied for maize and wheat, with exception of control, without fertilizer added. Fertilizers were applied at the end of October in 2013 and 2014, while sowing was performed during first half of April, dependently on meteorological conditions. All standard protection measures were applied.

After harvesting, grain yield was measured and calculated with 14% of moisture. The content of protein and oil was determined on infrared analyser (Infraneo, Chopin Technologies, France).

The experimental data were statistically processed by analysis of the variance (ANOVA) using Microsoft Excel and analysed by the LSD-test (5 %) and correlation (Pearson correlation).

Meteorological conditions. Two experimental years of 2014 and 2015 were opposite in precipitation level and monthly average temperature. The year 2014 was characterised with the double higher precipitation level (709.1 mm) compared to 2015, with 350.5 mm (Table 1).

Table 1. Average monthly temperature and precipitation sum during seasons of 2014 and 2015

Year	April	May	June	July	August	September	October	M/∑
	Temperature, °C							
2014	13.7	17.4	21.1	23.2	22.6	18	14.1	18.6
2015	12.9	19.1	22.1	26.4	25.7	20.2	12.4	19.8
	Precipitation, mm							
2014	84.8	192.5	71.2	187.4	41	75.6	56.6	709.1
2015	19.7	97.8	31.1	7.2	56	73.6	65.1	350.5

In 2015, precipitation were unequally distributed with the lowest value in July, of only 7.2 mm. This season was also characterised with higher average temperature (19.8 °C) and higher temperature during July, August and September for 3.2 °C, 3.1 °C and 2.2 °C respectively, compared to the same months in 2014. This means that drought was present during flowering and grain filling period of 2015 season.

Results and Discussion

The significant variations in maize grain yield were obtained by the influence of growing season (year), and interactions year x cropping system, year x fertilization regime, cropping system x fertilization regime and year x cropping system x fertilization regime, while single factors, like cropping system and fertilization regime were insignificant for yield variability (Table 1). Such situation could be closely tied to precipitation level, as well as drought present during 2015, when grain yield almost for half was lower than in 2014. Irrespective to insignificant influence of cropping system, the lowest yield was obtained in maize monoculture, what is averagely about 1.58 t ha^{-1} lower than in maize-field pea rotation. Three crop rotation system showed advantage during stressful 2015, with grain yield of 6.60 t ha^{-1}, what is 2.26 t ha^{-1} higher in comparison to monoculture, while in 2014, as a season with high precipitation level, the highest grain yield was achieved in maize-field pea rotation, with 12.03 t ha^{-1}, what is 1.44 t ha^{-1} higher than in monoculture. This means that in maize monoculture higher grain yield losses (in regard to rotation systems) are present during dry season, in relation to season with higher precipitation level *(Berzsenyi et al., 2000)*. The same authors indicated that increase in species number included in rotation reflects positively on maize grain yield, particularly when leguminous crop is prior to maize. Legume, as previous crop to maize in rotation has positive effect on grain yield increase and nutrient absorption. Legume crop increases soil N content by fixation, as well as mineralization, thus increasing yield in unfertilized filed *(Horst and Härdter, 1994)*, like F1 is in this experiment, what is particularly present during 2014. *Yusuf et al. (2009)* calculated separately the effect of N fixed by leguminous preceding crop, as well as rotation effect on maize yield and they obtained that increase in maize grain yield by 1.2-1.3 folds was supported by 124 to 279 kg ha^{-1} with fixed N and almost double by rotation effect, meaning that rotation with legume crop is complex and it has much more importance to yielding potential of maize than fertilization alone.

Table 2. Maize grain yield (t ha^{-1}) influenced by the different cropping system and fertilization regime

	2014					2015				
	MM	M-W	M-P	M-P-W	Aver.	MM	M-W	M-P	M-P-W	M.
F1	5.57	7.53	10.43	8.82	8.09	3.39	4.71	5.79	6.25	5.03
F2	10.54	12.93	12.69	11.79	11.99	4.29	4.76	6.57	6.31	5.48
F3	13.09	13.63	12.89	11.94	12.89	5.04	6.12	5.84	6.96	5.99
F4	13.16	13.23	12.10	11.63	12.53	4.62	4.91	5.97	6.89	5.60
M.	10.59	11.83	12.03	11.05	11.37	4.34	5.13	6.04	6.60	5.53
Year average						LSD 0.05				
F1	4.48	6.12	8.11	7.54	6.56	Year	1.873	Y x F	1.299	
F2	7.42	8.85	9.63	9.05	8.74	Fert.	3.388	Y x CS	1.762	
F3	9.07	9.88	9.37	9.45	9.44	Crop. Sys.	3.469	F x CS	3.439	
F4	8.89	9.07	9.04	9.26	9.06	Y x CS x F		0.82		
M.	7.46	8.48	9.04	8.82	8.45					

MM - maize monoculture; M-W - maize-wheat rotation; M-P - maize-field pea rotation, M-P-W - maize-field pea-wheat rotation; F1 - without fertilization; F2 -$N_{80}P_{60}K_{40;}$ F3 - $N_{120}P_{90}K_{60}$ and F4 - $N_{160}P_{120}K_{80}$

Increased fertilizer inputs are also important for maize yielding potential. The highest grain yield was obtained in F3 treatment in both years (12.89 t ha^{-1} in 2014 and 5.99 t ha^{-1} in 2015) (Table 2). When interactions of all three examined factors are considered, the differences between seasons emphasized that in 2014 the highest grain yield was achieved in combination of M-W with F3 fertilization regime (13.63 t ha^{-1}), while in 2015 the yield reached 6.96 t ha^{-1} in combination M-P-W rotation and also F3 fertilization regime. *Berzsenyi et al. (2000)* also achieved the higher grain yields with increased NPK fertilizer inputs, particularly in two year rotations.

Similarly to grain yield, the significant variations in protein content (Figure 1) and oil content (Figure 2) were obtained by the influence of year, interactions year x cropping system, year x fertilization regime, cropping system x fertilization regime and year x cropping system x fertilization regime, while factors as a cropping system and fertilization regime were insignificant for protein and oil variability. *Kaye et al. (2007)* likewise found that grain yield and N content in sorghum grain was significantly affected by interaction of cropping system and fertilization, increasing yield and grain quality by increased fertilizer supply and rotation with soybean, which adittionaly contributed to increased N level. The drought presence in 2015 affected not grain yield, but grain quality, too, increasing protein content and decreasing oil content in all applied treatments, when compared to 2014 (in average for about 1.97% and 0.27%, respectively).

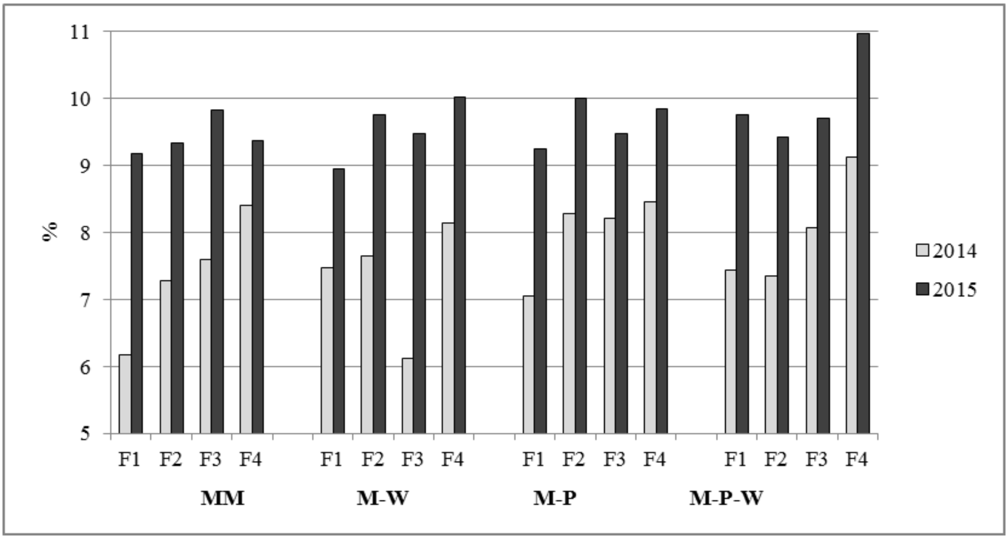


Figure 1. Protein content in maize grain influenced by different cropping system (MM - maize monoculture, M-W – maize-wheat rotation, M-P – maize-field pea rotation, M-P-W – maize-field pea-wheat rotation) and fertilization regime (F1 - without fertilization, F2 -$N_{80}P_{60}K_{40}$, F3 - $N_{120}P_{90}K_{60}$, F4 - $N_{160}P_{120}K_{80}$); LSD 0,05 for year=0.716; fertilization regime 1.179; cropping system 1.225; Y x F = 0.60; Y x CS = 0.697; F x CS = 1.238; Y x CS x F = 0.433)

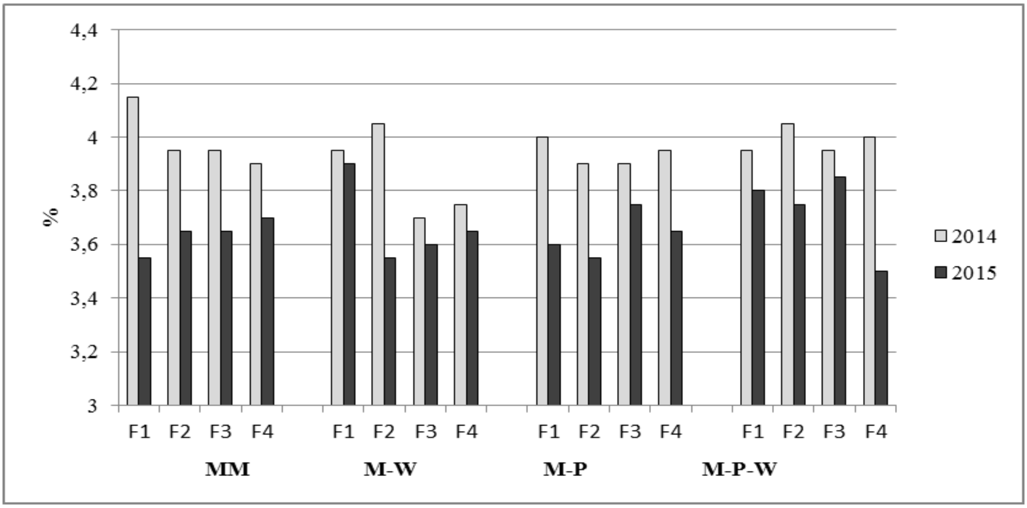


Figure 2. Oil content in maize grain influenced by different cropping system (MM - maize monoculture, M-W – maize-wheat rotation, M-P – maize-field pea rotation, M-P-W – maize-field

pea-wheat rotation) and fertilization regime (F1 - without fertilization, F2 -$N_{80}P_{60}K_{40}$, F3 - $N_{120}P_{90}K_{60}$, F4 - $N_{160}P_{120}K_{80}$); LSD 0,05 for year = 0.142; fertilization regime 0.199; cropping system 0.199; Y x F = 0.139; Y x CS = 0.142; F x CS = 0.210; Y x CS x F = 0.125)

Cropping system is important measure to increase maize grain quality, by increasing protein and oil content, which had the highest values in three crop, M-P-W rotation (on average 8.98% and 3.86%, respectively). Fertilization regime was correspondingly reflected on protein and oil content, with inverse results. This means that the highest average protein content was in F4 fertilization regime, while the highest oil content was in F1 fertilization regime. Contrary, the lowest protein content was in F1 and oil content in F4, denoting that increased NPK (mineral fertilizer) amounts are important for increased protein level in maize grain, but also it is negatively reflecting on oil synthesis, decreasing its content. *Montemurro et al. (2006)* indicated that increased amounts of soil N are important for increased N uptake, particularly after anthesis, thus positively reflecting on grain yield and grain N status (protein level). *Saha et al. (2008)* also confirmed that NPK mineral fertilizers, when applied in higher amounts reflect positive on maize grain protein level and grain yield in maize-wheat rotation system. The highest protein content in 2014 and 2015 was achieved in M-P-W x F4 treatment combination with values of 9.12% and 10.97%, respectively, while the highest oil content was achieved in 2014 in MM x F1 treatment combination and in 2015 in M-W x F1 combination. This means that the conditions provided by three year rotation and high fertilizer amounts enables increased protein synthesis, while the poorer conditions, with lower nutrient levels, such as monoculture and maize-wheat rotation, without fertilizer inputs are better basis for increased oil synthesis. This is opposed to results of *Rastija et al. (2006)* who obtained increased grain yield and oil content in soybean fertilized with ameliorative NPK fertilization.

The interdependence between fertilization regime and examined parameters obtained that increase in amount of applied fertilizer was significantly and positive followed mainly by the grain yield and in some lesser extent by the protein content, while correlation with oil content was also significant, but negative. Grain yield positively correlated with protein content and negative with oil content, too. *Montemurro et al. (2006)* and *Saha et al. (2008)* confirmed that increased fertilization positively affects yield and protein content in maize grain.

Table 3. Correlation between fertilization regime and examined traits: grain yield, protein content and oil content in maize grain

		Fertilization regime	Grain yield	Protein content
Grain yield	CC*	0.728		
	p**	0.001		
Protein content	CC	0.645	0.552	
	p	0.007	0.027	
Oil content	CC	-0.502	-0.483	-0.191
	p	0.047	0.058	0.479

*CC-correlation coefficient; the significant values at the level of significance of 0.05; **p-error

Conclusion

The significant variations in maize grain yield, protein and oil content were mainly obtained by the influence of growing season, as well as its interaction with cropping system and fertilization regime. That means that almost double lower yield, slightly lower oil content and about 1/3 higher protein content were obtained in dry 2015, in relation to 2014, as a season with high precipitation level and lower average temperature. Rotation with higher crop number included (M-P-W rotation) expressed the highest impact on maize yield increase, together with protein and oil content in grain, particularly during stressful 2015. Similar trend was observed in two crop rotation, where legume crop is prior to maize. What is more, increased fertilization level, mainly F3 and in some extent F4 treatment, were positively reflected on increase in yielding potential and protein content, but in parallel oil content in grain was decreased. Poorer growth conditions, present in maize monoculture revealed lower grain yield and protein content, with greater oil content, particularly in F1 treatment. This means that increased fertilization was followed significantly by yield and protein increase, but negatively by oil content in grain.

Sistemi gajenja i režimi đubrenja kao faktori kvaliteta zrna kukuruza

Vesna Dragičević, Milena Simić, Branka Kresović, Milan Brankov

Rezime

Sistemi gajenja kukuruza podrazumevaju kontinuirano gajenje (monokulturu), koje je jedan od najrasprostranjenijih sistema gajenja kukuruza u Srbiji i svetu, kao i plodored, koji podrazumeva gajenje useva različitih vrsta, obezbeđujući bolju kontrolu bolesti, štetočina i korova, kao i bolje iskorišćavanje vode i hraniva iz zemljišta, samim tim povećavajući prinose. Kada u plodoredu kukuruzu prethodi leguminoza, prinosi zrna kukuruza su znatno veći, nego u plodoredima gde druge biljne vrste prethode kukuruzu. Cilj eksperimenta je bio da se oceni uticaj različitih sistema gajenja (monokultura kukuruza (MM) i plodoredi kukuruz-ozima pšenica (M-W), kukuruz-jari stočni grašak (M-P) i kukuruz-jari stočni grašak-pšenica (M-P-W) i četiri režima đubrenja (kontrola, $N_{80}P_{60}K_{40}$, $N_{120}P_{90}K_{60}$ i $N_{160}P_{120}K_{80}$) na prinos kukuruza i kvalitet zrna (sadržaj proteina i ulja) tokom dve sezone suprotne po meteorološkim faktorima (2014. i 2015.). Sezona i njena interakcija sa sistemima gajenja i režimima đubrenja su osnovni faktori koji su uticali na variranje prinosa, sadržaj proteina i ulja u zrnu kukuruza. Duplo manji prinos, nešto niži sadržaj ulja i za oko 1/3 veći sadržaj proteina bio dobijen u sušnoj 2015. godini, u odnosu na 2014., kao sezonu sa više padavina i nižim prosečnim temperaturama. Plodored sa većim brojem useva (M-P-W sistem) je pokazao najveći uticaj na povećanje prinosa, sadžaj proteina i ulja, posebno u 2015. godini. Sličan trend je uočen i u plodoredu sa dva useva gde leguminoza prethodi kukuruzu. Takođe, povećan nivo đubriva se pozitivno odrazio na povećanje potencijala rodnosti i sadržaja proteina, ali je paralalno uticao na smanjenje sadržaja ulja u zrnu. Lošiji uslovi gajenja, koji su prisutni u monokulturi uticali su na smanjenje prinosa i sadržaja proteina, uz povećanje sadržaja ulja u zrnu i to posebno u tretmanu bez primene đubriva.

Ključne reči: đubrenje, prinos zrna, kukuruz, sadržaj ulja, sadržaj proteina, plodored

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31037.

References

BERZSENYI Z., GYŐRFFY B., LAP D.Q. (2000): Effect of crop rotation and fertilisation on maize and wheat yields and yield stability in a long-term experiment. European Journal of Agronomy, 13, 2–3, 225–244.

GOVAERTS B., MEZZALAMA M., UNNO Y., SAYRE K.D., LUNA-GUIDO M., VANHERCK K., DENDOOVEN L., DECKERS J. (2007): Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Applied Soil Ecology. 37, 1–2, 18–30.

HORST W.J., HÄRDTER R. (1994): Rotation of maize with cowpea improves yield and nutrient use of maize compared to maize monocropping in an alfisol in the northern Guinea Savanna of Ghana. Plant and Soil, 160, 2, 171–183.

KARLEN D.L., HURLEY E.G., ANDREWS S.S., CAMBARDELLA C.A., MEEK D.W., DUFFY M.D., MALLARINO A.P. (2006): Crop rotation effects on soil quality at three northern corn/soybean belt locations. Agronomy Journal, 98, 484-495.

KAYE M., MASON N.S.C., JACKSON D.S., GALUSHA T.D. (2007): Crop rotation and soil amendment alters sorghum grain quality. Crop Science, 47, 722-727.

KOVAČEVIĆ D. (2003): Opšte ratarstvo. Poljoprivredni fakultet, Univerzitet u Beogradu, 1-256.

KOVAČEVIĆ D., DOLIJANOVIĆ Ž., OLJAČA S. i JOVANOVIĆ Ž. (2008): Uticaj plodoreda u borbi protiv korova. Acta herbologica, 17, 45-51.

MONTEMURRO F., MAIORANA M., FERRI D., CONVERTINI G. (2006). Nitrogen indicators, uptake and utilization efficiency in a maize and barley rotation cropped at different levels and sources of N fertilization. Field Crops Research, 99, 2–3, 114–124.

RASTIJA M., KOVACEVIC V., VRATARIC M., SUDARIC A., KRIZMANIC M. (2006): Response of maize and soybeans to ameliorative fertilization in Bjelovar-Bilogora country. Cereal Research Communications, 34, 1, 641-644.

SAHA S., GOPINATH KA., MINA B.L., GUPTA H.S. (2008): Influence of continuous application of inorganic nutrients to a Maize–Wheat rotation on soil enzyme activity and grain quality in a rainfed Indian soil. European Journal of Soil Biology, 44, 5–6, 521–531. Special Section of the 7th International Apterygota Seminar 7th International Apterygota Seminar.

YUSUF A.A., IWUAFOR E.N.O., ABAIDOO R.C., OLUFAJO O.O., SANGINGA N. (2009): Grain legume rotation benefits to maize in the northern Guinea savanna of Nigeria: fixed-nitrogen versus other rotation effects. Nutrient Cycling in Agroecosystems, 84, 2, 129-139.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

CARBOHYDRATE CONTENT OF ALFALFA HARVEST AT DIFFERENT DEVELOPMENT STAGE IN THE SPRING GROWTH

Jordan Marković, Snežana Babić, Dragan Terzić, Vladimir Zornić, Tanja Vasić, Jasmina Milenković, Ivica Kostić

[1]Institute for Forage Crops Kruševac, 37251 Globoder
Corresponding author: E-mail: jordan.markovic@ikbks.com
Oroginal scientific paper

Abstract. The objective of this study was to investigate the effect of stage of maturity of alfalfa hay on carbohydrate content and carbohydrate fractions. Alfalfa was cut at full bud, early bloom and mid bloom stage of maturity. Alfalfa at full bud stage had the highest hemicellulose content and the lowest CHO (total carbohydrates), NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber) and lignin content. The content of CHO, NDF, ADF and lignin increased with advancing stage of maturity, whereas TESC (Total ethanol soluble carbohydrate) and NFC (Non fiber carbohydrates) stayed consisted with advancing maturity. The highest CHO fraction was CB_3 which represent available NDF and decreased from 395.7 g kg^{-1} DM to 375.6 g kg^{-1} DM with maturation. The CA fraction tended to decrease with advancing maturity, whereas CC fraction which represent unavailable NDF and lignin increased from 248.7 to 295.4 g kg^{-1} DM with advancing maturity. Various CHO fraction present in feed differ in rate and extent of ruminal degradation. These fractions influence the amount of CHO degraded in the rumen and escaping to the lower digestive tract. The knowledge of these CHO fractions and degradation is used in modern diet formulation programs to formulate ruminant diets.

Key words: alfalfa, carbohydrate fractions, stage of maturity

Introduction

Cultivated alfalfa (*Medicago sativa* L.) is one of the major forage crops in the world (*Hanson et al., 1988)* and the most important forage crop for dairy ration in Serbia. Alfalfa contains high nutrient levels, high digestibility and unique proportion of structural to non-structural components (*Yu et al., 2003*). Botanical traits, nutritive value and crude protein and carbohydrate (CHO) fractions of alfalfa are influenced by cultivar, stage of maturity (*Elizalde et al., 1999; Yu et al., 2003; Coblentz et al., 2008*), climate condition (*Lamb et al., 2003*) and cutting time due to accumulation of non-structural CHO during the day (*Burns et al., 2007; Brito et al., 2009*). CHO are the highest component of rations for lactating dairy cows, and can be partitioned into fiber (FC) and non-fiber (NFC) carbohydrates. Fiber CHO is the slowly digestible fraction of feeds that occupies space in the gastrointestinal tract and fiber CHO associated with lignin resists digestion and therefore does not contribute energy to the animal (*Mertens, 1997*). Balancing for an appropriate level and type of NFC and FC is a major challenge in ruminant ration formulation. Feeds vary widely in their amount and composition of NFC, and CHO fractions in NFC differ in rate and extent of fermentation and contribution to microbial CP production (*Hall and Herejk, 2001; Nocek and Tamminga, 1991*) and therefore to animal performance.

The objective of the present study was to quantify the main CHO fractions during the growing period of alfalfa. Determination of CHO fraction would improve balancing rations for animals, especially for dairy cows.

Materials and methods

The experiment was designed with three replication according to a randomized complete block. Alfalfa (*Medicago sativa* L.) – *cv* K 28 selected at Institute for forage crops, Kruševac was sampled at three stages of maturity – full bud (harevested on the 04th May), early bloom – 10-15% of flowering (harvested on the 21st May) and mid bloom – 50-60% of flowering (harvested on the 29th May).

Alfalfa samples were assayed for DM (Dry Matter) by oven drying at 60º C for 48 h. Standard procedures described by the AOAC (1990) were used to determine ash (AOAC, 942.05), Crude Protein (CP; AOAC 984.13) and ether extract (EE, AOAC 954.02), but ash, CP and EE were not presented in this paper. Total carbohydrates [CHO = 1000 – (CP + Ash + EE)] and Non-Fiber carbohydrates [NFC = 1000 – (aNDF + CP + Ash + EE)] were calculated according to NRC (2001). Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HCL) and lignin content were determined according to Van Soest et al. (1991). TESC (Total ethanol soluble carbohydrates – monosaccharides and disaccharides) were determined as total ethanol soluble carbohydrates according procedures described by Hall et al. (1999). Total CHO are divided into five fractions: instantaneously solubilizable CHO (CA, *i.e.* NSC); rapidly degradable CHO (CB_1, *i.e.* starch); intermediately degradable CHO (CB_2, *i.e.* NFC – NSC – starch); slowly degradable CHO (CB_3, *i.e.* ANDF – CC) and undegradable CHO (CC, *i.e.* aNDF × (Lignin / aNDF) × 2.4) composed of completely undegradable NDF (Lanzas et al., 2007).

The experimental data were analyzed by the analysis of variance for alfalfa samples using a model that accounted for the main effects of development stage. Effects were considered different based on significant ($p< 0.05$) F ratio. The significance of differences between arithmetic means was tested by LSD test (STATISTICA 6, Stat. Soft. 2006).

Results and discussion

Knowledge of changes in quality of alfalfa forage during growing season is important when preparing meals for ruminants, to ensure a satisfactory relationship between structural and non-structural carbohydrates (*Grubić and Adamović, 2003*). During the season as the stage of maturity advanced, results showed that the total carbohydrates (CHO) content increased from 679.2 to 743.8 g kg^{-1} DM (Table 1). Non-strucutural carbohydrates (Total ethanol soluble carbohydrates-TESC) concentrations altered by stage of growth increasing from 75.2 g kg^{-1} DM harvested at the FB stage to 85.4 g kg^{-1} DM harvested at the EBL stage, but after that decreased with flowering to 74.7 g kg^{-1} DM harvested at the MBL stage.

Table 1. Carbohydrate content of alfalfa harvest at different development stage in the spring growth

	FB	EBL	MBL
% of leaf in alfalfa hay	39.5	38.1	36.3
CHO, g kg^{-1} DM	679.2^{c}	731.4^{b}	743.8^{a}
TESC, g kg^{-1} DM	75.2^{b}	85.4^{a}	74.7^{b}
NDF, g kg^{-1} DM	458.4^{c}	493.5^{b}	517.3^{a}
ADF, g kg^{-1} DM	346.8^{c}	408.6^{b}	423.0^{a}
Hemic, g kg^{-1} DM	111.6^{a}	84.8^{b}	94.3^{b}
Lignin, g kg^{-1} DM	70.4^{c}	86.0^{b}	91.6^{a}
Lignin, g kg^{-1} NDF	153.5^{b}	174.3^{a}	177.0^{a}
NFC, g kg^{-1} DM	241.9^{b}	256.6^{a}	244.6^{b}
CA, g kg^{-1} CHO	110.7^{b}	116.7^{a}	100.4^{c}
CB_1, g kg^{-1} CHO	39.1^{a}	31.4^{b}	29.3^{c}
CB_2, g kg^{-1} CHO	205.8^{ns}	202.7^{ns}	199.2^{ns}
CB_3, g kg^{-1} CHO	395.7^{a}	366.9^{b}	375.6^{b}
CB, g kg^{-1} CHO	640.6^{ns}	601.0^{ns}	604.2^{ns}
CC, g kg^{-1} CHO	248.7^{b}	282.3^{a}	295.4^{a}

FB – Full Bud Stage; EBL – Early Bloom Stage; MB – Mid Bloom Stage; CHO – Total Carbohydrates; TESC – Total Ethanol Soluble Carbohydrates; NDF – Neutral Detergent Fiber; ADF – Acid Detergent Fiber; NFC – Non Fiber Carbohydrates; CA – Non structural CHO, instantaneously solubilizable; CB_1 – starch, rapidly degradable; CB_2 – NFC, intermediately degradable; CB_3 – available cell wall, slowly degradable; CC – undegradable CHO; Different letters (a, b, c) denote significant differences between different stages of development ($p< 0.05$); ns – non significance

The NDF and ADF concentrations of forage provide useful information about quality. The results of this study showed that content of cell wall increased with alfalfa maturation. During advancing maturity NDF increased from 458.4 g kg^{-1} DM at the FB stage to 517.3 g kg^{-1} DM at MBL stage and ADF increased from 346.8 g kg^{-1} DM at the FB stage to 423.0 g kg^{-1} DM at the MBL stage ($p< 0.05$). The highest increase of NDF and ADF content was recorded after the first development stage. Lignin content in DM of alfalfa also increased with advancing maturity from 70.4 to 91.6 g kg^{-1}. NDF lignification increased with plant growth and development, but the values were similar at the EBL stage and MBL stage and did not differ significantly. The highest content of hemicellulose was at the first development stage, whereas the highest content of NFC (Non-fiber carbohydrates) was at the EBL stage (256.6 g kg^{-1} DM).

Alfalfa cut at the EBL stage had a higher CA fraction (116.7 g kg^{-1} CHO) than when it cut at the FB and MBL stage ($p< 0.05$), and tended to have higher CC fraction (282.3 g kg^{-1} CHO) than alfalfa cut at the FB stage (248.7 g kg^{-1} CHO), but lower than alfalfa cut at the MBL stage (295.4 g kg^{-1} CHO). CB and CB_2 fraction had similar values and did not differ between stages of development. CB_1 fraction of CHO decreased from 39.1 to 29.3 g kg^{-1} CHO ($p< 0.05$), and CB_3 fraction decreased from the FB (395.7 g kg^{-1} CHO) to EBL stage (366.9 g kg^{-1} CHO).

The CA fraction tended to decrease with advancing stage of maturity, especially from the EBL to the MBL stage of maturity, wich was in agreement with the result of *Yu et al. (2012).* This might be due to reduced leaf : stem ratio with consequent increased cell wall CHO component and declined cellular CHO (*Yari et al., 2012*). The mean values of CHO fractions were comparable with those reported by *Yu et al. (2003)* and *Lanzas et al. (2007)*. Changes in CHO fractions with advancing stage of maturity were small which is in agreement with the results of *Elizalde et al. (1999)* for fresh alfalfa and *Yu et al. (2003)* for alfalfa hay.

The NFC fraction is very important for optimizing microbial protein synthesis in the rumen and supplying energy to the cow. The fibrous fraction is important for maintaining rumen health and also for supplying energy. Therefore, balancing these fractions is critical for optimizing dry matter intake and milk yield. On the other hand, feed efficiency is somewhat reflective of utilization of carbohydrate sources. Using data from 13 herds, *Britt et al., (2003)* observed a negative correlation with feed efficiency and either forage or NDF intake. Therefore, feeding management needs to focus on improving feed efficiency while also maintaining rumen health.

A maximum dietary level of 42 to 44% NFC has been suggested, but this concentration depends on concentration of NDF in the diet and the rate and extent of ruminal digestibility of the NFC fraction. Sugar supplementation to diets for lactating cows has received limited attention and has resulted in mixed responses. Optimization of ruminal fermentation may occur when the diet contains 4 to 5% sugars.

Conclusions

The CHO scheme for the CNCPS model that is outlined in this paper devides feed CHO in fractions that more accurately relate to ruminal fermentation characteristics. Potential nutrient supply to the animal reduced with advancing alfalfa maturity. With advancing maturity, leaf : stem ratio decreased and consequently, non-structural and non-fiber carbohydrates decreased, wile fiber fractions increased. Fraction CB_3, however, makes a higher contribution to total CHO at the FB stage than at the flowering stage. According data obtained in this investigation, we could conclude that it is possible to include very high proportion of alfalfa hay in the diets of high producing cows when this hay is at an early stage of maturity – FB stage.

Sadržaj ugljenih hidrata u lucerki košenoj u različitim fazama razvića tokom prolećene sezone iskorišćavanja

Jordan Marković, Snežana Babić, Dragan Terzić, Vladimir Zornić, Tanja Vasić, Jasmina Milenković, Ivica Kostić

Rezime

Cilj ovih istraživanja je bio da se utvrdi uticaj faze razvića na sadržaj ukupnih ugljenih hidrata i ugljenohidratnih frakcija u lucerki. Lucerka je košena u fazi pune butonizacije, početkom cvetanja i sredinom cvetanja. Lucerka košena u punoj butonizaciji je sadržala najveću količinu hemiceluloze i najniži nivo ukupnih ugljenih hidratat (CHO), NDF-a, ADF-a i lignina. Sadržaj CHO, NDF-a, ADF-a i lignina se povećavao sa rastom i razvićem biljaka, dok se sadržaj TESC i NFC nije značajno menjao. Najveća CHO frakciija je bila CB_3 koja predstavlja dostupni NDF i smanjuje se od 395,7 do 375,6 g kg^{-1} CHO sa napredovanjem faze razvića. CA frakcija pokazuje tendenciju opadanja, dok se CC frakcija koja predstavlja nedostupni NDF i lignin povećala od 248,7 do 295,4 g kg^{-1} CHO. Različite CHO

frakcije se razlikuju po stopi i brzini degradaciije u buragu. Ove frakcije određuju ukupnu količinu CHO koja će biti razgrađena u buragu i onih CHO koji će proći kroz burag nerazgrađeni. Poznavanje ovih ugljenohidratnih frakcija se koristi u savremenim sistemima ishrane za formulisanje obroka za preživare.

Ključne reči: lucerka, ugljenohidratne frakcije, faza razvića

Acknowledgement

The authors thank the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project TR-31057. Acknowledgements
The authors thank the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project TR-31057.

References

GRUBIĆ, G. AND ADAMOVIĆ, M. (2003): Ishrana visokoproizvodnih krava. Premis, Beograd, Srbija, 33-40.
BRITO, F., TREMBLAY, G. F., BERTRAND, A., CASTONGUAY, Y., BELANGER, G., MICHAUD, R., LAPIERRE, H., BENCHAAR, C., PETIT, H. V., QUELLET, D. R., BERTHIAUME, R. (2009): Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis inlate-lacatation dairy cows. Journal of dairy science, 92, 1092-1107.
BRITT, J. S., THOMAS, R. C., SPEER, N. C., HALL, M. B. (2003): Efficiency of converting nutrient dry matter to milk in Holstein herds. Journal of dairy science, 86, 3796-3801.
BURNS, J. C., FISHER, D. S., MAYLAND, H. F. (2007): Diurnal shifts in nutritive value of alfalfa harvested as hay and evaluated by animal intake and digestion. Crop science, 47, 2190-2197.
COBLENTZ, W. K., BRINK, G. E., MARTIN, N. P., UNDERSANDER, D. J., (2008): Harvest timing effects on estimates of rumen degradable protein from alfalfa forages. Crop science, 48, 778-788.
EASTRIDGE, M. L. (2007): Advancements in feeding carbohydrates. WCDS advances in dairy technology, 19, 63-74.
ELIZALDE, J. C., MERCHEN, N. R., FAULKNER, D. B. (1999): Fractionation of fiber and crude protein in fresh forages during the spring growth. Journal of animal science, 77, 476-484.

HALL, M. B. AND HEREJK, C. (2001): Differences in yields of microbial crude protein from *in vitro* fermentation of carbohydrates. Journal of dairy science, 84, 2486-2493.

HALL, M. B., HOOVER, W. H., JENNINGS, J. P., WEBSTER, T. K. M. (1999): A method for partitioning neutral detergent soluble carbohydrates. Journal of food science and agriculture, 79, 2079-2086.

HANSON, A. A., BARNES, D. K., HILL, R. R. (1988): Alfalfa and alfalfa improvement. Agronomy no. 29. The American Society of Agronomy, Madison, WI, USA.

LAMB, J. F. S., SHEAFFER, C. C., DEBROAH, A. (2003): Population density and harvest maturity effects on leaf and stem yield in alfalfa. Agronomy journal, 95, 635-641.

LANZAS, C., SNIFFEN, C. J., SEO, S., TEDESCHI, L. O., FOX, D. G. (2007): A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Animal feed science and technology, 136, 167-190.

MERTENS, D. R. (1997): Creating a system for meeting the fiber requirements of dairy cows. Journal of dairy science, 80, 1463-1481.

NOCEK, J. E., AND TAMMINGA, S. (1991): Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. Journal of dairy science, 74, 3598-3629.

NRC. (2001): Nutrient requirements of dairy cattle, 7th revised ed., National Academic Science, Washington, DC, USA.

VAN SOEST, P. J., ROBERTSON, J. B., LEWIS, B. A. (1991): Methods for dietary fiber, NDF and non starch polysaccharides in relation to animal nutrition. Journal of dairy science, 74, 3583-3597.

YU, P., CHRISTENSEN, D. A., MCKINNON, J. J., MARKERT, J. D., (2003): Effect of variety and maturity stage on chemical composition, carbohydrate and protein subfractions, *in vitro* rumen degradability and energy values of timothy and alfalfa. Canadian journal of animal science, 83, 279-290.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

BENEFITS OF MIXING GRASSES AND LEGUMES FOR FORAGE YIELD AND IMPACT OF DIFFERENT LEVELS OF NITROGEN FERTILIZATION

Zorica Bijelić[1], Violeta Mandić[1], Aleksandar Simić[2], Vesna Krnjaja[1], Dragana Ružić-Muslić[1], Bogdan Cekić[1], Nenad Mićić[1]

[1]Institute for Animal Husbandry, Belgrade-Zemun, 11080 Zemun, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author. Zorica Bijelić, zonesh@gmail.com
Original scientific paper

Abstract: Mixing various species in the mixtures, often increases the production of biomass owing to better utilization of the available resources. Therefore we study whether mixtures of grass and legumes would give higher herbage yield than monocultures under nitrogen fertilization. An experiment was carried out at experimental field at the Institute for Animal Husbandry, Belgrade, with two legume species *Trifolium pratense* and *Medicago sativa* and one grass *Lolium perenne*. Species were sown as a monocultures and binary mixtures with equal proportion of species (50:50) and with higher proportion of perennial ryegrass (70:30). Three levels of N (0, 50, 100 kgN ha^{-1}) were applied at the begining of vegetation. At higher N fertiliser application rate (100 kgN ha^{-1}) and with mixtures with higer legume proportion high yields of the mixture can be achieved. Mixtures achieve higher yields than the average yield of species in monoculture. Added N reduced overyielding from 59.3 % to 34.9 % for alfalfa mixtures and from 61.9 % to 44.9 % for red clover mixtures. Transgressive overyielding was evident just in second year and was higher for alfalfa mixtures than for red clover mixtures.

Key words: monocultures, mixtures, yield, overyielding, transgressive overyielding

Introduction

Production of forage crops in Republic of Serbia occupies an area of 865,372 ha. The most important forage crops are alfalfa, red clover monocultures, natural meadows and pastures while cultivation of forage mixtures is significantly lower. The main question is why this happened? Grass-legume mixtures have numerous features that can benefited production. This advantage include higher productivity, intra-annual yield stability, balanced yield, better use of land, water and air resources, what provide environmental benefit and reduces the cost of investment. Also, grass-legume mixtures have potentional to limit spread of pests and pathogens (*Ratnadass et al., 2012*) and growth of weeds (*Bijelić et al., 2017*). The yield of forage mixtures and impact of species diversity on it were thoroughly examened by many researchers. Forage mixtures of different species and different functional groups, are observed to have higher yields than would be expacted to have individual species in monoculture. This observation is based on the assumption that plant communities with higher species number better utilize available resources due to niche complenmentarity (*Ergon et al.,2016*). In research of *Nyfeler et al. (2009)* four species mixtures obtain twice the yield (overyielding) of the average of the individual species in pure crop. Also, *Picasso et al. (2011)* concluded that multispecies mixtures overyielding individual monocultures. How many and wich species in the mixtures can contribute to overyielding remains unclear. In meta-analysis of 44 independent experiments of multispecies swards and monocultures which were investigated species richness, *Cardinale et al. (2007)* concluded that net effect of plant richness on plant biomass was significantly positive and that the most diverse plant comunities have achieved 1.7 time the biomass of the average monoculture. Assessment of diversity is not reflected only on functional groups but can be a issue of different species or different varieties of the same species. For example, according to some outhors (*Loreau and Hector, 2001*), overyielding is the result of nitrogen fixing species while some other claim that overyielding exist in mixtures without nitrogen fixers (*van Ruijven and Berendse, 2003*). *Picasso et al. (2007)* study overyielding in multispecies mixtures and concluded that orchardgrass overyilding was highest of all examined species.

Besides the overyielding, sown mixtures of grassland species are frequently observed to have yield which exceeded that of its best-performing species when grown in monoculture. This phenomenon is called transgressive overyielding. In grass-legume mixture transgressive overyielding is not common. In order to yield more than monocultures it is necessary that species in the mixture are mutually complementary for resources. For transgressive overyielding degree of complementarity needs to be significantly higher (*Loreau, 2004*). According to *Cardinale et al. (2007)* transgressive overyielding occured only in 12% of the examined experiments even though *Finn et al. (2012)* claim that it existed in 79% of study mixtures. Level of trangressive overyielding ranged from 9-38% (*Ergon et al.,2016*), while in research of *Nyfeler et al. (2009)* mixtures were up to 57% more productive then the most productive monoculture.

Some common agricultural measurement can impact on mixture overyielding, like nitrogen fertilization and frequency of cutting. N fertilization decrease overyielding and transgressive overyielding up to 106% or 57% respectively (*Nyfeler et al., 2009*) while higher frequency of utilization has a positive impact (*Ergon et al., 2016*). In the light of climate change, an important fact is that the mixtures are more tolerant to drought compared to their pure crops. The size of overyielding under severe drought was very large for equal stand mixtures what suggests grass-legume mixtures as adaptation measure to compensate for yield losses under drought scenario (*Hofer et al., 2016*).

In the paper we study the dry matter yield, overyielding and transgressive overyielding in binary mixtures, sown in two species ration and fertilized with three different amount of nitrogen fertilizer over two years. The questions are: Whether mixtures of grass and legumes would give higher herbage yield than monocultures, is there a impact of applied doses of N on investigated parametres and how it change over the years?

Material and methods

A field experiment was conducted at the experimental field at the Institute for Animal Husbandry (44°49′N, 20°17′E, elevation 96 masl.), Serbia, on a silty clay loam with pH of 7.08. The average total annual precipitation is 622.5mm and average temperature 12.7°C. Weather condition in experimental years deviate from the long-term average. The first experimental year is characterized by higher average temperatures and less precipitation (13.2°C, 612.5mm), while the second one has a slightly lower temperature and higher precipitation (12.9°C, 722,7mm). Temperature and precipitation per mounths for study period are presented in Figure 1.

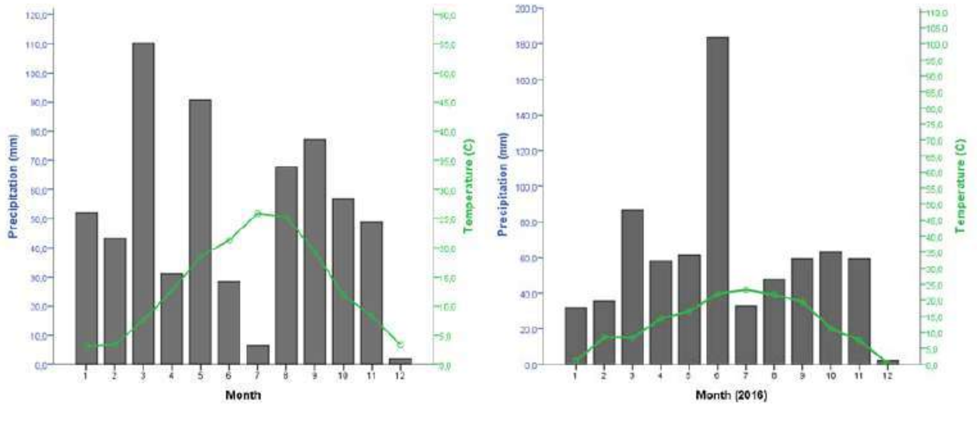


Figure 1. Average monthly temperature (°C) and sum of precipitation (mm) in two experimental year

Red clover (*Trifolium pratense*, cv. K-39) and Alfalfa (Medicago sativa, cv. NS Banat) were sown as a monocultures and in binary mixtures with perennial ryegrass (Lolium perenne, cv. Calibra). Mixtures were divided into equal stand mixtures with the same species proportion (50:50) and mixtures with higher proportion of perennial ryegrass (70:30). Nitrogen were applied at the begining of vegetation in a form of amonium nitrate. Each crop plot were fertilized with three N treatments: 0, 50, 100 kgN ha^{-1}. Plots were 10 m^2 in size, and species were sown in spring with 20 cm row distance and seeding rate of 25 kg ha^{-1} for grass and 20 kg ha^{-1} for legumes.

Experimental measurement were done at the first and secont production years. All plots were cut three times per studied year, each time the canopy maturity reached one third inflorescence of legume plants. After mowing, one kilogram samples of green mass were dried in the owen at 60 ° for 72 h and again measured. According to the measured values dry matter was determinate.

Overyielding and transgressive overyielding were calculated according to Nyfeler et al. (2009). Overyielding is the difference between the equal stand mixture yield and the average yield over the monocultures and transgressive overyielding as the difference between the highiest mixture yield and the highiest monoculture yield.

Data were analysed by the General lenear model (SPSS 20.0) for ANOVA to detect impact od N fertilization and year on DMY of the monocultures and mixtures. Shapiro-Wilk test was used to determine whether or not the observations themselves are normally distributed and Levene's Test for testing homogeneity of variances. Differences among means were detect with LSD at the probability level 0.05. Independent samples t test were used to detect whether there were differences in overyielding between equal stand mixture yield and average yield of monoculture or difference between the highiest mixture yield and the highiest monoculture yield.

Results

Examined years showed significant differensy in achieved biomass yield. Crop in second production year exceeded that of the previus year.

High significant difference was observed between mixtures and monocultures in each year. In first production year red clover and alfalfa as a pure crop and mixture of equal proportion of ryegrass and red clover show significatly higher yield than the others. In second investigation year equal stand mixtures yielded more than the monocultures and other mixtures. Mixtures with higher proportion of perennial ryegrass yielded less than red clover but more than alfalfa and perennial ryegrass in monoculture.

Over two years of investigation N fertilization show significant impact on monocultures and mixtures yield only in second year, namely treatment of 100 kgN ha^{-1} gave significantly higher yield than treatment with 50 kgN ha^{-1} and without N (Figure 1).

Strong positive overyielding was observed only in second investigation year (Table 1). The highiest estimated overyielding between monocultures and equal stand miyxtures of alfalfa and perennial ryegrass was achieved in treatments without N fertilization, 59.3 %. Additionally, tratments with 50 and 100 kgN ha^{-1} had smaller but significant overyielding of 38.9 and 34.9 %, respectively.

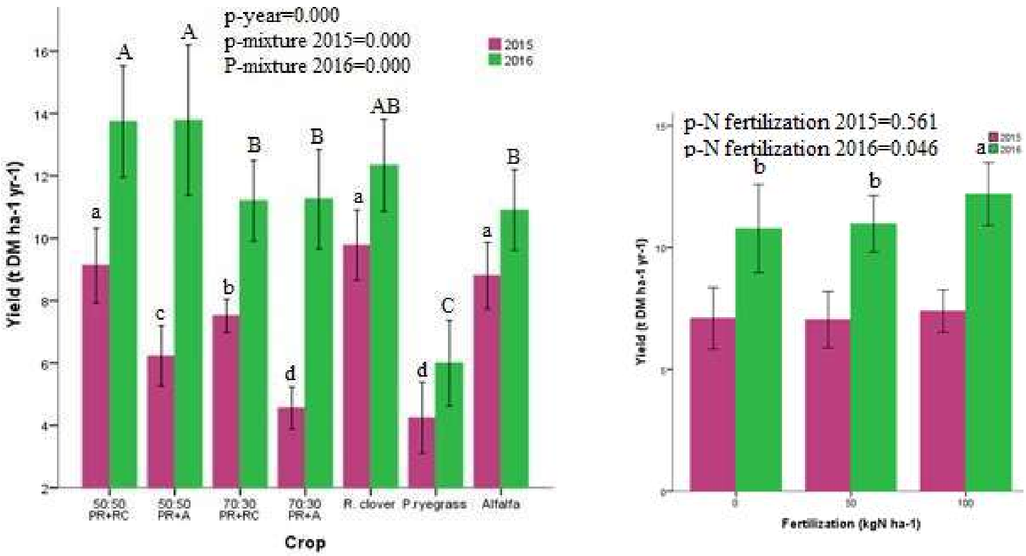


Figure 2. Dry matter yield of pure crops and grass-legume mixtures under different levels of nitrogen fertilization in two subsequent years

Overyielding level of red clover mixtures in first year was significant only for the contol, 35.2 %, and for all N treatmant in subsequent year of 61.9, 51.8 and 44.9, respectively. Observing the influence of the factors, year and the fertilization, we can concluded that overyielding was significantly higher in second production year and that N desrease the difference between the yield of monocultures and their equal stand mixtures.

Table 1. Dry mater yield of monocultures and yield and overyielding of equal stand mixtures for the three N treatments in two study years

		Monoculture yield (t DM ha^{-1})				Equal stand mixture		
	N kg ha^{-1}	*L. perenne*	*M. sativa*	*T. pratense*	average yield	yield (tDM ha^{-1})	Overyielding (t DM ha^{-1})	%
Year 1	N0	2.79	8.40		5.60	5.72	0.12^{ns}	2.0
	N50	4.26	8.00		6.13	6.20	0.07^{ns}	1.1
	N100	5.68	9.37		7.52	7.69	0.16^{ns}	2.1
	S.E.	0.50	0.33		0.35	0.43	0.18	
Year 2	N0	3.97	9.74		6.85	10.91	4.06*	59.3
	N50	6.96	11.67		9.32	12.95	3.63*	38.9
	N100	7.04	11.29		9.16	12.36	3.20*	34.9
	S.E.	0.59	0.56		0.38	1.04	1.02	
Year 1	N0	2.79		11.94	7.36	9.95	2.59*	35.2
	N50	4.26		10.97	7.61	8.57	0.95^{ns}	12.6
	N100	5.68		8.32	7.00	8.85	1.85^{ns}	26.4
	S.E.	0.50		0.49	0.24	0.73	0.82	
Year 2	N0	3.97		12.35	8.55	13.84	5.29*	61.9
	N50	6.96		11.94	9.45	14.35	4.90*	51.8
	N100	7.04		11.70	10.37	15.03	4.66*	44.9
	S.E.	0.59		0.64	0.40	0.77	0.87	

Table 2. Level of significante for impact of investigated factors on DMY of monoculture, equal stand mixtures and overyielding

	Avg. yield P.ryegrass. Alfalfa	Yield-equal alfalfa mix	Overyield. Alfalfa mix.	Avg. yield P.ryegrass. R.clover	Yield-equal R.clover . mix	Overyield. R.clover mix.
Year	***	**	**	***	***	**
Fertilization	*	*	*	*	*	*
Year x Fertilization	ns	ns	ns	*	ns	ns

The frequency of transgressive overyielding increase over time. In first year of study mixtures did not show transgressive overyielding (Table 3). In second year positive transgressive overyielding ranging from 5.40 to 10.30 % for alfalfa mixtures and from 3.43 to 6.52 % for red clover mixtures. Transgressive overyielding was observed with mixtures with bouth level of species proportion but was more common for the egual stand mixtures. N fertilization did not have significant impact on transgressive overyielding although there is a trend of decrease with the addition of nitrogen.

Table 3. Predicted highiest monoculture and mixture yield and transgressive overyielding of perennial ryegrass, alfalfa and red clover in two subsequent years

	species	N fertilization	Highest monocult. yield (t DM ha^{-1})	Mixture highest yield (t DM ha^{-1})	Transgressive overyielding (t DM ha^{-1})	%
Year 1	M. sativa	0	8.44	5.72	-2.72*	-
		50	8.58	5.60	-2.98**	-
		100	9.37	7.36	-2.01ns	-
Year 2	M. sativa	0	11.83	13.05	1.22ns	10.30
		50	11.67	12.35	0.67**	5.82
		100	9.45	9.96	0.51ns	5.40
Year 1	T.pratense	0	10.04	9.95	-0.85ns	-
		50	10.97	9.15	-1.82ns	-
		100	8.32	7.36	0.53ns	-
Year 2	T.pratense	0	13.80	14.70	0.90ns	6.52
		50	11.94	12.35	0.40ns	3.43
		100	11.46	11.96	0.50ns	4.36

Table 4. Level of significante for impact of investigated factors on highiest monoculture and mixture yield and transgressive overyielding of perennial ryegrass, alfalfa and red clover

	Highiest monocult. yield	Highiest alfalfa mix yield	Transgressive overyield. Alfalfa mix.	Highiest monocult yield	Highiest R.clover mix. yield	Transgressive overyielding R.clover mix.
Year	***	***	***	***	***	ns
Fertilization	ns	ns	ns	ns	ns	ns
Year x Fertilization	ns	ns	ns	ns	ns	ns

Disscusion

Yield of all crops in bouth examined years was strongly different. In second year yield of swards was 57.7% higher than in the first year. Climatic condition in first year are one of the important factors behind such a difference in the yield of monocultures and mixtures exist. Also in research of *Hennessy et al. (2012)* average herbage mass yield in first production year was significantly lower due to warmer climate during the year. In first year yield of monocultures was higher than the mixtures while in second year the opposite situation originated. Sown equal stand mixtures achieved higher yield than the mixtures where grass is dominant and monocultures. According to *Sanderson et al. (2013)* harvested biomass did not differ between mixtures dominated by one, two, or equal numbers of species. Likewise, mixtures dominated by legumes had higher yields than grass-dominated mixtures.

Nitrogen fertilization effect yield only in second production yield. The significant differences were only seen in the treatment with 100 kgN ha^{-1} at the level p<0.05. In study of *Hennessy et al. (2012)* N fertilization rate of 180 and 240 kgN ha^{-1} had great effect on herbage mass, while nitrogen dose of 60 and 120 kgN ha^{-1} did not significantly differ from the control treatment.

Overyielding in our research have appeared in both years, except that the differences between the mixtures yield and the average pure crop yields were very small and not significant in the first year of the study. Achieved values are lower than the values in study of *Picasso et al. (2008)* of 73% and *Nyfeler et al. (2009)* of 160%. Very similar results to ours got *Hofer et al. (2016)*. They assessed drought impact on production propertios of two and four key species mixtures and they argued that the level of overyielding in the rainfed contol condition was 38%, and in the treatment with severe drought 50%. Also, they found that extreme drought condition restricted growth of monocultures and mixtures, so, the overyielding was very small and non significant. Based on this finding, the existence of a very long dry season during vegetation in the first study year of our experiment caused small and non significant overyielding, especially for alfalfa mixtures.

In the literature there are different opinions about overyielding and transgressive overyielding. Overyielding is more common and higher then transgressive overyielding. In meta-analysis of *Cardinale et al. (2007),* showed that polycultures have generally achieved more biomass than the average species, but not more than the most productive species.

In our experiment, in first year we had no transgressive overyielding and there is no mixture that will outperform the most productive monoculture. In second year we have achieved transgressive overyielding but the differency was not significant. In contrast to our study *Nyfeler et al. (2009)* observed transgressive overyielding in first production year and it was 1.27 and 1.35 times the highiest mixture yield than the most productive species in monoculture. Also *Finn et al. (2012)* found a very high incidence of transgressive overyielding in four-species grass-clover mixtures. Some lower values of transgressive overyielding have been found in three years research of *Sturludottir et al. (2013)* of 9, 15, 7 %. In research of *Picasso et al. (2011)* mixtures of two, three, four and six species yielded more than the best mnoculture in all three investigating year, but the difference was significant only in the last year. The absence of transgressive overlielding in our experiment could be explained by the fact that the frequency of transgressive overyielding increases over time and that for some mixtures in certain agro-climatic conditions, takes a time to become evident (up to 5 years) (*Cardinale et al., 2007*). According to many researchers, species richness have a positive impact on grass-legume mixtures yields. For a short period more suitable are mixtures with low nuber of species while for longer time mixtures with large number of species are approved. In our experiment binary mixture did not achieved high transgressive overyield, tearfore combination of more then two species maybe better solution for this agroecological condition.

With N fertilization the relative abundance of each species in the mixture can be very disturbed , thus strengthening the competitive ability of certain species. Reduction of complementarity also reduces the potential for overyielding and transgressive overyielding (*Gross et al., 2007*). In our research, plants in control treatment acchieved the highiest overyielding and transgressive overyielding, while used nitrogen in doses of 50 and 100 desrease level of bouth investigation parametres.

Conclusion

Establishment od high yielding grass-legume mixtures, however, does not only require selection of adapted species that are compatible, but also optimum proportions of species and optimal agricultural measurements.

At higher N fertiliser application rate (100 kgN ha^{-1}) and with mixtures with higer legume proportion high yields of the mixture can be achieved. Drought significantly reduce the production of both grass and legume monocultures and their mixtures. Sown equal stand mixtures achieved higher yield than the mixtures where grass is dominant and monocultures. The mixtures achieve higher yields than the average yield of species in monocultures. Equal stand mixtures overyield significantly better in second production year. N treatmants, 0, 50, 100 kgN ha^{-1}, reduced overyielding from 59.3 % to 34.9 % for alfalfa mixtures and from 61.9 % to 44.9 % for red clover mixtures. Transgressive overyielding was evident just in second year and it range from 5.40 to 10.30 % for alfalfa mixtures and from 3.43 to 6.52 % for red clover mixtures.

Benefiti mešanja trava i leguminoza za prinos krme i uticaj različitih nivoa azotnog đubriva

Zorica Bijelić, Violeta Mandić, Aleksandar Simić, Vesna Krnjaja, Dragana Ružić-Muslić, Bogdan Cekić, Nenad Mićić

Rezime

Mešanje različitih vrsta biljaka u krmne smeše, često povećava proizvodnju biomase, zahvaljujući boljoj iskorištenosti dostupnih resursa. Zbog toga smo mi proučavali da li bi smeše trava i leguminoza dale veće prinos od njihovih monokultura u uslovima djubrenja azota. Na eksperimentalnom polju Instituta za stočarstvo, u Beogradu, izveden je eksperiment sa dve vrste leguminoza *Trifolium pratense* i *Medicago sativa* i jednom travom *Lolium perenne*. Vrste su posejane kao monokulture i binarne smeše sa jednakim udelom vrsta (50:50) i smeše sa većim udelom engleskog ljulja (70:30). Na početku vegetacije primenjena su tri nivoa N (0, 50, 100 kgN ha^{-1}). Kod veće količinama (100 kgN ha^{-1}) i sa smešama koje imaju veći udeo leguminoza mogu se ostvariti visoki prinosi smeša. Smeše postižu veće prinose od prosečnog prinosa vrsta u monokulturi. Dodavanje N smanjuje overyielding od 59,3% do 34,9% za lucerkine smeše i od 61,9% do 44,9% za smeše crvene deteline. Transgresivni overyielding bio je očigledan tek u drugoj godini istraživanja i bilo je veći za lucerkine smeše nego za smeše crvene deteline.

Ključne reči: monokulture, smeše, prinos, overyielding, transgresivni overyielding

Acknowledgements

The authors would like to thank the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project TR 31053.

Reference

BIJELIĆ Z., KRNJAJA V., MANDIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N., ŽIVKOVIĆ Ž., LAZAREVIĆ M. (2017): Weed abundance in alfalfa crop and alfalfa-grass mixtures under nitrogen fertilization. Journal of Mountain Agriculture on the Balkans, 20 (2), 211-219.

CARDINALE, B.J., WRIGHT, J.P., CADOTTE, M.W., CARROLL, I.T., HECTOR, A., SRIVASTAVA, D.S., LOREAU, M., WEIS, J.J. (2007): Impacts of plant diversity on biomass production increase through time because of species complementarity. Proc. Natl. Acad. Sci. U.S.A. 104, 18123–18128.

ERGON A., L. KIRWAN, M. A. BLEKEN, A.O. SKJELVAG, R.P. COLLINS, O.A. ROGNLI (2016): Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies: 1. dry-matter yield and dynamics of species composition. Grass and Forage Science, 71, 667-682.

FINN J.A., KIRWAN L., CONNOLLY J., SEBASTIA M.T., HELGADOTTIR A. AND LUCHER A. (2012) Four-species grass-clover mixtures demonstrate

transgressive overyielding and weed suppression in a 3-year continental-scale experiment. Grassland Science in Europe, 17, 186–188.
GROSS N., SUDING N.K., LAVOREL S., ROUMET C. (2007): Complementarity as a mechanism of coexistence between functional groups of grasses. Journal of Ecology, 95, 1296-1305.
HENNESSY D., ENRIQUEZ-HIDALGO D., O DONOVAN M., GILLILAND T. (2012): Effect of N fertiliser application rate on herbage production and sward clover content in grazed clover plots. Grassland Science in Europe, 17, 124-126.
HOFER D., SUTER M., HAUGHEY E., FINN A. J., HOEKSTRA J. N., BUCHMANN N., LUSCHER A. (2016): Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. Journal of Applied Ecology, 53, 1023-1034.
LOREAU M., HECTOR A. (2001): Partitioning selection and complementarity in biodiversity experiments. Nature, 5, 412(6842), 72-6.
LOREAU M., (2004): Does functional redundancy exist? Oikos, 104, 606-611.
NYFELER D., HUGUENIN-ELIE O., SUTER M., FROSSARD E., CONNOLLY J., LÜSCHER A. (2009): Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. Journal of Applied Ecology, 46, 683–691.
PICASSO V., E.C. BRUMMER, M. LIEBMAN, P.M. DIXON, B.J. WILSEY (2008): Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. Crop Science, 48, 331–342.
PICASSO D.V., BRUMMER C. E., LIEBMAN M., DIXON M. P., WILSEY J. B. (2011): Diverse perennial crop mixtures sustain higher productivity over time based on ecological complementarity. Renewable Agriculture and Food Systems, 26(4), 317-327.
RATNADASS A., F ERNANDES P., AVELINO J. AND HABIB R. (2012): Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agronomy for Sustainable Development, 32, 273–303.
SANDERSON, M.A., R. BRINK, R. G., STOUT, L. R. (2013): Grass–legume proportions in forage seed mixtures and effects on herbage yield and weed abundance. Agronomy Journal, 105(5), 1289-1297.
STURLUDOTTIR E., BROPHY C., BELANGER G., GUSTAVSSON M. A., JORGENSEN M., LUNNAN T., HELGADOTTIR A. (2014): Benefits of mixing grasses and legumes for herbage yield and nutritive value in Northern Europe and Canada. Grass and Forage Science, 69, 2, 229-240.
VAN RUIJVEN J., BERENDSE F. (2003): Positive effects of plant species diversity on productivity in the absence of legumes. Ecology Letters, 6, 170–175.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

FUNGAL CONTAMINATION OF CATTLE FEEDS

Vesna Krnjaja[1], Aleksandar Stanojković[1], Slavica Stanković[2], Zorica Bijelić[1], Violeta Mandić[1], Nenad Mićić[1], Miloš Marinković[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2]Maize Research Institute "Zemun Polje", Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
Original scientific paper

Abstract: In this study, the total fungal count and fungal contamination of 38 cattle feed samples that collected from animal farms in suburbs of Belgrade area during 2012-2014 were determined. The total fungal count was established using a dilution method, and standard mycological procedures were used to identify fungi especially toxigenic species. Analyses of the total fungal count in 17 samples of feed mixture for adult and in 21 samples of food mixtures for young cattle categories, the following values were determined: from 1 x 10^1 to 3.9 x 10^5 cfu g^{-1}. According to the Rulebook on the quality of animal feed of the Republic of Serbia, the total fungal count over permitted limit was established in 5.88% of samples of feeds for adult and in 38.10% of samples of feed mixtures for young cattle categories. Mycological analyses identified potentially toxigenic species from the genera *Aspergillus*, *Fusarium* and *Penicillium*. In the majority of samples of feed for adult categories, *Fusarium* species were isolated (88.24%), while *Aspergillus* species were isolated in most of the samples of feed mixtures for young cattle categories (85.71%). On average, *Aspergillus* species were isolated in most samples of cattle feed samples (81.09%), followed by *Fusarium* (77.46%) and *Penicillium* species (62.75%). The obtained results of the total fungal count indicate that the tested samples of feed mixtures do not satisfy the criteria of hygienic quality. Consequently, regular and constant controls of mycological quality are necessary preventive measures for the reduction and control of fungal contamination of food for cattle.

Key words: mixtures for cattle, total fungal count, toxigenic fungi

Introduction

Animal feed is an ideal medium for the growth and reproduction of microorganisms. Among the microorganisms filamentous fungi are considered as the most harmful because of their ability to produce toxic metabolites -

mycotoxins. Microscopic fungi and their mycotoxins are the most common contaminants of agricultural products before and after harvest, as well as in the time of transport or storage. Increased insect activity and favourable environmental factors for the development of toxic fungi contribute to the inevitable fungal contamination of animal feed (*Ghiasian and Maghsood, 2011).*

The most common toxigenic species of fungi isolated from animal feed belong to the genera *Aspergillus, Fusarium* and *Penicillium*. *Aspergillus* species that are producers of aflatoxins and ochratoxins are the most dominant in foods for dairy cows and other types of feed, while *Fusarium* species and their mycotoxins are important grain contaminants (*Placinta et al., 1999*). The most important fungal species and mycotoxins in maize grains as the most common livestock feed are *Aspergillus flavus* and aflatoxins, *Fusarium verticillioides* and *F. proliferatum* and fumonisins, *F. graminearum* and trichothecenes and zearalenone *(Chulze, 2010)*. Toxigenic fungi and their mycotoxins cause adverse effects on people, animals and crops causing diseases and economic losses (*Rosa et al., 2006*).

Given the prevalence of the most common types of toxin-producing fungi of the genera *Aspergillus*, *Fusarium* and *Penicillium* in Serbia, of particular importance to the health of animals is to consider the presence of mycotoxins as secondary metabolites of fungi. In cattle, mycotoxins can cause acute and chronic health disorders. Symptoms of the disease depend on mycotoxins and other stress factors in the herd of cattle. Aflatoxin B_1 (AFB_1) is considered the most important mycotoxin in various animal feeds. AFB_1 is a major hepatotoxin that causes various pathological effects on organs and tissues and the ingestion of AFB_1 by cows can lead to potentially harmful aflatoxin M_1 (AFM_1) residues in milk (*Kensler et al., 2011*). AFM_1 is a very toxic metabolite of aflatoxins B_1 and B_2 and is often contaminant of milk and milk products. It is also one of the most important hepatocarcinogens, mutagens, teratogens and immunosuppressors (*Smajlović et al., 2012*).

The aim of this paper was to determine the total fungal count and to identify potentially mycotoxigenic fungi genera in samples of cattle feed and also to assess the potential danger of the presence of these contaminants in the food chain.

Materials and Methods

The mycological quality of 38 samples of feed for cattle (17 samples for adult animals (dairy cows) and 21 samples of feed for young categories of cattle (young bull and calves)) were examined. Samples originating from different farms in the vicinity of Belgrade were collected during the three-year period (2012-2014). The size of laboratory sample was 1 kg. After laboratory admission, the samples were analysed for fungal contamination, immediately or were stored 2-3 days at controlled temperature prior the analysis. The moisture content of the tested maize kernel samples was determined using a laboratory moisture meter (OHAUS MB35, USA), and mycological analysis was performed according to the method ISO 21527-2 (2008).

Identification of toxigenic species and genera of fungi was performed according to *Watanabe (2002)*. The frequency of positive, i.e. samples contaminated by toxigenic fungi, was calculated according to the formula: Fr (%) = the number of samples were a fungal genus occurred/the total number of samples x 100.

Statistical analysis was performed with non-parametric test (Mann-Whitney Test), using the SPSS software (IBM, Statistic 20). The correlation among individual values for moisture content and total fungal count was determined using the Pearson correlation coefficient.

Results and Discussions

The total fungal count and identification of toxigenic fungi in animal feed are important indicators of hygienic quality.

The average moisture content in the samples of the tested feeds for adult cattle was 10.79% and for young cattle 11.23%. Mycological analysis of all tested cattle feed samples established the total fungal count in the range from 1 x 10^1 to 3.9 x 10^5 cfu g^{-1}. According to the Regulation on the quality of animal feed of the Republic of Serbia (*Official Gazette of the Republic of Serbia, 4/2010, and 27/2014 113/2012*), the total fungal count above the allowed limit (2 x 10^5 cfu g^{-1}) was recorded in 5.88% of the samples of feed for adult bovine animals, while the total fungal count above the allowed limit (5 x 10^4 cfu g^{-1}) in samples of feed for young categories of cattle was established in 38.10% of samples (Table 1). In the analysed groups of samples of feed for cattle, no statistically significant difference in the total fungal count was established (Table 2).

Similar to our results, in Argentina, *González Pereyra et al. (2012)*, by analysing 40 samples of mixtures for cattle collected from different feed lots have established the total fungal count varied from not detectable to 2.10 x 10^8 cfu g^{-1}. Likewise, in Brasil, *Rosa et al. (2006)*, by examining 133 animal feed samples, have found that in most samples the total fungal count was between 1.6 x 10^4 and 3.4 x 10^6 cfu g^{-1}. Furthermore, in Iran, by analysing 40 samples of feed for dairy cows and 35 samples of feed for beef cattle, *Rezaei et al. (2015)* have found that the average total fungal count in the beef cattle samples was greater (2.9 x 10^6 cfu g^{-1}) compared to the average total fungal count in feed sample for dairy cows (1.6 x 10^4 cfu g^{-1}).

Table 1. Level of fungal contamination of investigated cattle feed samples during 2012-2014

Fungal counts		Frequency (%)	
cfu g^{-1} *	log_{10}cfu	Feed for adult cattle	Feed for young cattle
2.1 x 10^5 – 3.9 x 10^5	5.32 – 5.59	5.88	4.76
5.1 x 10^4 – 2 x 10^5	4.71 – 5.30	52.94	38.1
1 x 10^1 - 5 x 10^4	2 – 4.7	41.18	57.14

*Colony forming units per g of sample

Table 2. Statistical analyses of total fungal counts (log_{10}cfu g^{-1}) in investigated cattle feed samples

Types of feed	Mean (log_{10}cfu g^{-1} ± S.D.)	Minimum (log_{10}cfu g^{-1})	Maximum (log_{10}cfu g^{-1})
Feed for adult cattle	4.45 ± 0.87	2	5.40
Feed for young cattle	4.11± 1.09	2	5.59
Level of significance	ns		

cfu g^{-1} - colony forming units per g of sample; * - significant - $P<0.05$; ns - not significant - $P>0.05$

Table 3. Frequency of contaminated cattle feed samples with potential toxigenic fungi from *Aspergillus, Fusarium* and *Penicillium* genera

Fungal genus	Frequency of fungal contaminated samples (%)		
	Feed for adult cattle	Feed for young cattle	Average
Aspergillus	85.71	76.47	81.09
Fusarium	66.67	88.24	77.46
Penicillium	66.67	58.82	62.75

In mycological analyses of the tested samples of feed for cattle, potentially toxigenic fungi species of the genera *Aspergillus*, *Fusarium* and *Penicillium* were most often isolated. *Aspergillus* species were identified in 85.71% of adult cattle feed samples, while *Fusarium* and *Penicillium* species were identified in the same number of samples (66.67%). In the group of samples of feed for young categories of cattle *Fusarium* species were identified in the largest number of samples (88.24%), while *Aspergillus* and *Penicillium* species were identified in 76.47% and 58.82% of the samples, respectively. The average for all feed samples showed *Aspergillus* species isolated in most samples (81.09%), while *Fusarium* and *Penicillium* species identified in 77.46% and 62.75% of the samples, respectively (Table 3).

Similar to our results, in Brazil, in the analysis of cow feed samples, *Rosa et al. (2008)* have isolated eight fungal genera, of which *Aspergillus* species were the most common (63%), followed by *Penicillium* (38%) and *Fusarium* (22%). Furthermore, in Iran, *Ghiasian and Maghsood (2011)* analysed 713 samples of different feed types and isolated *Aspergillus* species as the most dominant (37.4%), followed by *Penicillium* (23.7%) and *Fusarium* (17.5%). These authors also found that the mean fungal count of aflatoxigenic fungi in industrial-diary farms was significantly higher than in traditional-dairy farms.

Positive correlations were found in the tested samples between the total fungal count and moisture content, medium positive correlation (r=0.49) in samples of adult feed mixture and weak positive correlation (r=0.28) in samples of mixtures for young cattle categories. Similarly, by examining the correlation ratios between the relative humidity (RH) and the fungal count in maize grain samples, *Alptekin et al. (2009)* have established a positive correlation (r=0.378).

Conclusion

Subsequent to myological analyses of food samples for cattle, it can be concluded that the tested samples were contaminated most often with potentially toxigenic fungi from the genera *Aspergillus*, *Fusarium* and *Penicillium*. Of the toxic fungi genera, in the majority of samples of feed for young cattle categories, *Aspergillus* fungi were isolated, while the majority of samples of feed for adult bovine animals were contaminated with *Fusarium* species. Considering the values of the total fungal count, the tested samples did not completely meet the criteria of hygienic quality, especially for the young animal categories.

The results of these studies indicate the importance of monitoring because of the potential risk of contamination with mycotoxigenic fungi in the food chain. Therefore, information on the quality of food for humans and animals is of great general importance for preventing the danger to human and animal health

Gljivična kontaminacija hrane za goveda

Vesna Krnjaja, Aleksandar Stanojković, Slavica Stanković, Zorica Bijelić, Violeta Mandić, Nenad Mićić, Miloš Marinković

Rezime

U radu su ispitivani ukupan broj gljiva i gljivična kontaminacija 38 uzoraka smeša za ishranu goveda koji su sakupljeni na farmama u okolini Beograda tokom trogodišnjeg perioda (2012-2014). Primenom metode razređenja određivan je ukupan broj gljiva, dok su standardne mikološke metode korišćene za identifikaciju potencijalno toksigenih vrsta gljiva.

Analizama ukupnog broja gljiva u 17 uzoraka smeša za ishranu odraslih i u 21 uzoraka smeša za ishranu mladih kategorija goveda ustanovljeno je od 1×10^1 to $3{,}9 \times 10^5$ cfu g^{-1}. Prema Pravilniku Republike Srbije o kvalitetu hrane za životinje,

ukupan broj gljiva iznad dozvoljenog limita ustanovljen je u 5,88% uzoraka smeša za ishranu odraslih i u 38,10% uzoraka smeša za ishranu mladih kategorija goveda. Mikološkim analizama identifikovane su potencijalno toksigene vrste iz rodova *Aspergillus, Fusarium* and *Penicillium*. U najvećem broju uzoraka za ishranu odraslih kategorija goveda izolovane su *Fusarium* vrste (88,24%), dok su *Aspergillus* vrste izolovane u najvećem broju uzoraka smeša za ishranu mladih kategorija goveda (85,71%). U proseku za sve ispitivane uzorke hrane za goveda *Aspergillus* vrste su izolovane u najvećem broju uzoraka (81,09%), zatim slede *Fusarium* (77,46%) i *Penicillium* vrste (62,75%).
Dobijeni rezultati ukupnog broja gljiva ukazuju da ispitivani uzorci smeša za ishranu goveda ne zadovoljavaju u potpunosti kriterijume higijenskog kvaliteta. Zbog toga su redovne i stalne kontrole mikološkog kvaliteta neophodne preventivne mere za smanjenje i kontrolu gljivične kontaminacije hrane za goveda.

Ključne reči: gotove smeše za ishranu goveda, ukupan broj gljiva, toksigene gljive

Acknowledgment

Research was supported by Ministry of Education, Science and Technological Development, Republic of Serbia, projects TR 31023 and TR31053.

References

ALPTEKIN Y., DUMAN A.D., AKKAYA M.R. (2009): Identification of fungal genus and detection of aflatoxin level in second crop corn grain. Journal of Animal and Veterinary Advances, 8, 9, 1777-1779.
CHULZE S.N. (2010): Strategies to reduce mycotoxin levels in maize during storage: a review. Food Additives and Contaminants, 27, 5, 651-657.
GHIASIAN S.A., MAGHSOOD A.H. (2011): Occurrence of aflatoxigenic fungi in cow feeds during the summer and winter season in Hamadan, Iran. African Journal of Microbiology Research, 5, 5, 516-521.
ISO 21527-2:2008. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95, 1-13.
KENSLER T.W., ROEBUCK B.D., WOGAN G.N., GROOPMAN J.D. (2011): Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. Tox. Sci., 120, S28-S48.

PLACINTA C.M., D’MELLO J.P.F., MACDONALD A.M.C. (1999): A rewiev of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Technol., 78, 21-37.

ROSA C.A., RIBEIRO J.M., FRAGA M.J., GATTI M., CAVAGLIERI L.R., MAGNOLI C.E., DALCERO A.M., LOPES C.W. (2006): Mycoflora of poultry feeds and ochratoxin-producing ability of isolated *Aspergillus* and *Penicillium* species. Vet. Microbiol, 113, 89-96.

SLUŽBENI GLASNIK RS 4/2010, 113/2012 i 27/2014/ OFFICIAL GAZETTE OF RS 4/2010, 113/2012 i 27/2014. Pravilnik o kvalitetu hrane za životinje/ Regulation on the quality of animal feed.

SMAJLOVIĆ A., MUMINOVIĆ M., MUJEZINOVIĆ I., ĆUPIĆ V. (2012): Istraživanje degradacije aflatoksina M_1 u mlijeku. (Investigation of aflatoxin M_1 degradation in milk). Vet. glasnik, 66, 5-6, 387-394.

WATANABE T. (2002): Pictorial atlas of soil and seed fungi. In: Morphologies of cultured fungi and key to species. CRC Press, Boca Raton, London, New York, Washington D.C. pp. 486.

Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

FUNGAL SEED INFECTION OF SOME CULTIVATED GRASS SPECIES IN SERBIA

Ivana Stanojević[1], Snežana Andjelković[1], Snežana Babić[1], Darko Jevremović[2], Aleksandar Leposavić[2], Vladimir Zornić[1], Tanja Vasić[1]

[1]Institute for Forage Crops, Kruševac, Globoder, 37251 Kruševac, Republic of Serbia
[2]Fruit Research Institute, Kralja Petra I 9, 32000 Čačak, Serbia
Corresponding author: tanja.vasic@ikbks.com
Original scientific paper

Abstract: Grasses with its abundance of species and the presence of the fauna, is one of the most important factors of the biosphere. Grasses like perennial plants, are able to successfully produce organic matter in dense circuits more consecutive years, thus excluding her annual tillage. They can be directly exploited in livestock feed in various ways: grazing, production of hay, silage or industrial processing. There has not been a systematic research of grass mycoflora in Serbia. This research aims to present the results of preliminary research of mycopopulation of six different species of grass: K-11 (Perennial ryegrass), K-29 (Italian ryegrass), K-21 (Meadow fescue), K-20 (Tall fescue), K-13 (Italian ryegrass), K-40 (Cat grass), K-24 (Cat grass), K-14 (Red fescue). Total of 640 grass seeds has been examined and 10 genera of fungi were isolated: *Fusarium*, *Alternaria*, *Sclerotinia*, *Chaetomium*, *Aphanomyces*, *Trichoderma*, *Epicoccum*, *Cladosporium*, *Mucor* and *Penicillium*.

Key words: grass, fungi, seed

Introduction

Perennial grasses are very complex and diverse group of monocotyledonous plants, which belong to the subfamily Pooideae family Poaceae. The importance of perennial grasses is manifold, they are based on sustainable animal husbandry and livestock feed production, making the staple food of ruminants in regions with moderate climate as a component of natural and sown pasture, natural and sown meadows which may be used by means of cutting, haylage and cutter (*Tomić and Sokolović, 2007*). The most important species for animal feed are cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), Timothy grass (*Phleum pratense* L.) Italian ryegrass (*Lolium *multiflorum* Lam.), French ryegrass (*Arrhenatherum elatius* L., P. J. Beauv.ex C.Presl Presl.), red fescue (*Festuca rubra* L.), and smooth browen grass (*Bromus inermis* Leyss) (*Tomić and Soković, 2007*). Perennial grasses is characterized by high production of biomass, i.e. a dry matter content which is of high quality, forage rarely sown separately, but in order to supplement the growing area and the possible exploitation usually sown in mixtures with other legumes and grasses (*Stošić et al., 2005*).

A large range of diseases caused by microfungi, bacteria or viruses affect forage grasses and these pathogenic micro-organisms have diverse effects. Some micro-organisms decrease forage yield and some particularly aggressive parasites can even lead to the death of the plants that they attack. This is the case for *Microdochium nivale* and *Typhula incarnata* which cause Microdochium patch (pink snow mold) and Typhula blight (gray snow mold) on grasses (*Peeters, 2004*). Likewise, Pythium blight and Pythium root rot of grasses which caused by *Pythium aphanidermatum, Pythium aristosporum, Pythium graminicola, Pythium vanterpooli*, and other *Pythium* species and Brown patch on grasses which caused by *Rhizoctonia solani* Kuhn, *Ceratorhiza cerealis* (formerly *Rhizoctonia cerealis*). Than, anthracnose of turfgrass annual bluegrass and creeping bentgrass which causer is *Colletotrichum cereale* (formerly *Colletotrichum graminicola*), dollar spot on turfgrass which causer is *Sclerotinia homoeocarpa* and *Sclerotium rolfsii* Sacc. On the turfgrass, creeping red fescue, kentucky bluegrass, annual bluegrass, perennial ryegrass, tall fescue, and some varieties of bentgrass and bermudagrass occurrs leaf spot/melting-out on which caused by *Drechslera* spp. and/or *Bipolaris* spp. (*Couch, 1995; Allen et al., 2004*; *Tredway and Bupree, 2001)*. The diseases also influence forage quality by modifying the plant's chemical composition (protein, water-soluble carbohydrate and cellulose contents) and digestibility. Toxins detrimental to cattle are produced by some fungi (*Peeters, 2004*).

Due to its importance in the implementation of healthy seeds during the establishing of forage grass, it is important to study the occurrence and intensity of fungal diseases on grass seeds. Given the importance of grasses as a forage crop in Serbia, the aim of this paper is the determination of phytopathogenic fungi that cause diseases in grass for a clearer perception of problems (the extinction of plants, reducing yields, deterioration of the quality of feed and other) arising as a result of the presence those fungi.

Material and Methods

Research was conducted in the Laboratory for Plant Pathology of the Institute for Forage Crops in Kruševac. As a starting material six species of grass were used: K-11 Perennial ryegrass (*Lolium perenne* L.), K-29 Italian ryegrass

(*Lolium multiflorum* Lam.), K-21 Meadow fescue (*Festuca pratensis* huds.), K-20 Tall fescue (*Festuca arundinacea* Schreb.), K-13 Italian ryegrass (*Lolium multiflorum* Lam.), K-40 Cat grass (*Dactylis glomerata* L:), K-24 Cat grass (*Dactylis glomerata* L:), K-14 Red fescue (*Festuca rubra* L.). The samples origin originated from the harvest in 2016. Sampling was conducted during period of January-February 2017. Several samples were taken from each species and 80 seeds were used per sample in 8 Petri dishes, 10 seeds per Petri dish. The seeds were surface sterilized by the method of *Chi et al.(1964)*. Seeds were surface disinfected in 7% sodium hypochlorite (NaOCl) for 5 minutes, rinsed in sterile water and dried at room temperature. Then the seeds were put on potato dextrose medium (PDA). After ten days, the colonies were developed around the seed, and each colony was separately examined under the microscope for future identification and some of the cultures were transferred to potato-dextrose medium (PDA). Determination of *Fusarium* species was conducted based on macroscopic and microscopic traits on the PDA according to the methods of *Nelson et al. (1983)* and *Burgess et al. (1994)*, while other genera were determined by the method of *Watanabe et al. (1994).*

Microscopic examination was performed using microscopes Olympus CX31. Images were captured by the Olympus SC100 color camera on BX31 microscope (Olympus, Japan).

Results and Discussion

Mycosis of grasses can occur at the time of sowing or later after sowing The result of the fungal attack is poor germination, and then the poor overgrown (coverage) of the sward.

Microscopic study of colonies of fungi, isolated from grasses seed, showed varying degrees of infection (Table 1.). In the six different species of grasses 10 genera of fungi were isolated: *Fusarium*, *Alternaria*, *Sclerotinia*, *Chaetomium*, *Aphanomyces*, *Trichoderma*, *Epicoccum*, *Cladosporium*, *Mucor* and *Penicillium*. Among the identified genera, *Alternaria* was the most frequent on seed of Tall fescue – K20 (53.75%). *Fusarium* species were isolated from seed of four tested grasses in frequency of 2.5% (Italian raygrass - K13), 5% (Italian raygrass – K29 and Meadow fescue – K21) and 23.75% (Perennial raygrass – K11). *Sclerotinia* species were identified on seed of Perennial raygrass – K11 in frequency of 7.5%. Relative high percentage of *Aphanomyces* sp. was determned on seed of Meadow fescue – K21 (15%). *Penicillium* sp. was isolated on seed of Red fescue – K14 in frequency of 13.75%. Species from genera *Chaetomium, Trichoderma, Epicoccum, Mucor* and *Cladosporium* were isolated in some of tested grass species with different percentage frequency (Table 1).

According to the Serbian Regulation on the seed quality of grasses *(Službeni glasnik, 107/2008)* the frequency of *Alternaria* and *Fusarium* species of some tested unprocessed grass seeds above permitted limit (5%) were determined. Similarly to this, *Holmes (1983)* and *Raikes (1997)* have established that the *Fusarium* species were the most causal agent of grass diseases.

Table 1. Frequency of fungal species on tested grass seeds

Grass / Fungal species	K-11 Perennial ryegrass	K-29 Italian ryegrass	K-21 Meadow fescue	K-20 Tall fescue	K-13 Italian ryegrass	K-40 Cat grass	K-24 Cat grass	K-14 Red fescue
Fusarium sp.	23.75	5.0	5.0	0	2.5	0	0	0
Alternaria sp.	12.5	15.0	0	53.75	7.5	10.0	7.5	0
Sclerotinia sp.	7.5	0	0	0	0	0	0	0
Chaetomium sp.	13.55	5.0	0	8.75	2.5	0	20.0	12.5
Aphanomyces sp.	0	0	15.0	0	0	0	0	0
Trichoderma sp.	0	6.25	5.0	6.25	0	2.5	2.5	7.5
Epicoccum sp.	0	0	7.5	10.0	0	5.0	0	0
Mucor sp.	3.75	0	0	0	0	0	0	6.25
Penicillium sp.	0	0	0	0	0	0	0	13.75
Cladosporium sp.	0	0	10.0	0	8.75	7.5	5.0	8.75

The diseases caused by fungi are the most widespread. They develop on the surface or in the tissues of the plant. Many are present on the leaves where they often develop into spots of variable shapes and colours which help to identify them: *Puccinia* spp., *Rhynchosporium* spp., *Drechslera* spp., *Mastigosporium* spp. and *Cladosporium* spp. Snow mould is a common disease in regions where a heavy snow cover remains during winter (*Peeters, 2004*). This disease caused by a group of fungi including *Microdochium nivale* and *Typhula incarnata*, develops gaps in the sward by destroyng plants (*Ellis and Ellis, 1997*).

Therefore, it decreases sward productivity and longevity and induces the need for early costly resowing. The susceptibilitiy to this disease varies considerably from one species to another and, in regions where swards are prone to the disease, a resistent species grasses must be sown, for example *Phleum pratense* is the most resistant species and *Olium multiflorum* which is the most susceptible (*Ellis and Ellis, 1997*). *Pythium* species cause several diseases on cereals, grasses, vegetable crops, ornamental plants,fruit trees and forest trees. The type, symptoms and severity of these diseases depend on the pathogen species, their aggressiveness, the host plants, plant parts infected as well as the prevailing environmental conditions. Most *Pythium* species infect juvenile tissues of seedlings and plants, but can also attack feeder roots or root tips of older plants, stems and foliage of some grasses and fruits, resulting in damping- off, wilt, stunting, decline and fruit rot diseases (*Al-Sadi, 2012*). Pink snow mold or Microdochium patch is disease caused by *Microdochium nivale*. The causal organism of these diseases, *Microdochium nivale*, was formerly known as *Fusarium nivale*, and Microdochium patch is sometimes referred to as Fusarium patch (*Raikes, 1997*).

Conclusion

Curative treatments against diseases are not used in grasslands for economic reasons and so preventive means must be foreseen and utilized where possible in order to avoid the onset and development of the diseases. Choosing resistant species and varieties is essential when sowing a sward and, arguably, mixtures of species and varieties are preferable to pure sowings. A balanced fertilization associated with a suitable defoliation management system will help to maintain a healthy sward. Foliage fungi are favoured by nutrition stress in particular. However, the effect of fertilization on the development of diseases is a complex issue. It is obvious that the sward is especially attacked when the growing period long. Since nitrogen fertilization speeds up the growth of grass and reduces the interval between successive defoliations, in cutting as well as in grazing, nitrogen application helps to curb foliar diseases.

This paper presents the preliminary results of mycopopulations of six species of grass. Grasses is very important forage crop and its importance as animal feed is growing within our country. This work is the beginning of a more comprehensive study of phytopatogenic fungi on grass. So far, there were no significant researches in this direction in Serbia, so the future researches related to grasses will go in the direction of selection of genotypes with increased tolerance to diseases.

Gljivična infekcija semena nekih vrsta trava u Srbiji

Ivana Stanojević, Snežana Andjelković, Snežana Babić, Darko Jevremović, Aleksandar Leposavić, Vladimir Zornić, Tanja Vasić

Rezime

Trave svojom brojnošću vrsta i prisutnošću u biljnom pokrivaču, predstavljaju jedan od najvažnijih činilaca biosfere. Trave kao višegodišnje biljke, sposobne su da uspešno proizvode organsku materiju u gustim sklopovima više uzastopnih godina, čime se isključuje svakogodišnja obrada zemljišta. Mogu se

neposredno iskorišćavati u ishrani stoke na različite načine: ispašom, proizvodnjom sena, silaže ili industrijskom preradom.
Sistematskih istraživanja mikoflore trava u Srbiji do sada nije bilo. U ovome radu iznosimo rezultate preliminarnih istraživanja mikopopulacije 6 različitih genotipova trava: K-11 (engleski ljulj), K-29 (italijanski ljulj), K-21 (livadski vijuk), K-20 (visoki vijuk), K-13 (italijanski ljulj), K-40 (ježevica), K-24 (ježevica), K-14 (crveni vijuk). Ukupno je pregledano 640 semena sa kojih je izolovano 10 rodova gljiva: *Fusarium*, *Alternaria*, *Sclerotinia*, *Chaetomium*, *Aphanomyces*, *Trichoderma*, *Epicoccum*, *Cladosporium*, *Mucor* and *Penicillium*.

Ključne reči: trave, gljive, seme

Acknowledgment

Research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR 31057.

References

ALLEN, T.W., MARTINEZ, A., BURPEE, L.L. (2004): *Pythium* blight of turfgrass. The Plant Health Instructor. DOI:10.1094/PHI-I-2004-0929-01.
AL-SADI, Abdullah M. (2012): Efficacy of mefenoxam is affected by a lag period between application and inactivation of *Pythium* species. Phytopathologia Mediterranea 51, 2, 292−297.
BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory manual for Fusarium reaserch. University of Sydney and Royal Botanic Gardens, Sydney.
CHI C.C., CHILDERS W.R., HANSON E.W. (1964): Penetration and subsequent development of three *Fusarium* species in alfalfa and red clover. Phytopathology, 54, 434-437.
COUCH, H.B. (1995): Diseases of Turfgrasses. 3rd ed. Krieger Publishing Company, Malabar, FL.
ELLIS, M.B., ELLIS, J.P. (1997): Microfungi on land plants: an identification handbook. Slough, UK, Pichmond Publishing. pp 868.
HOLMES S.J.I. (1983): The susceptibility of agricultural grasses to pre-emergence damage caused by *Fusarium culmorum* and its control by fungicidal seed treatment. Grass and Forage Science, 38, 209-214.

NELSON P.E., TOUSSOUN T.A., MARASAS W.F.O (1983): *Fusarium* species, an illustrated manual for identification. Pennsylvania State University Press, Unervesity Park and London.

PEETERS, A. (2004): Wild and sown grasses. Published by the Food and agriculture organization of the united nations and Blackwell publishing, 5-42.

RAIKES, C. (1997): Fusarium Patch Disease on Winter Sports Turf in the UK**.** Journal of Turfgrass Management, 2, 3, 35-50.

SLUŽBENI GLASNIK RS (107/2008): Pravilnik o zdravstvenom pregledu useva i objekata za proizvodnju semena, rasada i sadnog materijala i zdravstvenom pregledu semena, rasada i sadnog materijala / Regulation on the seed quality of agricultural plants.

STOŠIĆ, M., LAZAREIĆ, D., DINIĆ, B., TERZIĆ, D., SIMIĆ, A. (2005): Natural grasslands as basic of livestock development in hilly –mountainousregions of central Serbia. 8th international symposium „Modern Trends in Livestock Production“. Biotehnology in Animal Husbandry. Vol 21, Book 1., 265-273.

TOMIĆ, Z., SOKOLOVIĆ, D. (2007): Oplemenjivanje višegodišnjih trava-metode, kriterijumi i rezultati u Srbiji. Zbornik radova Instituta za ratarstvo i povrtarstvo, Novi Sad, Srbija, vol. 44, No I, 51-69.

TREDWAY, L.P., BUPREE, L.L. (2001): *Rhizoctonia* diseases of turfgrass. The Plant Health Instructor. DOI: 10.1094/PHI-I-2001-1109-01

WATANABE T. (1994): Pictorial atlas of soil and seed fungi. Morphologis of cultured fungi and key to species. Lewis Publishers. Boca Raton.

SPONSOR AND DONOR

AIK Banka

Извор: локална база података COBISS.SR/NBS

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

636/638(082)(0.034.2)
631/635(082)(0.034.2)

INTERNATIONAL Symposium Modern Trends in Livestock Production (11 ; 2017 ; Belgrade)
Proceedings [Elektronski izvor] / 11th International Symposium Modern Trends in Livestock Production, 11 - 13 October 2017, Belgrade ; [editor in chief Milan M. Petrović]. - Belgrade : Institute for Animal Husbandry, 2017 (Belgrade : Institute for Animal Husbandry). - 1 USB fleš memorija ; 1 x 1 x 3 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovnog ekrana. - Tiraž 200. - Bibliografija uz svaki rad.

ISBN 978-86-82431-73-2

a) Сточарство - Зборници b) Пољопривреда - Зборници
COBISS.SR-ID 245942028

<----------------------------------
1

11th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCKPRODUCTION

11th - 13th October 2017 - Belgrade, Serbia

P R O C E E D I N G S

INSTITUTE FOR ANIMAL HUSBANDRY

Autoput 16, P. Box 23, 11080, Belgrade, Serbia

www.istocar.bg.ac.rs