BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

BIOTECHNOLOGY
IN ANIMAL HUSBANDRY

THE APPLICATION OF MODERN MOLECULAR TECHNIQUES IN ANIMAL SELECTION

Milan P. Petrović[1], Marina I. Selionova[2], Violeta Caro Petrović[1], Dragana Ružić Muslić[1], Nevena Maksimović[1], Zoran Z. Ilić[3], Ivan Pavlović[4]

[1] Institute for Animal Husbandry, Belgrade-Zemun, Serbia
[2] All Russian Institute for Sheep and Goat Breeding, Stavropol, Russia
[3] Faculty of Agriculture, University of Pristina, Kosovska Mitrovica, Serbia
[4] Scientific Institute for Veterinary of Serbia, Belgrade, Serbia
Corresponding author: milanppet@yahoo.com
Review paper

Abstract: Some problems of modern analysis of the genome of domestic animals and the possibility of applying the results of the research in the selection have considered. Molecular genome analysis has progressed rapidly. Today, using modern DNA analysis, the domestic animals breeding value can be safely foreseen. Based on the scientist's intuition of early-20th century, after half a century, genetic markers had discovered. Thus, the traditional selection method has integrated with molecular techniques of selection by applying marker-assisted selection (MAS). Microsatellites have attracted great attention from scientists because they are widespread in the genome and have a high level of polymorphism. Thousands of SNPs have discovered with their exact position in the genome. This very reliable method occurred for analyzing the genome which shows the change of only one nucleotide base into the DNA molecule. All modern achievements of molecular genetics have opened the way for practical application in the selection of all kinds of domestic animals. Today, a combination of molecular or genomic selection combined with a traditional is very reliable method for a faster, more accurate assessment of the breeding value of animals.

Key words: domestic animals, selection, molecular techniques, genetic markers

Introduction

The primary goal of modern selection is the association of all known sources of animal information (phenotype, origin and genetic markers) in order to obtain the highest reliability of the estimated breeding value (EBV) and ensure

genetic improvement *(Petrovic 2000; Petrovic et al., 2001; Petrović and Pantelić, 2015; Petrovic et al., 2017a; Petrovic et al., 2017b).* Genomics offers ample opportunities; it's not only for genetic improvement of animals but can also be used as an important tool for the assessment of genetic diversity of local sheep and goat breeds *(Rupp et al., 2016).* The genome of domestic animals contains 20,000-35,000 useful-useful genes. Generally, selection by using of molecular techniques should involve the process of DNA molecular analysis in order to search for markers or individual nucleotide polymorphisms (SNPs) to obtain information on the genetic potential of the product's characteristics of the individual, which will serve as a selection criterion. From the first ideas of DNA molecular analysis for this purpose, many procedures have applied of such; a number of genetic markers, methods and in practice is necessary to accept a common platform, the name of which this would be a genomic selection. The term "genomic selection" was first introduced by *Haley and Visscher (1998). Meuwissen (2001)* developed and presented a methodology for the analytical estimation of the value of breeding by using a map of markers covering the whole genome.

Genomic selection (GS) is at the present level of science, a method by which high density markers cover the entire genome. This leads to individual genetic evaluation - genomic estimated breeding values (GEBV). Genomic selection can increase the precision of selection, shorten the generation interval by selecting individuals at an early stage of life, and accelerate genetic progress *(Christensen et al., 2014).*

Practical work in genomic selection involves taking a sample of biological material (blood, seed, hair) from which the DNA is isolated in the laboratory. The procedure for isolating the pure DNA is very complex and includes cutting restriction endonucleases, radioactive or non-radioactive labeling, ligation, use of special detergents, bases, organic solvents and enzymes. Following the isolation of the DNA, a genotyping procedure is performed using the Illumina SNP50K chip. Genotyping is a method by which genetic variations are determined without the sequencing of the whole genome. The result of genotyping is the signals for each of the 50,000 SNPs that are calculated in the SNP genotype (AA, AB or BB). Thus, for the genotyping individual, the result is (more precisely, the genotype) for each of the 50,000 SNPs. The fact that we have come to know about the genotype of a large number of SNPs does not give a complete answer to the breeding value of the animal. In order to solve this, it is necessary to evaluate the influence of the individual SNP by using the SNP equation. Thus, the genomic breeding value (GBV) of a genotyped animal is the sum of the effects of all SNPs, obtained using the SNP equation *(Špehar, 2013).*

In many countries, genomic selection becomes the leading method of genetic evaluation *(Hayes et al., 2009, 2012).* Especially for GS cows GS can soon replace the traditional system of genetic evaluation of animals.

Development of molecular techniques

Genomic technologies allow deciphering the genotype of the animal at birth. This can be done at the same time by selecting breeding animals. SNP as the latest technology has been designed to further improve the accuracy and reliability of selection and assessment of the breeding value of animals. The SNP genome predecessors are selective markers, which make it possible to determine the presence or absence of certain genes (alleles) of the carrier of quantitative traits in the animal genome.

A. Serebrovski, a Russian Scientist was first given the idea and the theory of the use of markers in breeding during the second decade of the 20th century. The term "marker" (then called "signal" while the English term "keeper") began to use later. According to Serebrovsky, the marker is an allele gene with pronounced phenotype, localized next to another allele responsible for an economically significant trait, but there is no clear phenotypic expression. Thus, the selection of the phenotypic expression of this allele-signal is possible by selecting related alleles that determine the manifestation of the observed trait. However, the direct marker is far more reliable than the associated marker for the prediction of phenotypic variations of the particular character of domestic animals (*Dekkers, 2004, Scherbatov et al., 2013*). Genetic markers were initially based on morphological characteristics (phenotypic markers).

However, quantitative traits are the result of a complex inheritance process. The markers used and environment conditions determined their manifestation. Therefore, its use is limited. Polymerase chain reaction (PCR) and sequencing (DNA nucleotide assay) DNA occupies a significant place in genomic animal selection *(Avise, 2004). Buisson et al. (2014)* conclude a genomic breeding scheme (GS) appears technically and economically relevant in French dairy sheep breeds. The α_{s1} casein genotype had a significant effect on milk yield, fat content and protein content (*Carillier-Jacquin et al., 2016).* In recent years, several new molecular techniques have been developed, in addition to genetic markers such as microsatellites, the leading site has taken the polymorphism of single nucleotides (SNP - Single Nucleotide Polymorphism), which allows the selection options to expand considerably and deepen.

The application of DNA technology and genetic markers in livestock breeding began in the 1980s. Molecular genetic markers have used for programs for the conservation of genes of endangered breeds of domestic animals. Also, markers have found application for solving the problem of origin and parenthood of the distribution of species, genetic causes of hereditary diseases. The application of markers was positive to accelerate the selection of individual characteristics - resistance to certain factors from the impact on productive performance.

The traditional methods of farm animals' selection can be integrated with molecular techniques of selection by applying marker-assisted selection (MAS) (*Lande and Thompson, 1990, Ollivier 1998, Caro Petrovic et al., 2017).*

Modern molecular methods in selection

Microsatellites have attracted great attention from scientists because they are widespread in the genome and have a high level of polymorphism. Microsatellites, such as SSR (Simple Sequence Repeats) or STR (Simple Tandem Repeats), repeated several times in the tandem by the genome of more than one hundred thousand loci *(Weber and May, 1989).*
Both of the mentioned about are highly polymorphic and suitable for individual DNA typing, or the production of a genetic profile of the individual. They are considered to be the most informative genetic markers.

Microspheres are non-coding parts of DNA, but some studies reveal their role in gene expression (*Moxon and Wills, 1999*). It is known that the variants of microsatellites differ in the number of repetitions of the underlying motive, which is actually the result of mutations. The structure of microsatellites can have several forms:
• filled (CACACACACACA),
• interrupted (CACACACAggggCACACA) or
• assembled (CACACACAGTGTGTGTCACA).

Their multiplication is carried out by the chain reaction of polymerase - PCR technique. Microspheres are relatively short compared to other genetic markers, as they generally have 50 and 150 pb.

Their advantage is that very small amounts of DNA are required for duplication so in similar research have been used for these reasons. It is also extensively used to examine the genetic distance between individual animal populations *(Cunningham et al., 2001; Teneva et al., 2010; 2013).*

Some countries have developed a genetic test system. In addition to a number of advantages, microsatellites insufficiently map some specific genome areas. On the other hand, the high cost of equipment and reagents and the development of automatic methods using SNP chips slowly take their place. A very practical method for analyzing the genome is the SNP polymorphism of a nucleotide, which shows the change of only one nucleotide base into the DNA molecule.

Thousands of SNPs have discovered with their exact position in the genome. Such differences in the molecule, are often encoded with letters, e.g., A and B. Possible combinations are AA (homozygote saturated A), AB (heterozygote) and BB (homozygote for allele B) for each SNP marker. However, for many SNPs, it is not known whether they cause some changes in the

manifestation of individual traits or maybe they are just near a genome *(Spehar, 2013).*

SNPs are point mutations that may occur as a result of spontaneous mutations or the action of mutagens. The difference in only one base can cause characteristic changes. SNPs are widespread in the human and animal genome. In addition, SNPs have a low rate of mutation per generation ($\sim$ 10-8) as opposed to microsatellites, making them suitable markers for population genetic analysis *(Scherbatov et al., 2013).*

The SNP great advantage also allows the ability to use automatic detection methods, for example, using DNA strings. In the world, databases are already being formed, testing a large number of animals to reveal the connections between known point mutations and productivity. Massive numbers of polymorphic variants of the gene are currently defined, as well as their impact on animal production characteristics. Some genetic tests that use markers that define production qualities are already publicly available for use in breeding. We'll list some of these markers (*Shcherbatov et al., 2013*):

• Meat and meat production efficiency markers - MC4R, HMGA1, CCKAR, POU1F1
• Fertility markers: ESR - estrogen receptor gene, EPOR - erythropoietin receptor gene
• Markers of disease resistance - ECR F18 receptor gene

Through the results of markers, can estimate the frequency of desired and undesired alleles in the population and continue the selection in the future, so that all animals get only desirable gene alleles.

Genomic selection - is a powerful tool for future use. Currently, the effectiveness of genomic selection is limited in various interactions between the locus of quantitative traits, the variability of quantitative properties, and the influence of the environmental factors. However, the results of the research in many countries have confirmed that the use of standard statistical methods together with genomic selection increases reliability in predicting breeding values of animals.

That in today's conditions of science development, the goal of genomic selection of domestic animals is to connect all known sources of information (phenotype, origin and genetic markers) to obtain the highest accuracy of the assessed breeding value (EBV) and ensure genetic improvement.

Animal Breeding Assessment is a very complex process. It supports the fact that the selection with statistical methods in some areas such as resistance to disease, meat quality, fertility, is characterized in low efficiency due to the following factors (*Petrovic and Pantelic, 2015*):

• the low heritability of traits
• significant influence of environmental factors

• sex restrictions
• the manifestations of properties only under the influence of certain factors
• when the manifestation of the characteristics is relatively late
• the fact that the trait is difficult to measure (for example, health specifics)
• the presence of hidden carriers of traits

In the above cases and mentioned limitations, in the application of the standard statistical procedure and for a more accurate assessment it is necessary to analyze the offspring, which is necessary to wait until breeding maturity. However, the use of DNA markers makes it possible to analyze the genotype immediately after birth, without waiting for the manifestation of the trait or appearance of the progeny, which greatly accelerates the selection. On the other hand, if the selection was direct to properties with a high degree of inheritance, then the genomic selection will not bring significant benefits.

It has concluded that genomic selection should not negate the traditional approaches to the assessment of the nursing value of the animal. Statistical analysis and genomic selection technology complement each other. The use of genetic markers makes it possible to accelerate the animal selection process, and standard methods assess the effectiveness of this selection.

In practice, genomic selection will be a reliable "prognosis" for livestock production and will accelerate the process of selecting the most valuable animals. The main advantages of genomic selection are:

1. High accuracy of testing
2. New evidence and assessment characteristics
3. Very quick selection
4. Accelerated genetic improvement of animal husbandry

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 give the opportunity to identify genetic variations that produce different production performances *(Bai Y. et al.,2012)*. This could also increase the chances of resistance to pathogens that slow down the production of animals.

These findings can also provide useful information in the production of healthy food for human consumption *(Bai X. et al., 2012)*. The first genome sequencing has done in livestock *(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*). In the study of *Suárez-Vega et al. (2015)* the quality scores of the extracted RNA samples for each breed from four animals were sequenced for time points D10, D50 and D150,and three biological replicates

were sequenced for D120 have acquired a total of 1,116 million paired-end reads from the transcriptome sequencing of the 30 milk samples analyzed. The alignment of the reads to the Ovis aries genome Oar_v3.1 genome build have yielded a mean of 985.05 million reads (88.10%) that aligned to unique locations in the ovine genome per RNA-seq sample; a mean of 1.47 million reads (4.01%) per sample that aligned to multiple locations in the genome; however, a mean of 2.84 million reads (7.65%) per sample that did not align to any genome location.

The achievements of molecular techniques in practice

Xu et al. (2017) noted that selection intensity can be heightened significantly through improvements in the scale and precision of genotyping and phenotyping, that the breeding cycle time could shorten by accelerating breeding procedures through integrated breeding approaches such as marker-assisted selection and doubled haploid development. Genomic resources also provide a basis for genetic improvements of economically important traits in sheep with the identification of functional genes and variants associated with these traits helps facilitate traditional breeding techniques using quantitative approaches and molecular breeding techniques are cost-effective and time-saving compared to conventional crossbreeding schemes (*Song-song and Meng-hua 2017)* . In sheep and goat farming, traditional methods of selection are increasingly complemented by the modern DNA analysis, to detect the gene that affects the expression of particular production traits or are located near the site responsible for the given property *(Carillier et al., 2013, 2014).* As is well known, the use of genetic markers enabled the detection of genes responsible for expressing significant properties or determining their approximate location in the genome. Therefore, genetic markers are not genes that define production or other properties, but rather indicate a specific site in the genome that potentially finds these genes. In recent years, *Kijas et al. (2012); Zhang et al. (2013)* identified the genes that are responsible for some of the economically important traits of sheep and goats. Since the eighteenth decade of the twentieth century, the development of molecular genetics methods, sheep breeding has been among the first to meet modern selection procedures. Experimentally and in practice, all known genetic markers were then used. A special echo in the selection of sheep and goats had the application of microsatellites to characterize individual breeds and determine their genetic distances, or the population structure. These researches are actual today *(Kijas et al., 2009;Jevšinek Skok et al., 2015; Murital et al., 2015; Yilmaz et al.,2015; Zinovieva et al., 2015; Ocampo et al., 2016; Seilsuth et al., 2016; Souheil Gaouar and Samiakdidi 2016; Edea et al., 2017; Deniskova et al.,2018; Selepe et al., 2018).* Likewise the contribution of microsatellites markers in the clarification of the origin, genetic risk factors, and implications for conservation of

sheep breed has studied *(Sassi-Zaidy et al.2016).* Since 2010, the international consortium for the exploration of the genome of 23 sheep breeds developed Illumina Ovine SNP50K chip, a new phase of genomic selection of sheep begins. In recent years, more than 50,000 SNPs have tested to see their connection with individual production characteristics. Otherwise, the Illumina SNP50K chip is a small glass tile that has 12 panels where 50,000 SNPs have tested for each animal. This reveals which nucleotide was present in a particular SNP position. The signals received for each of the 50,000 SNP markers have calculated as "SNP marker genotype" (AA, AB or BB). In this way, the final result is in the form of a genotype for 50,000 SNP markers for each genotyped sheep. Since 2011, chips have used in copiers, and the Illumina SNP50K chip has been developed using the genome of 25 goats. For a relatively short period since finding this method to date, thousands of SNPs for which the position of the genome is accurately known and the consequence of the change in the nucleotide base have discovered. For example, some studies have shown that sheep with a heavy run in a particular SNP have a base of adenine (A), where the animals with a lighter run in the same SNP have a guanine base (G) *(Petrovic and Pantelic, 2015).* The application of genomic selection in sheep farming is increasingly taking place. Among the leading countries are Australia, New Zealand, Russia, China, and more precisely the countries with the most developed sheep breeding. When it comes to European countries, France is most prominent, especially in the Lacone breed. The SNP s58995.1 (rs420767326 A>G) in MEF2B gene and OAR3_115712045.1 (rs401775061 A>C) in TRHDE gene, which significantly associated with the post-weaning gain in sheep have discovered (*Zhang et al. 2013;2016*). The MEF2B gene encodes the protein from the MEF2 family of which the interaction of proteins from the MEF2 family with the promoter myostatin gene of sheep has a stimulating effect on the expression of myostatin; a protein that limits muscle growth in mammals *(Du et al., 2007).* Therefore, the mutation of the MEF2B gene can have a significant effect on the production of sheep meat through the change in the production of myostatin which was confirmed in some other studies *(Chen et al., 2015).*The MEF2B is a new promising candidate gene defining the productive qualities of sheep *Trukhachev et al. (2016).*

The production and properties of milk in the Spanish breed of sheep Churra by *Garcia-Gamez et al. (2012)* and found a link to certain genes. Similar research was carried out by *Gras et al. (2016)* found a positive association between LGB and milk yield and composition which recommend this candidate gene like the marker for a future MAS program. Diacylglycerol O-Acyltransferase 1 (DGAT1) a gene turned out to be a functional and positional candidate gene for a major region on chromosome 14 closely associated with fat content *(Martin et al., 2017).*

Carillier et al. (2015) stated that the availability of SNP54k chips for goats has made it possible to genotype alpine and saanen goats in France. In both breeds,

genomic selection can improve annual genetic improvement by reducing the length of the father-son interval. The quality of predicting the value of an individual in this way is sensitive to the size of the reference population, which can affect the accuracy of genomic indicators.

Kim et al. (2016) had use caprine and ovine 50K SNP BeadChips from Barki goats that have identified several candidate regions under selection that spanned 119 genes. A majority of the genes were involved in multiple signaling and signal transduction pathways in a wide variety of cellular and biochemical processes. In particular, selection signatures spanning several genes that directly or indirectly influenced traits for adaptation to hot arid environments, such as thermo-tolerance (melanogenesis) (*FGF2*, *GNAI3*, *PLCB1*), body size and development (*BMP2*, *BMP4*, *GJA3*, *GJB2*), energy and digestive metabolism (*MYH*, *TRHDE*, *ALDH1A3*), and nervous and autoimmune response (*GRIA1*, *IL2*, *IL7*, *IL21*, *IL1R1*) were also identified. Genomic selection in sheep concentrates on all genetic aspects and production directions. However, it should point out that genomic selection in sheep farming, and especially in the case of chewing, is taking place slower in comparison with cattle breeding. However, this stated conditionally, since each of these animal husbandry branches has own significance, objectives and selection requirements depending on the different natural and social influences. In the study of *Nan et al.(2014)* have revealed thousands of differentially expressed genes, of which most were possibly associated with wool growth and several potential gene families might participate in hair growth regulation.

Results of SNP genotypes can be applied to determine the history and diversity of the population of sheep and goats in the framework of our country and the world. It is also possible to determine the origin of individuals, genetic variations associated with certain diseases, with some properties of carcasses, etc.
In the end, we can summarize how the genomic selection of sheep and goats has the following benefits:
• More precisely, predicting the genetic value for the desired breeding goal
• With properties that are usually difficult to improve
• For features that are difficult or expensive to measure
• For features that cannot be measured early
• Characteristics with low heritability, of such as yield and quality characteristics of meat, production of wool, reproductive rate, resistance to parasites.

Conclusion

Selection as the principle of improving animal production characteristics is a permanent process. By this developing science, the techniques of selecting animals are changing and improving. In addition to traditional selection, the development of molecular genetics has opened up a new era. Today, animal

science has gained a powerful tool called genomic selection. Combined with the traditional assessment of the breeding value of domestic animals through molecular indicators at the level of the genome, is the path to faster and more efficient genetic progress in the future.

Primena savremenih molekularnih tehnika u selekciji životinja

Milan P. Petrović, Marina I. Selionova, Violeta Caro Petrović, Dragana Ružić Muslić, Nevena Maksimović, Zoran Z. Ilić, Ivan Pavlović

Rezime

Razmotreni su i neki problemi savremene analize genoma domaćih životinja i mogućnosti primene rezultata istraživanja u selekciji. Analiza molekularne osnove domaćih životinja napredovala je brzo. Danas, koristeći savremenu analizu DNK, može se sa značajnom pouzdanošću predvideti odgajivačka vrednost domaćih životinja. Na osnovu intuicije naučnika početkom 20. veka, a posle pola veka otkriveni su genetski markeri. Na taj način je, tradicionalni metod selekcije integrisan sa molekularnim tehnikama i počelo se sa primenom selekcije pomoću markera (MAS). Mikrosateliti su privukli veliku pažnju naučnika jer su rasprostranjeni u genomu i imaju visok nivo polimorfizma. Nakon toga, hiljade SNP-a su otkriveni sa svojom tačnom pozicijom u genomu. Ovaj vrlo pouzdan metod za analizu genoma pokazuje promenu samo jedne nukleotidne baze u molekulu DNK. Savremena dostignuća molekularne genetike otvorila su put za praktičnu primenu u selekciji svih vrsta domaćih životinja. Danas je kombinacija molekularne ili genomske selekcije kombinovana sa tradicionalnom veoma pouzdanom metodom za bržu i preciznu procenu odgajivačke vrednosti životinja.

Ključne reči: domaće životinje, selekcija, molekularna tehnika, genetski markeri

Acknowledgement

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 Genomic, 11, 438.

AVISE J.C. (2004): Molecular Markers, Natural History, and Evolution (Second Edition). Sinauer, Sunderland, MA. (684pp.)

BAI X., WU X., WANG X., GUAN Z., GAO F., YU J., YU L., TANG B., LIU X., SONG Y., WANG X., RADU B., BOIREAU P., WANG F., LIU M. (2012): Regulation of cytokine expression in murine macrophages stimulated by excretory/secretory products from Trichinella spiralis in vitro. Molecular and Cellular Biochemistry, 360, 79-88.

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.

BUISSON D., LAGRIFFOUL G., BALOCHE G., AGUERRE X., BOULENC P., FIDELE F., FRÉGEAT G. , GIRAL-VIALA B., GUIBERT P., PANIS P., SOULAS C., ASTRUC J.M., BARILLET F. (2014): Genomic breeding schemes in French Lacaune and Manech dairy sheep: design and expected genetic gain. Proceedings, 10th World Congress of Genetics Applied to Livestock Production http://www.wcgalp.org/system/files/proceedings/2014/genomic-breeding-schemes-french-lacaune-and-manech-dairy-sheep-design-and-expected-genetic-gain.pdf.

BURT D.W. (2005): Chicken genome: current status and future opportunities. Genome Research, 15 (12), 1692-1698.

CARILLIER C., HÉLÈNE LARROQUE H., **ROBERT-GRANIÉ C. (2014):** Comparison of joint versus purebred genomic evaluation in the French multi-breed dairy goat population. *Genetics Selection Evolution,* **46**:67.

CARILLIER C., LARROQUE H., PALHIERE I., CLEMENT V., RUPP R., ROBERT –GRANIE C. (2013): A first step toward genomic selection in the multi-breed French dairy goat population. Journal of Dairy Science, 96 (11): 7294-305.

CARILLIER, C., LARROQUE, H., ROBERT-GRANIÉ, C. (2015)*:* Comparison of multi-breed and within-breed genomic evaluation in French dairy goats. International Plant and Animal Genome, San Diego, USA, 2015-01-10-2015-01-15.

CARILLIER-JACQUIN C., LARROQUE H., ROBERT-GRANIÉ C. (2016): Including αs1 casein gene information in genomic evaluations of French dairy goats. *Genetics Selection Evolution*, 48:54 DOI 10.1186/s12711-016-0233-x.

CARO PETROVIĆ V., PETROVIĆ M.M., MAKSIMOVIĆ N., LAZAREVIĆ M., STANOJKOVIĆ A., MIĆIĆ N., MARINKOVIĆ M. (2017): Analysis of Beta-Lactoglobulin in Small Herd of Cattle by PCR-RFLP Trends and Challenges in the

Genetic Improvement of Farm Animals. Proceedings of the 11th International Symposium Modern Trends in Livestock Production October 11-13, 2017, 43-49.

CHEN L., CHENG B., LI L., ZHAN S., WANG L., ZHONG T., CHEN Y., ZHANG H. (2015): The molecular characterization and temporal-spartial expression of myocyte enhancer factor 2 genes in the goat and their association with myofiber traits.Gene 555(2): 223-230. doi: 10.1016/j.gene.2014.11.007.

CHRISTENSEN O.F., MADSEN P., NIELSEN B., SU G. 2014): Genomic evaluation of both purebred and crossbred performances. Genetics Selection Evolution, 46:23, 1-9.

CUNNINGHAM E.P., DOOLEY J.J., SPLAN R.K., BRADLEY D.G. (2001): Microsatellite diversity, pedigree relatedness and the contributions of founder lineages to Thoroughbred horses. Animal Genetics, 32, 360-364.

DEKKERS J.C.M. (2004): Commercial application of marker and gene assisted selection in livestock: strategies and lessons. Journal of Animal Science, 82, E313-E328.

DENISKOVA T.E., DOTSEV A.V., SELIONOVA M.I., KUNZ E., MEDUGORAC I., REYER H., WIMMERS K., BARBATO M., TRASPOV A.A., BREM G., ZINOVIEVA N.A.(2018): Population structure and genetic diversity of 25 Russian sheep breeds based on whole-genome genotyping. *Genetics Selection Evolution,* 50:29, 1-16.

DU R., AN X.R., CHEN Y. F., QIN J. (2007): Some motifs were important for myostatin transcriptional regulation in sheep (Ovis aries). Journal of biochemistry and molecular biology, 40 (4): 547-553. PMID: 17669271.

EDEA Z., DESSIE T., DADI H., DO K.-T., KIM K.-S. (2017): Genetic Diversity and Population Structure of Ethiopian Sheep Populations Revealed by High-Density SNP Markers. Frontiers in Genetics, 8 (18), 1-14.

GARCIA-GAMEZ E., SAHANA G., GUTIERREZ-GIL B., ARRANZ J.J. (2012): Linkage disequilibrium and inbreeding estimation in Spanish Churra sheep. BMC Genetics., 13:43. https://doi.org/10.1186/1471-2156-13-43.

GRAS, MA, PISTOL, GC, PELMUS, RS, LAZAR, C, GROSU, H, & GHITA, E. (2016): Relationship between gene polymorphism and milk production traits in Teleorman Black Head sheep breed. Revista MVZ Córdoba, 21(1), 5124-5136.

HALEY C.S., VISSCHER P.M. (1998): Strategies to utilize marker – quantitative trait loci associations. Journal of Dairy Science, 81 (2): 85-97.

HAYES B.J., BROWMAN P.J., CHAMBERLAIN A.C.,VERBYLA K., GODDARD M.E. (2009): Accuracy of genomic breeding values in multi-breed dairy cattle population. Genetics Selection Evolution., 41 (1): 51.

HAYES B.J., LEWIN H.A., GODDARD M.E. (2012): The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity , and adaption. Trends in Genetics., 29, 206-214.

INTERNATIONAL SHEEP GENOMICS CONSORTIUM, 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): Animal Genetics, 41 (5): 449-53. doi: 10.1111/j.1365-2052.2010.02100.x.

JEVŠINEK SKOK D., ŠALAMON D., DŽIDIĆ A., BOJKOVSKI D., SIMČIČ M. (2015): Genetic diversity of Bela Krajina Pramenka compared to three Croatian sheep breeds – a preliminary study. Poljoprivreda 21, (1) Supplement, 142-145.

KIJAS J.W., TOWNLEY D., DALRYMPLE B.P., HEATON M.P., MADDOX J.F., et al. (2009): A Genome Wide Survey of SNP Variation Reveals the Genetic Structure of Sheep Breeds. PLoS ONE 4(3): e4668. doi:10.1371/journal.pone.0004668.

KIJAS JW, LENSTRA JA, HAYES B, BOITARD S, PORTO NETO LR, SAN CRISTOBAL M, et al. (2012) Genome-Wide Analysis of the World's Sheep Breeds Reveals High Levels of Historic Mixture and Strong Recent Selection. PLoS Biol 10(2): e1001258. https://doi.org/10.1371/journal.pbio.1001258.

KIM E-S., ELBELTAGY A.R., ABOUL-NAGA A.M., RISCHKOWSKY B., SAYRE B., MWACHARO J.M., ROTHSCHILD M.F. (2016): Multiple genomic signatures of selection in goats and sheep indigenous to a hot arid environment. Heredity, 116, 255–264.

LANDE R., THOMPSON R. (1990): Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics vol.124, 743-756.

MARTIN P., PALHIÈRE I., MAROTEAU C., BARDOU P., CANALETABET K., SARRY J., WOLOSZYN F., BERTRAND-MICHEL J., RACKE I., BESIR H., RUPP R., TOSSER-KLOPP G. (2017): A genome scan for milk production traits in dairy goats reveals two new mutations in Dgat1 reducing milk fat content. Scientific reports, 7:1872 doi:10.1038/s41598-017-02052-0.

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.

MOXON E.R., WILLS C. (1999): DNA Microsatellites: Agents of Evolution? Scientific American, 72-77.

MURITAL I., AFOLAYAN O., BEMJI M.N., DADI O.1, LANDI V., MARTÍNEZ A., DELGADO J.V., ADEBAMBO O.A., AINA A.B.J., ADEBAMBO A.O. (2015): Genetic diversity and population structure of Nigerian indigenous goat using DNA microsatellite markers. Archivos de Zootecnia. 64 (246): 93-98.

NAN LIU , HEGANG LI, KAIDONG LIU, JUANJUAN YU, RAN BU, MING CHENG, WEI DE, JIFENG LIU, GUANGLING HE JINSHAN ZHAO (2014): Identification of skin-expressed genes possibly associated with wool growth regulation of Aohan fine wool sheep. BMC Genetics, 15:144 https://doi.org/10.1186/s12863-014-0144-1.

OCAMPO R., CARDONA H., MARTÍNEZ R. (2016): Genetic diversity of Colombian sheep by microsatellite markers. Chilean Journal of Agricultural Research 76(1) 40-47.

OLLIVIER L. (1998): The accuracy of marker-assisted selection for quantitative traits within populations in linkage equilibrium. Genetics vol.148, 1367-1372.

PETROVIC P.M. (2000): Genetika i oplemennjivanje ovaca (Monografija). Naucna Knjiga. Beograd, 365 pp.

PETROVIC P.M., CARO PETROVIC V., RUZIC MUSLIC D., MAKSIMOVIC N., CEKIC B., ILIC Z. Z , KURCUBIC V. (2017a): Strategy for Sustainable Development and Utilization of Sheep and Goat Resources in Serbia. KnE Life Sciences, pages 11–21. DOI 10.18502/kls.v2i6.1015

PETROVIC P.M., PANTELIC V. (2015): Modern selection on farm animals /in Serbian/. Institut za stočarstvo, Beograd-Zemun,320pp.

PETROVIC P.M., RUŽIC MUSLIC D., CARO PETROVIC V., MAKSIMOVIC N., CEKIC B., YULDASHBAEV A.Y., SELIONOVA M.I. (2017b): Trends And Challenges in the Genetic Improvement of Farm Animals. Proceedings of the 11th International Symposium Modern Trends in Livestock Production October 11-13, 2017 pages 1-14.

PETROVIĆ P.M., ŽUJOVIĆ M., NEGOVANOVIĆ D., STRSOGLAVEC S., RUZIĆ D. (2001): The importance of new selection methods in modern system of sheep breeding. International Symposium. Biotechnology in Animal Husbandry 17 (5-6), 159-167.

RUPP R., MUCHA S., LARROQUE H., MCEWAN J., CONINGTON J. (2016): Genomic application in sheep and goat breeding. Animal Frontiers, 6,1, 39-44. doi:10.2527/af.2016-0006.

SASSI-ZAIDY Y. B., MARETTO F., CHARFI-CHEIKHROUHA F., MOHAMED-BRAHMI A., CASSANDRO M. (2016): Contribution of microsatellites markers in the clarification of the origin, genetic risk factors, and implications for conservation of Tunisian native sheep breeds. Genetics and Molecular Research 15 (1): 1-11. gmr.15017059.

SEILSUTH S., SEO J.H., KONG H.S., JOO JEON A.G. (2016): Microsatellite Analysis of the Genetic Diversity and Population Structure in Dairy Goats in Thailand. Asian-Australasian Journal of Animal Science, 29 (3): 327–332.

SELEPE MM, CECCOBELLI S, LASAGNA E, KUNENE NW (2018): Genetic structure of South African Nguni (Zulu) sheep populations reveals admixture with exotic breeds. Plos One 13(4): e0196276. https://doi.org/10.1371/journal.pone.0196276.

SHCHERBATOV V.I., ACES I.N., DIKAREV A.G., MUZYKANTOVA L.V. (2013): Methods for integrated assessment and early diagnosis of agricultural livestock productivity. FGBOU VPO "Kuban State Agrarian University", 293 pp.

SONG-SONG X., MENG-HUA L. (2017) : Recent advances in understanding genetic variants associated with economically important traits in sheep (*Ovis aries*)

revealed by high-throughput screening technologies. Frontiers of Agricultural Science and Engineering, 4(3): 279-288. doi: 10.15302/J-FASE-2017151.

SOUHEIL GAOUAR S.B., SAMIAKDIDI, L. O. (2016): Estimating population structure and genetic diversity of five Moroccan sheep breeds by microsatellite markers. Small Ruminants, volume 144, 23-27. *Abstract.*

SPEHAR M. (2013): Genetsko vrednovanje i uvođenje genomske selekcije u govedarstvo Republike Hrvatske. Mljekarski list, 50, 6, 8-11.

SUÁREZ-VEGA A., GUTIÉRREZ-GIL B., KLOPP C., ROBERT-GRANIE C., TOSSER-KLOPP G., JOSÉ ARRANZ J. (2015): Characterization and Comparative Analysis of the Milk Transcriptome in Two Dairy Sheep Breeds using RNA Sequencing. Scientific Reports | 5:18399 | DOI: 10.1038/srep18399

TENEVA A., DIMITROV K., CARO PETROVIC V., PETROVIC P.M., DIMITROVA I., TYUFEKCHIEV N., PETROV N. (2013): Molecular genetics and ssr markers as a new practice in farm animal genomic analysis for breeding and control of disease disorders. Biotechnology in Animal Husbandry, 29, 405-429.

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

TRUKHACHEV V., BELYAEV V., KVOCHKO A., KULICHENKO A., KOVALEV D., PISARENKO S., VOLYNKINA A., SELIONOVA M., AYBAZOV M. , SHUMAENKO S., OMAROV A., MAMONTOVA T., YATSYK O., KRIVORUCHKO A., PETROVIC P.M., PANTELIC V., CARO PETROVIC V. (2016): MEF2B Gene SNP Markers of Meat Productivity in Severokavkazskaya Sheep Breed. Genetika, Vol. 48, No.1, 97-108.

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.

WEBER J.L., MAY P.E. (1989): Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. American Journal of Human Genetics, 44,388-396.

XU Y., LI P., ZOU C., LU Y., XIE C., ZHANG X., PRASANNA B.M., OLSEN M.S.(2017): Enhancing genetic gain in the era of molecular breeding, *Journal of Experimental Botany*, Volume 68, Issue 11, 2641–2666, https://doi.org/10.1093/jxb/erx135.

YILMAZ O., SEZENLER T., SEVİM S., CEMAL I. (2015): Genetic relationships among four Turkish sheep breeds using microsatellites. Turk J Vet Anim Sci., 39: 576-582.

ZHANG L., LIU J., ZHAO F., REN H., XU L., LU J., ZHANG S., ZHANG X., WEI C., LU G., ZHENG Y., DU L.(2013): Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep. PLoS ONE 8(6): e66569. doi:10.1371/journal.pone.0066569.

ZHANG L., MA X., XUAN J., WANG H., YUAN Z., WU M., et al. (2016)

Identification of MEF2B and TRHDE Gene Polymorphisms Related to Growth Traits in a New Ujumqin Sheep Population. PLoS ONE 11(7): e0159504. doi:10.1371/journal. pone.0159504.

ZIMIN A., DELCHERA., FLOREA L., KELLEY D., SCHATZ M., PUIU D., ET AL. (2009): A whole genome   assembly of the domestic cow, Bos Taurus. Genome Biology, 10 (4), R42.

ZINOVIEVA N.A., SELIONOVA M.I.,   GLADYR E.A., PETROVIC P.M., CARO PETROVIC V., RUZIC MUSLIC D., PETROVIC M.M. (2015): Investigation of Gene Pool and Genealogical Links Between Sheep Breeds of Southern Russia by Blood Groups and Dna Microsatellites. Genetika, Vol. 47, No.2, 395-404.

INFLUENCE OF SOME FACTORS ON THE FERTILITY OF HYBRID SOWS

Ivelina Zapryanova[*], Radka Malinova

Agricultural University, 12 Mendeleev Blvd., 4000 Plovdiv, Bulgaria
*Corresponding author: ivelina_z@abv.bg
Original scientific paper

Abstract: The purpose of this study was to establish the influence of the boar and the age category of sows as factors for the fertility of hybrid sows ((Tai Zumu x Landrace) x Large White). The mother is a reliable source of variation on the number of the alive pigs ($p<0.05$) and the total number of the delivered ones in a farrow ($p<0.01$). The difference between the gilts and the sows is significant in the number of the alive and the total number of pigs in a farrow ($p<0.05$). The total number of delivered pigs from the sows are within the range of 10.6 ± 0.4 to 11.4 ± 1.2, while with the gilts ones they are from 8.9 ± 0.6 to 11 ± 1.8. High positive correlation was established between the live-born pigs and the total number of pigs born by gilts (rp= 0.858 $p<0.01$), as well as with the next parity (rp= from 0.702 to 0.861, $p<0.01$). The connection between the stillborn and the total number of born pigs, we reported a moderate and reliable correlation, both with the gilts (rp= 0.381, $p<0.01$) and the sows (rp = from 0.408 to 0.584, $p<0.01$)

Key words: litter size, alive and stillborn piglets, correlation, reproduction, prolificacy, gilts, sows

Introduction

The size of the farrow is one of the basic factors characteristic for the fertility of pigs and has a priority significance for pig farming. The increased number of delivered pigs by sow per year leads to reduction of production costs which results in the increased efficiency of pig farming.

A number of conditions like fertilization season, age, and sequence of farrowing of the mothers, etc., have influence both on the total number of delivered pigs and on the alive and stillborn pigs. The size of the farrow can increase in generation F1 as a result from the heterosis effect of crossing breeds and lines *(Pusic et al., 2014).*

The significance of male breeders in pig farming, especially after the widely-spread introduction of artificial insemination, is undeniable. However, the efficiency of production is significantly influenced by the reproduction ability of

the breeders because not all boars and their ejaculates have identical fertilizing abilities. Regardless of the mass application of analyses for defining the quality of semen, they usually turn out to be insufficient for the prediction of the relative fertility of male breeders *(Flowers 1997; Alm et al. 2006).*

The purpose of this study was to follow the influence of some factors on the fertility of gilts and sows, as well as to establish the relation between the basic indicators of the litter of hybrid sows.

Materials and Methods

The study included a total of 843 litters of the hybrid 'Y' ((Tai Zumu x Landrace) x Large White), for the period of 2010-2017, and bred in a pig farm located in the region around the town of Plovdiv, Bulgaria, under extensive conditions of breeding.

The stock was inseminated twice during the estrus, with sperm of imported terminal boars 'D' (Large White x Pietrain). The first insemination was not done earlier than the time of the first registered estrus after reaching sexual maturity.

The following indicators were studied: the average total number pigs (LS±SE); the average number of live pigs (LS±SE), and the average number of stillborn pigs (LS±SE), with both age categories – gilts and sows, depending on the boars.

During the course of the study, the influence of the father and mother as factors, influencing the fertility of pigs was analyzed, as well as that of the age category of female breeders (gilts and main sows) on the studied indicators.

When processing the data and establishment of the influence of some factors on the studied traits we used univariate (Model 1,2..5) and multivariate dispersion analysis (Model 6 and 7) as the linear model had the following statistical type:

$Y_{ijk} = \mu + S_i + A_j + e_{ijk}$

$Y_{ijk} = \mu + S_i + A_j + CS_{ij} + e_{ijk}$,

where: Y_{ijk} – observation vector; μ - overall average constant; S_i, A_j are fixed effects corresponding to the boar (i=7); age class (j=2); CS_{ij} is random effect of interaction boar* age class; e_{ijk}..- residual variance.

In data processing, we used a multi-factor dispersion analysis and Pearson's coefficient of correlation was used for bivariate correlation analysis, and were performed with SPSS software product version 24.

Results and Discussion

Table 1 shows that the mother is a reliable source of variation between the number of live ($p<0.05$) and the total number of pigs in a farrow ($p<0.01$). The difference between gilts and sows is significant only in the number of stillborn and the total number of pigs in a farrow ($p<0.05$).

The season of fertilization of pigs does not have any influence on the number of dead ($p<0.001$), and the live ($p<0.01$) pigs, while the year – on the number of the dead pigs in a farrow ($p<0.05$).

A number of indicators characterizing the reproduction ability of pigs depend on environmental factors. The season, the temperature, and the duration of daytime, have influence not only on the sexual cycle and the estrus, but also on the fertilization of pigs *(Nikolova, 2012)*. In her studies with pigs of Danube White and Landrace breed, the author established reliably better reproduction indicators with the animals fertilized and farrowed during the winter compared to the summer months of the year.

In our previous research, we established that the month and season of insemination influences reliably the number of the pigs born ($p<0.01$ and $p<0.001$, respectively) and the farrowing interval ($p<0.01$ and $p<0.05$, respectively). The smallest number of pigs are born after insemination in the summer – June, July, August, and the pigs inseminated in the winter have the most numerous farrowings *(Zapryanova, 2017)*

Table 1. The effect of some factors on the number of piglets at birth

F-criterion and degree of reliability				
Model	Factors	Traits		
		Alive piglets	Stillborn piglets	Total number of piglets
1	Boar	1.156	0.905	0.683
2	Mother	1.272*	0.840	1.465**
3	Season of fertilization	4.125**	6.457***	1.897
4	Year of fertilization	1.592	2.316*	1.479
5	Age category of sows	3.54*	2.16	7.05**
6	Boar	1.067	0.913	0.578
	Age category of sows	2.963*	2.189	6.298*
7	Boar	1.049	0.759	0.79
	Age category of sows	1.079	2.204	4.06*
	Boar*Age category of sows	0.519	1.044	0.58
***P<0,05, **P<0,01, ***P<0,001**				

One of the main goals of selection of pigs is achieving of more numerous farrows. However, there is a negative dependency between the number and the live mass of the animals, especially those, bred in less favourable conditions *(Nogaj et al., 2006)*. At the same time, *Rydhmer (1992)* established that the weight at birth influences the survival rate of the pigs in the first weeks of their life.

The number of alive born pigs in a litter determines significantly the number of animals for slaughtering, which is why this indication has too big economic significance. According to *Dimitrov (2015)* gilts and the ones after the sixth farrow, as a rule, have smaller plurality farrows. *Legault (1983)* defines the capacity of the uterus, which is not large enough for the early pregnancies, as a reason for the smaller values of the examined indicators at the beginning of the reproductive life. In experiments with Swedish Landrace, Duroc sows and their crossbreeds *Pusic et al. (2014)* establish reliable increase of the number of the live-born pigs until the fifth farrow. In the same experiment, the authors find out that the sequence of farrowing has a significant effect on the indications of fertility, with the exception of the number of stillborn pigs in the farrow.

In the conditions of the conducted experiment, the smallest number of animals is born by the gilts, fertilized by father No. 985 – 8.8±1.3 pigs, and the highest is from father No. 995 – 11±1.8 pigs, who is with the highest fertility among the main sows (11.4±1.2) (Fig.1). We report the biggest difference (2.1 pigs) between the two categories of sows at their fertilization with boar No. 985, and the smallest one – with fathers No. 943 and No. 982. The highest number of live born pigs are in the farrows of the sows, inseminated by boar 982- 10.5±0.7 piglets, and the smallest number in the farrows with father No. 995 (8.9±0.9).
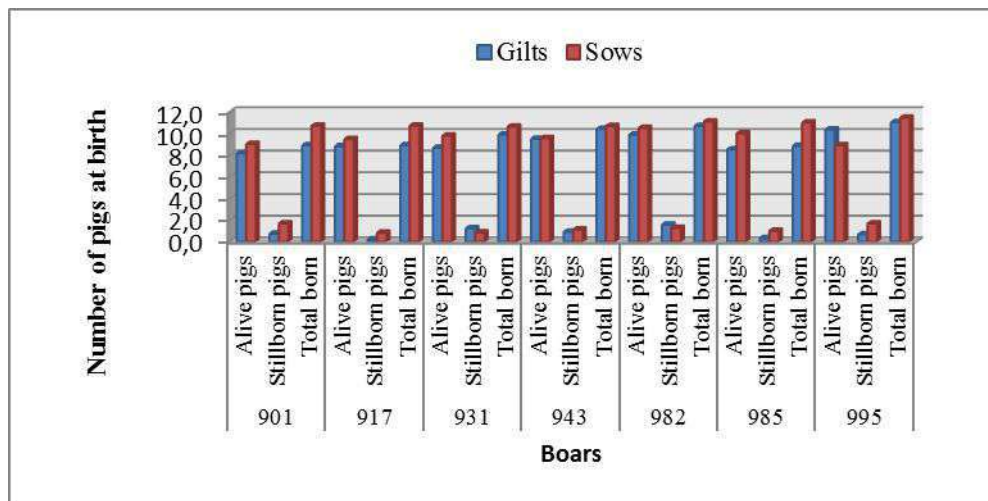
Figure 1. Litter size of gilts and sows depending on the boar

The effectiveness of pig-farming production depends on a number of factors, some of which refer to the reproduction ability of the male breeders, because not all boars and their ejaculates have the same fertilizing ability, and also on the number of live-born, stillborn and total number of pigs in a farrow

Figure 2 shows the distribution of the pigs in categories depending on their father. Highest total fertility is displayed by father No. 995 (11.3±0.9), but the level of stillborn pigs is too high as well (1.4±0.5 pigs). The difference between it and the boar with the lowest total number of piglets (No.917) is 1.6 pigs, and in the category of the stillborn ones the difference is almost 1 pig. The highest number of live pigs is in the litter of boar No. 982 (10.3±0.6), followed by the fathers with numbers 985 and 943- 9.7±0.5 and 9.5±0.3 pigs, respectively.
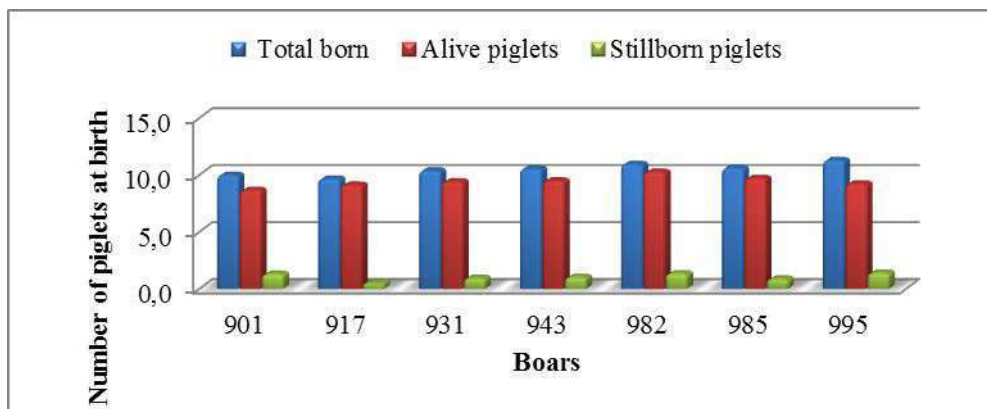
Figure 2. Distribution of pigs by category according to the boars

Table 2. Phenotypic correlation between the traits of litter size depending on the parity number

Parity number	Traits	Alive piglets	Stillborn piglets
First parity (n=166)	Stillborn piglets	-0.148	-
	Total number of piglets	0.858**	0.381**
Second parity (n=169)	Stillborn piglets	-0.233**	-
	Total number of piglets	0.790**	0.412**
Third parity (n=154)	Stillborn piglets	-0.114	-
	Total number of piglets	0.861**	0.408**
Fourth parity (n=204)	Stillborn piglets	-0.130	-
	Total number of piglets	0.822**	0.457**
Fifth parity (n=116)	Stillborn piglets	-0.005	-
	Total number of piglets	0.809**	0.584**
Sixth parity (n=34)	Stillborn piglets	-0.219	-
	Total number of piglets	0.702**	0.584**

**p<0.01

Without a doubt, the reproductive process is the most important process for continuation and refinement of species. The improvement of the reproduction abilities of the pigs is essential because it affects the number of the delivered pigs and their productive potential *(Miclea et al. 2007, 2009)*. Pig fertility is a main characteristic of this type of animals, which, together with the high growth intensity, defines the effectiveness of pig-farming production. According to *Radović et al. (2016)* the fertility of the pigs depends on a number of indicators, which can be divided to reproductive indicators and indicators, related to the farrow. The authors assign the age of the first oestrus and insemination to the first group, as well as the age of the first farrow, and to the second group – size and mass of the farrow at birth and at weaning.

Table 2 shows the phenotype correlations between the traits of the litter size at the first and the following parity. There is a high positive correlation between the live-born pigs and the total number of pigs born by gilts (rp= 0.858 p<0.01), as well as with the next parity (rp= from 0.702 to 0.861, p<0.01). The values of the phenotype correlation between the total number of born ones and the number of live-born pigs in the conditions of our experiment are lower, but in a unison with what *Lukač et al. (2016); Radović et al. (2016),* and others found.

Regarding the connection between the stillborn and the total number of born pigs, we reported a moderate and reliable correlation, both with the gilts (rp= 0.381, p<0.01) and the sows (rp = from 0.408 to 0.584, p<0.01). The correlation between stillborn and piglets born alive is negative and not reliable, with the exception of the significant value between these traits on second parity (rp= -0.233, p<0.01).

Conclusion

The mother is a reliable source of variation on the number of the live pigs (p<0.05) and the total number of the delivered ones in a farrow (p<0.01).

The difference between the gilts and the sows is significant in the number of the live and the total number of pigs in a farrow (p<0.05). The total number of delivered pigs from the sows are within the range of 10.6±0.4 to 11.4±1.2, while with the gilt ones they are from 8.9±0.6 to 11±1.8.

High positive correlation was established between the live-born pigs and the total number of pigs born by gilts (rp= 0.858 p<0.01), as well as with the next parity (rp= from 0.702 to 0.861, p<0.01). The connection between the stillborn and the total number of born pigs, we reported a moderate and reliable correlation, both with the gilts (rp= 0.381, p<0.01) and the sows (rp = from 0.408 to 0.584, p<0.01).

Uticaj nekih faktora na plodnost hibridnih krmača

Ivelina Zapryanova, Radka Malinova

Rezime

Cilj ovog istraživanja bio je utvrđivanje uticaja nerasta i starosne kategorije krmača kao faktora plodnosti hibridnih krmača (Tai Zumu x landras) x jorkšir). Majka je pouzdan izvor varijacije na broj živorođene prasadi (p <0,05) i ukupan broj u leglu (p <0,01). Razlika između nazimica i krmača je značajna u broju živorođenih i ukupnom broju svinja u leglu (p <0,05). Ukupan broj prasadi od krmača je u opsegu od $10,6 \pm 0,4$ do $11,4 \pm 1,2$, a kod nazimica od $8,9 \pm 0,6$ do $11 \pm 1,8$. Uspostavljena je visoka pozitivna korelacija između živorođene prasadi i ukupnog broja prasadi nazimica (rp = 0,858 r <0,01), kao i sa sledećim paritetom (rp = od 0,702 do 0,861, p <0,01). Između broja mrtvorođenih i ukupnog broja prasadi, zabeležili smo umerenu i pouzdanu korelaciju, kao i kod nazimica (rp = 0.381, r <0.01) i krmača (rp = od 0.408 do 0.584, p <0.01)

Ključne reči: veličina legla, živo i mrtvo rođena prasad, korelacija, reprodukcija, plodnost, nazimice, krmače

References

ALM K., PELTONIEMI O.A., KOSKINEN E., ANDERSSON M. (2006): Porcine field fertility with two different insemination doses and the effect of sperm morphology. Reprod Domestic Animal, 41, 210–213.
DIMITROV S. (2015): Relationship between litter size and parity number with gestation length of sows. Bulgarian Journal of Animal Husbandry. LII, 6, 3-6 (Bg)
FLOWERS WL. (1997): Management of boars for efficient semen production. Journal of Reproduction and Fertility Supplement, 52, 67–78.
LUKAČ D., VIDOVIĆ V., VASILJEVIĆ T., STANKOVIĆ O. (2016): Estimation of genetic parameters and breeding values for litter size in the first three parity of Landrace sows. Biotechnology in Animal Husbandry, 32, 3, 261-269.
LEGAULT C. (1983): Breeding for larger litters in swine. In: Pork Industry Conference, University of Illinois. Urbana
MICLEA V., ZAHAN M., MICLEA I., VAJDA I. (2007): Influence of harvest frequency on the quality of boar semen. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 63-64: 95-98.

MICLEA V., ZAHAN M., ROMAN I., MICLEA I., NEGRESCU B. (2009): The influence of sow age on gestation duration and number of piglets. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 66, (1-2), 90-93.

NIKOLOVA T. (2012): Influence of season on some reproductive traits in group-housed Danube white and Landrace sows. Bulgarian Journal of Animal Husbandry. XLIX, 1, 30-35 (Bg).

NOGAJ J., JARCZYK A., KOWALEWSKI D. (2006): The effect of selected factors on litter and piglet weight at the age of 21 days. Animal Science Papers and Reports, 24. Supplement 1, 93-101.

PUSIC M., RADISIC R., POPOVIC N.,. BESKOROVAJNI R., TOLIMIR N. (2014): The effect of genotype and the number of farrowing on sow fertility traits. Proceedings. Fifth International Scientific Agricultural Symposium „Agrosym 2014", 874-878.

RADOVIĆ Č., PETROVIĆ M., BRKIĆ N., PARUNOVIĆ N., RADOJKOVIĆ D., SAVIĆ R., GOGIĆ M. (2016): Correlation of litter size traits. Biotechnology in Animal Husbandry, 32 (4), 331-339.

RYDHMER L. (1992): Relations between piglet weights and survival. Animal Production, 15, 183-184.

ZAPRYANOVA I. (2017): Study of the traits, characterizing the reproductive activity of hybrid sows. Proceedings of Scientific conference with international participation animal science „Challenges and innovations" 1 – 3 November, Sofia, Bulgaria, 243-250.

THE QUALITY OF PORK HAM - TISSUE YIELD DEPENDING ON INDIVIDUAL FACTORS

Čedomir Radović[1*], Marija Gogić[1], Nenad Parunović[2], Dragan Radojković[3], Radomir Savić[3], Aleksandar Stanojković[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
[3]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000, Belgrade, Serbia
[*]Corresponding author: cedomirradovic.izs@gmail.com
Original scientific paper

Abstract: The study included the progeny of three boar-sires breeds (SL - Swedish Landrace; LW – Large White and P - Pietrain). A total of 201 progeny of both sexes (93 female and 108 male castrated animals), originating from 16 boar-sires, were tested. The study included the progeny of 10 SL boar-sires (sires nuRWer: 1, 2, 3, 7, 8, 9, 15, 16, 17 and 18), progeny of 3 LW sires (sires nuRWer: 4, 5 and 6) and 3 P boar-sires (sires nuRWer 14, 19 and 20), born in four seasons (winter, spring, summer and autumn). Studies have shown that, with an mean weight of a warm carcass side of 81.20 kg, the highest mean values for ham weight (RW; 10.456 kg), mass of intermuscular fatty tissue (RINT; 0.477 kg), ham bone (RB; 0.837 kg) and muscle tissue RMT, 7,939 kg) have progeny of the sires of Pietrain breed (P) compared to SL and LW sires. In comparison to animals sired by SL and LW boars, the progeny of P sires had less skin and subcutaneous fat tissue (RSFT) by 30 and 549 grams. Studies have shown that we have progeny of sires 7 and 9 of SL breed which have the lowest LSMean values for the yield of skin and subcutaneous fat tissue (869 and 876 g), which is below the mean for breed by 364 and 357 g. In addition, when it comes to intermuscular fatty tissue, the lowest established value was recorded in the progeny of sire no. 8 of SL breed (182 g), which is by 220 g less than the general mean and by 132 g below the mean of the sire breed. The animals originating from sires n. 19 and 20 showed the highest weight of muscle tissue (RMT) (8.489 and 8.118 kg) in the ham, which is by 2.853 and 2.482 kg more meat compared to the progeny of sire no. 5 of LW breed. The total weight of the ham and the ham muscle yield were influenced by ($P < 0.01$ and $P < 0.001$) sire breed, sires within the breed, gender and season of birth. A very significant ($P < 0.001$) influence of the weight of warm carcass sides on the ham weight and tissue yield was determined.

Key words: breed, sire, sex, birth, progeny

Introduction

The quality traits of pig carcass sides and pig meat varied under the influence of a large number of factors. The influence of genetic and non-genetic factors (genotype of sire, sire, sex, and farm) on quality of carcass side was investigated by *Petrović et al. (2006).* They have found, in two examined farms, that the sire, sex and the weight of the warm carcass side affected (P <0.01) the thickness of the bacon, the yield and the proportion of muscle tissue in the carcass sides. Pigs, of all species of domestic farm animals, have the highest level of accumulation of fat in the carcass. In the newborn piglets, the content of fat tissue in the carcass is only about 2% and its share in the carcass increases with the increase of the age of the animal. In the course of life, pigs mostly accumulate subcutaneous fat tissue, which on mean accounts for 60 to 70% of the total fatty tissue in the body, the fat tissue of the body cavities makes up 10 to 15%, and intermuscularl fat 20 to 35%. The content of intramuscular fat tissue in most industrial pig genotypes is between 2.5 and 3.5% (*Karolyi, 2007*). Selection may influence the content of intramuscular fatty tissue, or the content of fat in meat (*Dević and Stamenković, 2004*). Statistically significant differences (P <0.001) for intramuscular fat content in ML (2.31: 3.32%) between the progeny of the Landrace and Duroc sires have been found in the study by *Mason et al. (2005).* In regard to the effect of sex on the quantity of fat tissue in the pig carcasse, *Čepin and Žgur (2003)* state that uncastrated male animals, compared to females, have a much lower percentage of fat under the same diet regime. They also state that the selection is a powerful tool for reducing fat content, and that adequate animal nutrition provides the ability to reduce fat content and fatty acid changes. Observing the share of certain tissues in the four primary parts of the carcass side (ham, loin, the shoulder and the rib part) in gilts and castrates, *Kušec et al. (2006)* have found that the highest proportion of muscle tissue is in the ham, with the highest proportion of fatty tissue in the rib part of the carcass, while the highest share of the bone tissue is found in the loin. By comparing the share of the main tissues of the basic carcass parts in the examined pigs, statistically significant differences between the sexes were not determined (*Kušec et al., 2006*). Distribution of fatty tissue, as well as the total amount of fat in the carcass, varies considerably between pigs of different breeds. For example, there is a clear difference in the content of intramuscular fat between different pig genotypes. It is generally higher in pig of Duroc breed than in Landrace or Large White pigs, although differences in the content of subcutaneous and abdominal fat tissue between pigs of different genotypes may be small. Distribution of the fat tissue in the pig carcass can be changed relatively easily through selection. Variations in energy intake through diet are the decisive factor in the distribution of fatty tissue, as well as the total amount of fat in the pig carcass within the appropriate genotype (*de Lange et al., 2003*). In animals intended for meat production, the estimated heritability for fat content is relatively high (between 0.3 and 0.6). This means that

the selection is a powerful tool for reducing fat content. An even better option for reducing the fat content and changing fatty acids is adequate nutrition, especially in non-ruminants, as these animals absorb fatty acids in unchanged form (*Čepin and Žgur, 2003*).

Materials and Methods

The study included the progeny of three breeds of sires (SL - Swedish Landrace, LW – Large White and P - Pietrain). A total of 201 progeny of both sexes (93 female and 108 male castrated animals) originating from 16 boar-sires, were tested. The test included the progeny of 10 SL sires (sires number 1, 2, 3, 7, 8, 9, 15, 16, 17 and 18), 3 LW sires (sires number: 4.5 and 6) and 3 P sires (sires number 14, 19 and 20). The minimum number of progeny per sire was 9. The examined animals were born in four seasons (winter, spring, summer and autumn). The ham was separated from the loin (loin-rump section) part and the belly part with a right angle cut in relation to the longitudinal axis between the 5th and 6th lumbar vertebrae and from the lower leg by a flat cut through the knee joint.
The cutting and separation of individual tissues of the ham is shown in Figure 1.

Figure 1. Separation and dissection of the ham (Photo: Č. Radović)

The tissues were separated by a knife, as precisely as possible. Removing the subcutaneous surface fatty tissue with the skin implied removing the entire fatty tissue over the outer muscle layers. After the subcutaneous fatty tissue was separated, the bone and cartilage parts were extracted and the smallest parts of the soft tissue were removed from them (with a knife). Fases, aponeuroses (tendons) were separated together with muscle tissue. In the intermuscle areas there is a lower or higher content of intermuscular fatty tissue. Intermuscular fatty tissue was removed by separation of muscle parts and individual muscles with the greatest possible precision. According to the set goal, the research was carried out on the farm, experimental slaughterhouse and in the laboratory of the Institute for Animal Husbandry, Zemun-Belgrade. Data was processed by applying the adequate software package "LSMLMW and MIXMDL, PC-2 VERSION" (Harvey, 1990), i.e. by using the procedure of the Least Square Method in order to determine the significance (P<0.05) of systematic influences on traits of meat quality. Model included: sire breed, sires within the breed, gender, gender within the breed, birth season of progeny and carcass side mass (linear effect).

$$Y_{ijklm} = \mu + R_i + O_{j:i} + P_k + P_{k:i} + S_l + b_3 (x_3 - \overline{x}_3) + \varepsilon_{ijklm}$$

where: Y_{ijklm} - expression of trait of m individual animal, of i boar breed, of j sire within i breed, of k sex and l birth season; μ = general population average, R_i – effect of sire breed (i=1, 2, 5); $O_{j:i}$ – effect of sire within the breed ($j:i_1$ =1, 2, 3, 7, 8, 9, 15, 16, 17, 18; $j:i_2$ =4, 5, 6; $j:i_3$=14, 19, 20); P_k – effect of sex/gender (k =1,2); $P_{k:i}$ – effect of sex/gender within the breed; S_l – effect of offspring birth season (l =1, 2, 3, 4); b_3 – linear regression effect of the warm carcass side weight (x_3); ε_{ijklm} – random error (residue).

Results and Discussion

Table 1 shows the variations in the total weight of the ham and the yield of individual tissues in it under the influence of the sire breed and sire within the breed. By correcting the total ham weight (RW) to the mean weight of the warm carcass side (WCSW = 81.20 kg), the progeny of (P) sires had the highest mean values for this trait (10,456 kg) compared to other sires. They also had the highest mean weight of intermuscular fatty tissue (RINT; 0.477 kg), of bones in the ham (RB; 0.837 kg) and muscle tissue (RMT; 7.939 kg). In relation to the animals originating from Swedish landras (SL) and Large White (LW) sires, progeny of (P) sires had less skins and subcutaneous fat tissue (RSFT) by 30 and 549 grams. The lowest LSMean values for the yield of skin and subcutaneous fat tissue (RSFT) were recorded for the progeny of sires no. 7 and 9, of the SL breed (869 and 876

g), which is less in relation to the breed mean by 364 and 357 g. In addition, when it comes to intermuscular fatty tissue, the lowest established value was recorded for progeny of sire no. 8 of SL breed (182 g), which is by 220 g less than the general mean and 132 g less than the mean for the sire breed. The animals originating from sires n. 19 and 20 had the highest amount of muscular tissue (RMT) (8.489 and 8.118 kg) in the ham, which is by 2.853 and 2.482 kg more meat compared to the progeny of sire no. 5 of breed LW. A very significant (P <0.001) influence of the weight of warm carcass sides on the weight of the ham and tissue yield was determined.

Table 1. The influence of the sire breed and sire within the breed on the quality of the ham/leg in progeny (LSMean ± S.E.)

Source of variation		RW[2]. kg	RSFT. kg	RINT. kg	RB. kg	RMT.kg
μ ± S.E.		9.519±0.06	1.396±0.04	0.402±0.01	0.780±0.01	6.940±0.07
SB[1]	Sire no.					
Swedish Landrace	1	9.054±0.12	1.520±0.08	0.392±0.03	0.742±0.02	6.401±0.16
	2	8.911±0.13	1.183±0.09	0.383±0.03	0.753±0.02	6.592±0.17
	3	8.654±0.13	1.472±0.09	0.447±0.03	0.756±0.02	5.979±0.17
	7	9.101±0.17	0.869±0.11	0.266±0.04	0.708±0.03	7.258±0.21
	8	9.189±0.16	1.187±0.10	0.182±0.04	0.730±0.03	7.089±0.20
	9	9.274±0.17	0.876±0.11	0.233±0.04	0.717±0.03	7.448±0.22
	15	9.227±0.16	1.406±0.10	0.388±0.04	0.831±0.03	6.602±0.20
	16	9.341±0.15	1.347±0.10	0.302±0.04	0.796±0.02	6.896±0.19
	17	9.400±0.15	1.123±0.10	0.279±0.03	0.802±0.02	7.196±0.19
	18	9.303±0.14	1.344±0.09	0.272±0.03	0.817±0.02	6.869±0.18
	Mean	9.145±0.07	1.233±0.05	0.314±0.02	0.765±0.01	6.833±0.09
Large White	4	9.065±0.14	1.506±0.09	0.388±0.03	0.753±0.02	6.418±0.17
	5	8.876±0.14	2.060±0.09	0.433±0.03	0.747±0.02	5.636±0.17
	6	8.925±0.13	1.691±0.09	0.423±0.03	0.717±0.02	6.094±0.17
	Mean	8.955±0.09	1.752±0.06	0.415±0.02	0.739±0.01	6.049±0.11
Pietrain	14	9.550±0.10	1.230±0.06	0.312±0.02	0.801±0.02	7.208±0.12
	19	10.937±0.18	0.975±0.12	0.579±0.04	0.894±0.03	8.489±0.23
	20	10.880±0.18	1.405±0.12	0.539±0.04	0.817±0.03	8.118±0.23
	Mean	10.456±0.11	1.203±0.07	0.477±0.03	0.837±0.02	7.939±0.14
WCSW (b)		0.111***	0.027***	0.004***	0.007***	0.074***

[1]SB-sire breed, WCSW (b)- linear effect of the weight of warm carcass side (WCSW=81,20 kg); [2] RW- the weight of the ham, RSFT– the weight of the skin and subcutaneous fat tissue of the ham, RINT- the weight of the intermuscular fat tissue of the ham, RB- the weight of bones of the ham, RMT- the weight of muscles of the ham

By observing according to sexes (Table 2), it can be seen that higher LSM values were recorded for RW (+ 293g, P <0.001), RB (+35g, P <0.01) and RMT (+568g; P <0.001) in female progeny relative to male animals, while lower values were determined for RSFT (-260 g, P <0.001) and RINT (-48 g, P <0.01). The highest meat yield (RMT) was recorded in animals born in winter and autumn (7.512 and 7.233 kg). These animals also had a higher RW (9.696 and 9.717 kg) compared to animals born in spring and summer. In our study, a higher proportion of muscle in the ham in the pure landrace breed (74.71%) was observed in relation to the value of 64.59% determined by *Kosovac et al. (2009)*. In this study of five genotypes, *Kosovac et al. (2009)* have found the highest value of muscular tissue in the progeny crosses sired by Pietrain breed 69.67%. The established proportion of muscle tissue in the ham in our study for the progeny of Pietrain breed sires was significantly higher and amounted to 75.92% of the muscle tissue in the total weight of the ham. Depending on sex, *Kušec et al. (2006)* have found a lower proportion of fatty tissue in the castrated male animals compared to the females (20.44: 21.93%), which is in contrast to our results (subcutaneous fat tissue in castrated males was 16.28%, in females 13.10%). By examining the different levels of threonine, *Barowicz et al. (2009)* have not recorded statistically significant impact on the weight of the ham (8.13 to 9.26 kg) nor on the share of meat in the ham, which was similar in all groups and varied from 72.82 to 73.85%. In our research, the proportion of meat in the ham was 72.91%.

Table 2. The effect of sex and season of birth on the quality of the ham in progeny (LSMean ±S.E.)

Sources of variation		RW[2]. kg	RSFT. kg	RINT. kg	RB. kg	RMT.kg
Sex	M[1]	9.372±0.07	1.526±0.04	0.426±0.02	0.763±0.01	6.656±0.08
	F	9.665±0.07	1.266±0.05	0.378±0.02	0.798±0.01	7.224±0.09
Saeason	Winter	9.696±0.19	1.076±0.13	0.321±0.05	0.787±0.03	7.512±0.24
	Spring	9.196±0.07	1.712±0.05	0.525±0.02	0.759±0.01	6.199±0.09
	Summer	9.466±0.09	1.471±0.06	0.401±0.02	0.776±0.01	6.817±0.11
	Autumn	9.717±0.08	1.325±0.05	0.361±0.02	0.799±0.01	7.233±0.10

[1]M- male castrates, F–females; [2] RW- the weight of the ham, RSFT– the weight of the skin and subcutaneous fat tissue of the ham, RINT- the weight of the intermuscular fat tissue of the ham, RB- the weight of bones of the ham, RMT- the weight of muscles of the ham

Table 3 shows the statistical significance of the factors on the tested properties. The total weight of the ham (RW) was influenced by the (P <0.001) breed of the sire, sires within the breed, sex, and the genotype of the animal, while the season showed significant influence (P <0.01). Other factors did not show significant influence (P> 0.05) on this trait. Factors included in the model (the impact of the sire breed, sires within the breed, sex, season of birth, sex within the

breed and the weight of the warm carcass side) explain 93.6% of the variability of the RW trait. All of the included factors showed significant impact on the yield of skin and subcutaneous fatty tissue (RSFT) as well as on muscle (RMT), (P <0.05; P <0.01 and P <0.001), only for the interaction of sex within the sire breed and sex within the genotype no influence was determined (P> 0.05). According to *Renaudau et al., (2005), Renaudeau and Mourot, (2007), Serrano et al., (2007); Radović et al., (2008),* the dependance of presence of muscular and fatty tissue on gender is in accordance with our research.

Table 3. Statistical significance (level of significance) of the effect of factors included in the model in the analysis of quality traits of the pork ham

Sources of variation (impact)[1]	RW[2]	RSFT	RINT	RB	RMT
RO	***[3]	***	***	***	***
O:SL	NS	***	***	***	***
O:LW	NS	***	NS	NS	**
O:P	***	**	***	**	***
Pol	***	***	**	**	***
Sezona	**	***	***	NS	***
Pol:RO	NS	NS	NS	NS	NS
WCSW (b)	***	***	***	***	***
R^2	0,936	0,693	0,668	0,658	0,830

[1]SB-sire breed, S:SL – sires within the breed Swedish Landrace; S:LW – sires within the breed Large White; S:P – sires within the breed Pietrain; Sex:SB-sex of the progeny within the sire breed; [2] RW- the weight of the ham, RSFT– the weight of the skin and subcutaneous fat tissue of the ham, RINT- the weight of the intermuscular fat tissue of the ham, RB- the weight of bones of the ham, RMT- the weight of muscles of the ham [3] NS=P>0,05; *=P<0,05; **=P<0,01; ***=P<0,001

Conclusion

With the mean weight of the warm carcass side of 81.20 kg, the highest mean values for the weight of the ham (RW; 10.456 kg), the weight of intermuscular fat tissue (RINT; 0.477 kg), the bones of the ham (RB; 0.837 kg) and muscle tissue (RMT; 7.939 kg) were recorded in the progeny of Pietrain sires (P) in relation to sires of other breeds. In comparison to the animals originating from the Swedish Landrace (SL) and Large White (LW) sires progeny of the (P) sires had less skin and subcutaneous fat tissue (RSFT) by 30 and 549 grams. The study showed that the progeny of SL sires no. 7 and 9, had the lowest LSMean values for the yield of skin and subcutaneous fat (869 and 876 g), which was less than the mean for the breed by 364 and 357 g. In regard to the intermuscular fatty tissue, the lowest established value was recorded in progeny of SL sire no. 8 rase (182 g), which was by 220 g less than the general mean and by 132 g less than the mean for

sire breed. The animals originating from sires no. 19 and 20 had the highest amount of muscular tissue (RMT) (8.489 and 8.118 kg) in the ham, which was by 2.853 and 2.482 kg more meat compared to the progeny of LW sire no. 5. The total weight of the ham and the muscle yield of the ham were influenced by (P <0.01 and P <0.001) sire breed, sires within the breed, sex and season of birth. A very significant (P <0.001) influence of the weight of warm carcass sides on the weight of the ham and tissue yield was determined.

Kvalitet buta-prinos tkiva u butu u zavisnosti od pojedinih faktora

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

Rezime

Ispitivanjem su bili obuhvaćeni potomci tri rase nerasta-očeva (SL - švedski landras; LW - veliki jorkšir i P - pijetren). Ukupno je ispitano 201 potomak oba pola (93 ženska i 108 muška kastrirana grla) koji su vodili poreklo od 16 očeva-nerasta. Ispitivanjem su bili obuhvaćeni potomci 10 nerasta rase SL (očevi broj: 1, 2, 3, 7, 8, 9, 15, 16, 17 i 18), 3 nerasta rase LW (očevi broj: 4,5 i 6) i 3 nerasta rase P (očevi broj: 14, 19 i 20) rođeni u četiri godišnje sezone (zima, proleće, leto i jesen). Istraživanja su pokazala da, pri prosečnoj masi tople polutke od 81.20 kg, najveće prosečne vrednosti za masu buta (RW; 10,456 kg), masu intermuskularnog masnog tkiva (RINT; 0,477 kg), kostiju buta (RB; 0,837 kg) i mišićnog tkiva (RMT; 7,939 kg) imaju potomci očeva rase pijetren (P) u odnosu na očeve rase SL i LW. U odnosu na grla koja potiču od očeva SL i LW potomci očeva rase P imala su manje kože i potkožnog masnog tkiva (RSFT) za 30 i 549 grama. Istraživanja su pokazala da imamo potomke očeva nerasta br.7 i 9, rase SL koji imaju najmanje LSMean vrednosti za osobinu prinosa kože i potkožnog masnog tkiva (869 i 876 g) što je manje u odnosu za prosek rase za 364 i 357 g. Pri tom, kada je reč o intermuskularnom masnom tkivu najmanju utvrđenu vrednost imali su potomci oca br. 8 rase SL (182 g), što je za 220 g manje od opšteg proseka i za 132 g od proseka rase očeva. Grla koja potiču od očeva br. 19 i 20 imala su najviše mišićnog tkiva (RMT) (8,489 i 8,118 kg) u butu što je za 2,853 i 2,482 kg više mesa u odnosu na potomke nerasta br. 5 rase LW. Na ukupnu masu buta i prinos mišića u butu uticali su (P<0,01 i P<0,001) rasa oca, očevi unutar rasa, pol i sezona rođenja. Utvrđen je vrlo visoko značajan (P<0,001) uticaj mase toplih polutki na masu buta i prinos tkiva.

Acknowledgment

References

ČEPIN S., ŽGUR S. (2003): Meat production technologies with reduced fat and cholesterol contents in meat. Tehnologija mesa, 44, 117-130.

BAROWICZ T., PIETRAS M., PIESZKA M., MIGDAŁ W., ŽIVKOVIĆ B. (2009): The effect of dietary synthetic threonine suplementation on fattening traits, slaughter traits and meat quality of fatteners. Biotechnology in Animal Husbandry, 25, 5-6, 871-877.

De LANGE C. F. M., MOREL H.C. P., BIRKETT H. S. (2003): Modelling chemical and physical body composition of the growing pig. Journal of Animal Science, 81, E. Suppl. 2, E159–E165.

DEVIĆ B., STAMENKOVIĆ T. (2004): Sadržaj intramuskularne masti u mišićima svinja. Tehnologija mesa, 45, 125-128.

KAROLYI D. (2007): Masti u mesu svinja. Meso, 6, 335-340.

KOSOVAC O., ŽIVKOVIĆ B., RADOVIĆ Č., SMILJAKOVIĆ T. (2009): Quality indicators: Carcass side and meat quality of pigs of different genotypes. Biotechnology in Animal Husbandry, 25, 3-4, 173-188.

KUŠEC G., ĐURKIN I., PETRIČEVIĆ A., KRALIK G., MALTAR Z. (2006): Utjecaj spola na distribuciju tkiva u svinjskim polovicama. Krmiva, 48, 131-142.

MASON M. L., HOGAN A. S., LYNCH A., O'SUSLIVAN K., LAWLOR G. P., KERRY P. J. (2005): Effects of restricted feeding and antioxidant supplementation on pig performance and quality characteristics of longissimus dorsi muscle from Landrace and Duroc pigs. Meat Science, 70, 307–317.

PETROVIĆ M., PUŠIĆ M., RADOJKOVIĆ D., MIJATOVIĆ M., KOSOVAC O., RADOVIĆ Č. (2006): The effect of breed, sire and sex on the quality of carcass sides. Biotechnology in Animal Husbandry, 22, 1-2, 79-88.

RENAUDEAU D., HILAIRE M., MOUROT J. (2005): A comparison of growth performance, carcass and meat quality of Creole and Large White pigs slaughtered at 150 days of age. Animal Research, 54, 43–54.

RENAUDEAU D., GIORGI M., SILOU F., WEISBECKER J. L. (2006): Effect of breed (lean or fat pigs) and sex on performance and feeding behaviour of group housed growing pigs in a tropical climate. Asian-Australasian Journal of Animal Science, 19, 4, 593–600.

RENAUDEAU D., MOUROT J. (2007): A comparison of carcass and meat quality characteristics of Creole and Large White pigs slaughtered at 90 kg. BW. Meat Science 76, 165–171.

RADOVIĆ Č., PETROVIĆ M., ŽIVKOVIĆ B., KOSOVAC O., PARUNOVIĆ N. (2008): The effect of different fixed factors on quality traits of pig carcass. Journal of Mountain Agriculture on the Balkans, 11, 4, 649-659.
SERRANO M. P., VALENCIA D. G., NIETO M., LAZARO R., MATEOS G. G., (2007): Influence of sex and terminal sire line on performance and carcass and meat quality of Iberian pigs reared under intensive production systems. Meat Science, 78, 420–428.

EFFECT OF GENOTYPE AND SEX ON CARCASS CHARACTERISTICS OF CHICKENS RAISED IN EXTENSIVE SYSTEM

Lidija Perić[1], Vladimir Strugar[2], Snežana Bogosavljević-Bošković[3], Mirjana Đukić Stojčić[4]

[1]University of Novi Sad, Faculty of Agriculture, Novi Sad
[2]NS Seme, Institute for Field and Vegetable Crops, Novi Sad
[3]University of Kragujevac, Faculty of Agronomy, Čačak
Corresponding author: lidija.peric@stocarstvo.edu.rs
Original scientific paper

Abstract: The aim of this work was to examine the possibility of using different poultry breeds for meat production in extensive systems, concerning their slaughter traits. Total of 200 birds of two genotypes were used in this trial: fast-growing hybrid Ross 308 and pure breed White Rock. Chicks were raised indoors until the age of 4 weeks and subsequently they were enabled to access the range. The trial lasted for 13 weeks. At the end of the trial, 10 male and 10 female chicks per breed were sacrificed for the evaluation of slaughter traits – dressing percentage, yield and share of carcass parts. It was established that both genotype and sex significantly affected carcass traits while genotype affected production traits. Chickens of the Ross 308 hybrid had higher body weights, better feed conversion ratio and higher mortality rate. Chickens of the Ross 308 hybrid had dressing percentage compared to the White Rock breed, while the male chickens had higher dressing percentage compared to the female ones. Chickens of the Ross 308 hybrid had a significantly larger weight and share of breast meat, whereas those of the White Rock breed had a larger share of drumsticks and thighs. A significant influence of the genotype was determined on the amount of abdominal fat in the carcass. Chickens of the White Rock breed had a significantly lower fat content compared to the Ross 308 hybrid, which essentially makes them the genotype which is more suitable for the extensive fattening longer than 10 weeks.

Key words: broilers, White Rock, sex, carcass traits, free-range

Introduction

Intensive production of poultry meat has led to a significant improvements of the production characteristics, but also to the deterioration of poultry welfare

and meat quality. This has further lead to the revitalization of the alternative systems which implies raising chickens on a free range during longer periods of time. Poultry meat from these systems seems to be more acceptable for the consumers, because the food safety and quality have become crucial for them *(Rodić et al., 2006; Bogosavljević-Bošković et al.,2012)*. The modern consumers demand meat which has a low percentage of fats, which is healthier and tastier than broiler meat *(Bogosavljević-Bošković et al., 2008; Castellini et al., 2008; Li et al., 2017)*.

The key factor which affects the production and slaughter traits of broilers is the genotype *(Berri et al., 2005)*. It is quite questionable whether the fast-growing hybrids are suitable for raising in alternative systems because they are exposed to greater challenges, particularly concerning their health *(Muhammad et al., 2017)*. Although the pure breeds are suitable for rearing in extensive systems, the problem is whether their relatively poor production traits could meet the demands of the market-oriented production. There is no precise data on how large the share of meat from the slow-growing genotypes on the European market is, but it is estimated that it ranges between 5 and 10% *(Forte et al., 2018)*. The available data for Serbia shows that over 65% of the total meat production originate from highly productive meat type hybrids *(Milošević et al., 2005)*.

Present research is conducted to compare the carcass characteristics of pure poultry breeds with those of fast-growing hybrids raised in extensive system.

Materials and methods

The trail was performed on the Experimental farm of Faculty of Agriculture, University of Novi Sad. The farm was adapted for a raising chickens in free range system. The total of 200 chickens of two genotypes were used in this trial: fast-growing hybrid Ross 308 (100 birds) and pure breed White Rock (100 birds). Chicks were raised in the poultry house until the age of 4 weeks (10 birds/m^2) and subsequently they were enabled to access the range covered with grass (1.5 m^2 of range/bird). The trial lasted for 13 weeks.

Chicks were fed for 2 weeks with starter feed, from 2-4 weeks with grower feed and until the end of the trial with finisher feed (Table 1). Feed and water were provided *ad libitum*.

Table 1. Composition of starter, grower and finisher diets

Ingredients, %	Starter	Grower	Finisher
Corn	51.00	53.50	65.50
Soybean meal	30.00	22.50	19.00
Sunflower meal	5.00	8.00	6.00
Yeast	4.00	-	-
Barley	-	5.00	-
Dehydrated alfalfa meal	2.00	3.00	3.00
Soybean oil	4.00	4.00	2.50
Dicalcium phosphate	2.00	2.00	2.00
Limestone	0.80	0.80	0.80
Salt	0.20	0.20	0.20
Premix	1.00	1.00	1.00
Total	100.00	100.00	100.00
Chemical composition			
Dry matter, %	89.78	89.70	89.26
Crude protein, %	22.12	19.11	16.68
Metabolizable energy, MJ/kg	12.384	12.466	12.704
Crude fat, %	6.17	6.33	4.99
Crude fibre, %	4.82	5.14	5.21
Ca, %	0.97	0.96	0.94
P (total), %	0.79	0.74	0.72

At the end of the trial, 10 male and 10 female chicks per breed were sacrificed for the evaluation of carcass traits – dressing percentage, and yield and share of carcass parts. Dressing percentages were evaluated as "standard processing" (whole carcass), "ready to roast" (carcass without head and edible giblets) and "ready to grill" (carcass without head, neck, legs and edible giblets). Thereafter, the dressed cold carcasses were dissected into primal cuts (breast, drumstick, thigh, wing and back).

Data were analyzed by ANOVA and means were separated by Duncan's post hoc test using StatSoft computer package (STATISTICA 13, 2014).

Results

The results showed a significant influence of genotype on production traits of birds. Chickens of hybrid Ross 308 achieved significantly (P<0.01) higher body weight compared to the White Rock chickens (4104 g vs. 1791 g). The feed conversion ratio was much higher in the White Rock breed (3.16) compared to the Ross 308 (2.54). On the other hand, mortality rate was only 4% for the White Rock and 7% for the Ross 308.

The influence of the genotype and sex on the processed carcass weight is presented in Table 2. It was determined that the influence of sex and genotype on the live weight of chickens and on processed carcass weight was highly significant (P<0.01). For all the examined parameters, the female chickens had significantly lower weight compared to the male ones, while the White Rock chickens had significantly lower weight compared to the Ross 308 hybrid. There was no significant interaction between the sex and genotype determined.

Table 2. The weight of the processed carcasses, g ($\bar{x}\pm$SD)

Weight, g	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	S x G
Standard processing	Male	1739 ± 82.35 [A]	3492 ± 188.56 [B]	0.000	0.000	NS
	Female	1228 ± 78.73 [A]	3085 ± 215.95 [B]			
	Average	**1483 ± 274.55 [A]**	**3288 ± 427.44 [B]**			
Ready to roast	Male	1582 ± 74.93 [A]	3234 ± 190.66 [B]	0.002	0.000	NS
	Female	1124 ± 72.02 [A]	2876 ± 178.93 [B]			
	Average	**1353 ± 249.01 [A]**	**3055 ± 498.57 [B]**			
Ready to grill	Male	1392 ± 66.08 [A]	2882 ± 129.69 [B]	0.004	0.000	NS
	Female	978 ± 62.71 [A]	2577 ± 131.43 [B]			
	Average	**1185 ± 225.66 [A]**	**2730 ± 367.21 [B]**			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01)
NS – No statistical significance S-sex; G-genotype; S x G- the interaction sex x genotype

The influence of the sex on dressing percentage was statistically significant (P<0.05) (Table 3). Generally, the fast-growing hybrids are highly selected for the uniformity of all production traits, especially for the yield and carcass quality. For this reason, the differences between males and females were smaller when the carcass yield was expressed in relative values. The influence of the genotype was not statistically significant for "standard processing", but it was significant for "ready to roast" and "ready to grill" ones (P<0.05). A significant interaction (P<0.05) between sex x genotype was observed in "standard processing" dressing.

Table 3. Dressing percentage, % ($\bar{x}\pm$SD)

Dressing, %	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	S x G
Standard processing	Male	82.35 ± 2.31	82.18 ± 2.80	0.035	NS	0.028
	Female	78.73 ± 0.84 [A]	80.68 ± 3.21 [B]			
	Average	**80.54 ± 2.36**	**81.43 ± 2.68**			
Ready to roast	Male	74.93 ± 2.31 [A]	76.09 ± 2.26 [B]	0.019	0.000	NS
	Female	72.02 ± 1.13 [A]	75.22 ± 2.79 [B]			
	Average	**73.48 ± 2.17 [A]**	**75.66 ± 2.35 [B]**			
Ready to grill	Male	66.08 ± 2.34 [A]	67.83 ± 2.33 [B]	0.012	0.007	NS
	Female	62.71 ± 1.06 [A]	67.41 ± 2.18 [B]			
	Average	**64.40 ± 2.32 [A]**	**67.62 ± 2.06 [B]**			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01)
NS – No statistical significance; S-sex; G-genotype; S x G- the interaction sex x genotype

Concerning the weight of the head, neck, legs and edible giblets, we found highly significant differences (P<0.01) between genotypes and sexes for all the examined parameters (Table 4). Chickens of the Ross 308 hybrid and male chickens had a significantly larger weight of the listed carcass parts.

Table 4. The weight of the head, neck, legs, edible giblets and abdominal fat, g ($\bar{x}$±SD)

Parameter	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	S x G
Head weight, g	Male	70.90 ± 6.67 [A]	92.65 ± 7.50 [B]	0.000	0.000	NS
	Female	49.90 ± 3.28 [A]	84.13 ± 7.99 [B]			
	Average	**60.40 ± 11.93** [A]	**88.39 ± 15.02** [B]			
Neck weigh,. g	Male	90.20 ± 8.76 [A]	151.30±11.81 [B]	0.000	0.000	NS
	Female	62.13 ± 7.37 [A]	123.51±9.50 [B]			
	Average	**72.92 ± 16.09** [A]	**137.41±30.31** [B]			
Legs weight, g	Male	88.40 ± 3.27 [A]	165.75±14.91 [B]	0.000	0.000	NS
	Female	56.00 ± 4.56 [A]	124.28±8.08 [B]			
	Average	**72.20 ± 17.06** [A]	**145.01±24.68** [B]			
Edible giblets weight, g	Male	94.70 ± 8.75 [A]	199.75 ±18.45 [B]	0.000	0.000	NS
	Female	82.50 ± 7.72 [A]	175.12 ±15.06 [B]			
	Average	**88.60 ± 0.18** [A]	**187.44 ±22.49** [B]			
Abdominal fat, g	Male	0.0 [A]	48.02 ±3.32 [B]	0.000	0.000	NS
	Female	0.0 [A]	55.83 ±4.84 [B]			
	Average	**0.0** [A]	**51.92 ±7.34** [B]			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01)
NS – No statistical significance; S-sex; G-genotype; S x G- the interaction sex x genotype

What was particularly significant was the yield of abdominal fat which was not even detected in White Rock breed, while in the Ross 308 hybrid it was smaller than expected, considering that the chickens' live weight was over 4 kg. This might have been the result of increased chicken's activity on a range, but also of the feed which contained the lower level of energy compared to the feed which the broiler chickens are regularly fed.

When the weight of the head, neck, legs, edible giblets and abdominal fat of the chickens is expressed in percentages of the live weight, the values are significantly more balanced, both between sexes and between genotypes (Table 5).

Table 5. The share of the head, neck, legs, edible giblets and abdominal fat in the live weight of chickens, % ($\bar{x}$±SD)

Parameter	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	S x G
Head,%	Male	3.16±0.16[A]	2.17±0.13[B]	NS	0.003	NS
	Female	3.18±0.32 [A]	2.20±0.18 [B]			
	Average	**3.17±0.26 [A]**	**2.18±0.19 [B]**			
Neck, %	Male	4.27±0.31 [a]	3.56±0.28 [b]	NS	0.015	NS
	Female	3.98±0.34	3.53±0.26			
	Average	**4.09±0.39 [a]**	**3.39±0.26 [b]**			
Legs, %	Male	4.26±0.32	3.99±0.32	NS	NS	NS
	Female	3.53±0.18	3.25±0.29			
	Average	**3.81±0.44**	**3.57±0.34**			
Edible giblets, %	Male	4.58±0.17	4.71±0.22	0.036	0.028	NS
	Female	5.33±0.44[a]	4.58±0.34[b]			
	Average	**5.04±0.52**	**4.64±0.36**			
Abdominal fat, %	Male	0.00 [A]	1.05±0.09 [B]	NS	0.007	NS
	Female	0.00 [A]	1.25±0.11 [B]			
	Average	**0.00 [A]**	**1.15±0.12 [B]**			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01); [a-b] (P<0.05); NS – No statistical significance S-sex; G-genotype; S x G- the interaction sex x genotype

The Ross 308 chickens had a significantly lower share of the head and neck in live weight compared to the White Rock chickens. Significant difference (P<0.05) between genotypes was determined in the share of edible giblets, but only in female chickens. Differences in the share of abdominal fat were the most prominent, because in the carcasses of the White Rock breed there was no abdominal fat, whereas the share of it in the carcasses of the Ross 308 hybrid was 1.05% for male and 1.25% for female chickens.

Table 6. The yield of the basic carcass parts, g (x̄±SD)

Parameter	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	S x G
Drumsticks weight, g	Male	235.12±8.15 [A]	441.67 ± 60.34 [B]			
	Female	156.88±9.48 [A]	376.89 ± 35.30 [B]	0.000	0.000	NS
	Average	**186.92±41.56** [A]	**409.28 ± 75.22** [B]			
Thighs weight, g	Male	272.80±18.59 [A]	497.31 ± 50.18 [B]			
	Female	187.50±18.29 [A]	428.97 ± 35.34 [B]	0.000	0.000	NS
	Average	**220.3±46.65** [A]	**463.14 ± 60.93** [B]			
Breast weight, g	Male	327.80±19.98 [A]	940.42 ± 60.72 [B]			
	Female	253.38±30.34 [A]	879.59 ± 85.44 [B]	0.000	0.000	NS
	Average	**282.04±44.62** [A]	**910.01 ± 95.33** [B]			
Wings weight, g	Male	173.60±6.54 [A]	356.91 ± 19.12 [B]			
	Female	126.38±8.79 [A]	317.60 ± 18.77 [B]	0.000	0.000	NS
	Average	**144.54±25.12** [A]	**337.26 ± 22.41** [B]			
Back weight, g	Male	386.40±55.38 [A]	643.47 ± 68.49 [B]			
	Female	254.63±26.21 [A]	577.72 ± 55.28 [B]	0.000	0.000	NS
	Average	**305.31±76.65** [A]	**610.60 ± 75.63** [B]			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01)

NS – No statistical significance S-sex; G-genotype; S x G- the interaction sex x genotype

Concerning the yield of the most quality parts of the carcass, the obtained results showed that this trait was under the significant influence of the sex (P<0.01) and genotype (P<0.01) (Table 5). Male chickens had a significantly larger weight of the basic parts of the carcass compared to the female ones, as well as the chickens of the Ross 308 hybrid compared to the White Rock breed.

When the weight of the basic parts of the carcass is expressed in relative values, we obtain different results (Table 7). Namely, the sex had a significant influence only on the share of the breast (P<0.05), and the genotype did so on all parts of the carcass except wings. The greatest difference was determined in the share of the breast, which was almost 10% larger in the Ross 308 hybrid. This is a consequence of an intensive selection on the yield of breast meat.

Table 7. The share of the basic parts of "ready to grill" carcass, % (x̄±SD)

Parameter	Sex	Genotype		P values		
		White Rock	Ross 308	S	G	P x G
Drumsticks,%	Male	16.84 ± 0.72[a]	15.32 ±0.62**[b]**			
	Female	16.06 ± 0.76 [a]	14.62 ±0.75 **[b]**	NS	0.041	NS
	Average	**16.36 ± 0.81**[a]	**14.97 ±0.84 [b]**			
Thighs, %	Male	19.54 ± 0.39[A]	17.25 ±0.43 [B]			
	Female	19.16 ± 0.10[A]	16.64 ± 0.31 [B]	NS	0.000	NS
	Average	**19.31 ± 0.82**[A]	**16.94 ± 0.78** [B]			
Breast, %	Male	23.58 ± 1.97 [A]	32.62 ± 2.30 [B]			
	Female	25.84 ± 1.35 [A]	34.12 ± 1.87 [B]	0.023	0.000	NS
	Average	**24.97 ± 1.91** [A]	**33.37 ± 2.78** [B]			
Wings, %	Male	12.46 ± 0.61	12.38 ± 0.54			
	Female	12.95 ± 0.86	12.32 ± 0.61	NS	NS	NS
	Average	**12.76 ± 0.78**	**12.35 ± 0.75**			
Back, %	Male	27.57 ± 2.47 [A]	22.32 ± 1.14 [B]			
	Female	25.99 ± 1.34 [A]	22.41 ± 1.22 [B]	NS	0.000	NS
	Average	**26.60 ± 1.89** [A]	**22.36 ± 1.45** [B]			

[A-B] Values which have a different letter in superscript are statistically significant (P<0.01) [a-b] (P<0.05); NS – No statistical significance; P-sex; G-genotype; P x G- the interaction sex x genotype

Discussion

In presented research we found significant differences in production results between fast-growing hybrid Ross 308 and White Rock breed, which were expected. Other authors also reported that fast-growing hybrids achieved much higher body weights compared to the slow-growing hybrids (*Nielsen et al. ,2003; Wang et al. 2009)* or pure breed (*Tang et al. 2009).* However, they all concluded that fast-growing hybrids are less suitable for rearing in extensive systems.

Carcass weight and dressing percentage were under the significant influence of the genotype and sex, which was also confirmed in the research of *Shahin and Elazeem (2005)* and *Pathak et al. (2009). Batkowska et al. (2015)* examined how suitable were the cross breeds for the extensive fattening and they compared them to the commercial Cobb hybrid. The highest dressing percentage was determined in the Cobb 500 chickens (around 75%), while cross breeds had lower dressing percentage for 5.6% (Cobb x Green-legged Partridgenous), or 7.2% (Cobb x Sussex). *Rajkumar et al. (2016)* stated that the chickens of the pure Aseel breed had smaller dressing percentage compared to the commercial broiler lines under the conditions of the extended fattening. As opposed to these authors,

Fanatico et al. (2008) did not found a significant influence of the genotype on dressing percentage when comparing slow-growing and fast-growing hybrids under the conditions of extensive rearing.

Mikulski et al. (2011) concluded that the yield of carcass parts was largely affected by the genotype and raising system, which was also confirmed by *Pavlovski et al. (2007)* and *Đukić Stojčić et al. (2016). Almasi et al. (2015)* determined a significant influence of the genotype on the yield of the parts of the carcass in slow-growing and medium-growing hybrids. In our research, the weight of all parts of the carcass was larger in the Ross 308 hybrid, but the share of almost all parts of the carcass was larger in the White Rock breed, with the exception of the share of breast meat. This implies the significant differences in the composition of the carcass which are the consequence of the selection of hybrids on the increased yield of breast meat *(Milošević et al., 1999; Castellini et al., 2002). Bogosavljević-Bošković et al. (2009; 2011)* determined that the broilers which had access to the free ranges had a lower share of breast meat compared to the broilers reared indoor.

What is particularly significant when we observe the quality of the carcass is the fat percentage. An increased amount of fat in broiler chicken carcasses is a problem which had been indicated by *Milošević and Supić (1995)*, as well. They stated that the percentage of total fat in broiler chicken carcasses could range even up to 10%, depending on the diet, and the percentage of abdominal fat can range up to 3%.

The differences in the weight of abdominal fat were precisely the most prominent part of this experiment, because there was no abdominal fat in the carcasses of the White Rock breed. It is certain that the amount of abdominal fat was influenced by the effect of chickens' activity on the ranges, as well as the feed which contained the lower level of energy. The results of the other authors, when the percentage of abdominal fat is concerned, vary considerably. *Milošević et al. (2005)* stated that, in the free range rearing system, the percentage of abdominal fat in the chickens of the Hybro genotype which were 56 days old was 1.95%. Within the same rearing system, but up to 70 days, *Perić et al. (2003)* stated that the percentage of abdominal fat in the Ross 308 genotype was 0.65%. *Rajkumar et al. (2016)* also marked the low percentage of abdominal fat in the pure Aseel breed (0.73-0.78%). *Wang et al. (2009)* determined that the breeding of slow-growing hybrids on a free range significantly decreased the amount of abdominal fat, while having no effect on the yield and the weight of the basic parts of the carcass. *Milošević et al. (2005)* stated that it is possible to lower the amount of fat in the carcasses of fast-growing hybrids used in the extensive production, as well, by applying a suitable feeding regime, which would significantly contribute to the quality of the poultry meat.

Conclusion

Based on the results of this research, it can be concluded that both genotype and sex significantly affected carcass traits while genotype affected production traits. Chickens of the Ross 308 hybrid had higher body weights, better feed conversion ratio and higher mortality rate. Chickens of the Ross 308 hybrid had higher "ready to roast" and "ready to grill" dressing percentage compared to the White Rock breed, while the male chickens had higher dressing percentage compared to the female ones. Chickens of the Ross 308 hybrid had a significantly larger weight and share of breast meat, whereas those of the White Rock breed had a larger share of drumsticks and thighs. A significant influence of the genotype was determined on the amount of abdominal fat in the carcass. Chickens of the White Rock breed had a significantly lower fat compared to the Ross 308 hybrid, which essentially makes them the genotype which is more suitable for the extensive fattening longer than 10 weeks.

Uticaj genotipa i pola na klanične karakteristike pilića u ekstenzivnom sistemu gajenja

Lidija Perić, Vladimir Strugar, Snežana Bogosavljević-Bošković, Mirjana Đukić Stojčić

Rezime

Cilj ovog rada bio je da utvrdi mogućnost uzgoja brzorastućeg hibrida Ross 308 i čiste rase vajtrok u uslovima ekstenzivnog tova, posebno kada su u pitanju klanične osobine. Po 100 pilića od svakog genotipa odgojeno je unutar objekta do 4 nedelje starosti, a posle toga su pilići puštani na zatravljeni ispust do 13. nedelja starosti, koliko je trajao tov. Na kraju tova, 10 muških i 10 ženskih pilića po genotipu je žrtvovano i određene su klanične osobine – klanični randmani, masa i udeo pojedinih delova trupa u konfekciji.

Rezultati su pokazali da postoji visoko značajan uticaj genotipa i pola pilića na klanične osobine, kao i značajan uticaj genotipa na proizvodne osobine. Pilići hibrida Ross 308 imali su značajno veću masu, bolju konverziju hrane i veći mortalitet. Imali su i veći randman "spremno za pečenje" i "spremno za roštilj" u odnosu na piliće rase vajtrok, dok su muški pilići imali veće randmane u odnosu na ženske. Hibrid Ross 308 imao je značajno veći prinos i udeo belog mesa u trupu, dok je rasa vajtrok imala veći udeo bataka i karabataka. Posebno je značajan uticaj

genotipa na sadržaj abdominalne masti, koje kod pilića rase vajtrok bio značajno niži, što ovu rasu čini pogodnijom za uzgoj u uslovima produženog tova.

Ključne reči: brojleri, vajtrok, genotip, pol, klanične osobine, ispust

Acknowledgement

References

ALMASI A., ANDRASSYNE B.G., MILISITS G., KUSTOSNE P.O., SUTO Z. (2015): Effects of different rearing systems on muscle and meat quality traits of slow-and medium-growing male chickens, British Poultry Science, 56:3, 320-324.
BATKOWSKA J., BRODACKI A., ZIĘBA G., HORBANCZUK J.O., ŁUKASZEWICZ M. (2015): Growth performance, carcass traits and physical properties of chicken meat as affected by genotype and production system. Archives Animal Breeding, 58, 325–333.
BERRI C., LEBIHAN-DUVAL E., BAÉZA E., CHATRIN P. PICGIRAAD L., JEHL N., PICKARD M., QUENTIN M., DUCLOS M.J. (2005): Further processing characteristics of breast and leg meat from fast, medium and slow growing commercial chickens. Animal Research, 54, 123-134.
BOGOSAVLJEVIĆ-BOŠKOVIĆ S., MITROVIĆ S., PETROVIĆ M., ĐOKOVIĆ R., DOSKOVIĆ V. (2008): Uticaj uzrasta i sistema držanja na odabrane parametre kvaliteta mesa pilića u tovu. Savremena poljoprivreda, 57, 3-4, 137-143.
BOGOSAVLJEVIĆ-BOŠKOVIĆ S., PETROVIĆ D., DOSKOVIĆ V., ŠARANČIĆ D.(2009): Prinos osnovnih delova trupa pilića u zavisnosti od dužine tova i sistema gajenja. Biotechnology in Animal Husbandry, 25, 5-6, 1039-1044.
BOGOSAVLJEVIĆ-BOŠKOVIĆ S., DOSKOVIĆ V., MITROVIĆ S., RAKONJAC S., PETROVIĆ M.D. (2011): Broiler meat quality: The effect of rearing system and length of fattening period, Biotechnology in Animal Husbandry, 27, 4, 1635-1642.
BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., DOSKOVIĆ V., PETROVIĆ M. D. (2012): Broiler rearing system: a review of major fattening results and meat quality traits. World Poultry Science Journal, 68: 217-228.
CASTELLINI C., MUGNAI C., DAL BOSCO A. (2002): Meat quality of three chicken genotypes reared according to the organic system. Italian Journal of Food Science, 14:411–412.

CASTELLINI C., BERRI C., LE BIHAN-DUVAL E., MARTINO G. (2008): Qualitative attributes and consumer perception of organic and freerange poultry meat, World's Poultry Science, 64, 500-513.

ĐUKIĆ STOJČIĆ M., PERIĆ L., LEVART A., SALOBIR J. (2016): Influence of rearing system and nettle supplementation (Urtica dioica) on the carcass traits and fatty acid composition of Redbro broilers. European Poultry Science, 80. DOI: 10.1399/eps.2016.145.

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.

FORTE C., MANUALI E., ABBATE Y., PAPA P., VIECELI L., TENTELLINI M., TRABALZA-MARINUCCI M., MOSCATI L. (2018): Dietary Lactobacillus acidophilus positively influences growth performance, gut morphology, and gut microbiology in rurally reared chickens. Poultry Science, 97, 3, 930–936.

LI Y., LUO CH., WANG J., GUO F (2017): Effects of different raising systems on growth performance, carcass, and meat quality of medium-growing chickens. Journal of Applied Animal Research, 45, 1, 326-330.

MIKULSKI D., CELEJ J., JANKOWSKI J., MAJEWSKA T., MIKULSKA M. (2011): Grow performance, carcass traits and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access. Asian-Australian Journal Animal Science, 24: 1407–1416.

MILOŠEVIĆ N., SUPIĆ B. (1995): Genetski aspekti proizvodnje brojlerskog mesa. Savetovanje živinara Jugoslavije, Živinarstvo, 7-8, 125-136.

MILOŠEVIĆ N., SUPIĆ B., PERIĆ L., PAVLOVSKI Z., MITROVIĆ S. (1999): Pravci genetskog napretka u proizvodnji živinskog mesa i jaja. Savremena poljoprivreda, 1-2, 57-64.

MILOŠEVIĆ N., PERIĆ L., STRUGAR V. (2005): Gajenje tovnih pilića na ispustu. Biotechnology in Animal Husbandry, 21, 1-2, 65-72.

MUHAMMAD I.K., FAISAL F.K., MAJID M., MOHAMMAD U.K., ALI S.M., NIKOLAI P., PIGOREV I., GLINUSKIN A., LAISHEVTCEV A. (2017): Free range poultry husbandry and physiochemical quality of meat: A review. International Journal of Research Ayurveda Pharm, 8 (Suppl 1):74-79.

NIELSEN B.L., THOMSEN M.G., SORENSEN P., YOUNG J.F. (2003): Fed and strain effects on the use of outdoor areas by broilers. British Poultry Science, 44, 2, 161-169.

PATHAK V., BHAT Z.F., BUKHARI S.A.A., AHMAD S.R. (2009): Carcass quality parameters of Vanaraja chicken. Indian Journal Poultry Science, 44, 1, 97-99.

PAVLOVSKI Z., ŠKRBIĆ Z., CMILJANIĆ R., LUKIĆ M., TOMAŠEVIĆ D. (2007): Uticaj sistema gajenja i bioloških faktora na konformaciju trupa i klanične osobine pilića u tovu. Biotechnology in Animal Husbandry, 23, 3-4,59-66.

PERIĆ L., MILOŠEVIĆ N., ORLIĆ D., SUPIĆ B. (2003): Raising chickens on a free range, 1. Production parameters and health status, ZFB Lucrari Stiintifice, Timisoara, XXXVI, 360-363.

RAJKUMAR U., MUTHUKUMAR M., HAUNSHI S., NIRANJAN M., RAJU M.V.L.N., RAMA RAO S.V., CHATTERJEE R.N. (2016): Comparative evaluation of carcass traits and meat quality in native Aseel chickens and commercial broilers. British Poultry Science, 57:3, 339-347.

RODIĆ V., PERIĆ L., VUKELIĆ N., MILOŠEVIĆ N. (2006): Consumer's attitudes towards chicken meat produced in extensive systems. World's Poultry Science Journal, XII European Poultry Conference, Book of Abstracts, 62, 186-187.

SHAHIN A.K., ELAZEEM A.F. (2005): Effect of breed, sex and diet and their interactions on carcass composition and tissue weight distribution of broiler chickens. Archiev fur Tierzucht, 48, 612-26.

TANG H., GONG Y. Z., WU C. X., JIANG J., WANG Y., LI K. (2009): Variation of meat quality traits among five genotypes of chicken. Poultry Science, 88:2212-2218.

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(10):2219-2223.

REARING CONDITIONS AND HEALTH STATUS OF CALVES ON SMALL RURAL FARMS

Nenad Stojiljković[1], Nenad Mićić[1], Marija Gogić[1], Vladimir Živković[1], Bogdan Cekić[1], Jožica Ježek[3], Renata Relić[2]

[1]Institute for Animal husbandry, Autoput 16, P.Fax 23, 11080 Belgrade, Serbia
[2]University of Belgrade, Faculty of agriculture, Nemanjina 6, 11080 Belgrade, Serbia
[3]University of Ljubljana, Veterinary faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia
Corresponding author: Nenad Stojiljković, zake9103@gmail.com
Original scientific paper

Abstract: Good rearing conditions are the key factor for health and welfare of calves. Diarrhea, respiratory and navel infections are commonly referred to as the most common health problems in calves. The aim of this study was an observation of rearing conditions and the occurrence of the most common health problems in calves on small rural farms in Danube Region of Serbia. Data were colected from 30 farms by surveying the farmers. The questionary consisted of 75 questions pertaining to the following: general information on the farm, and on calving, nutrition, weaning, housing, hygiene of space for cows and calves, on calf diseases and diseases prevention measures. According to the answers, the average number of all categories of cattle at the fams is 18 heads. Calves are separated from their mothers immediately after birth at 67% farms. They receive colostrum up to two hours after birth at 47% of the farms and immediately after birth at 20% of the farms. Average age of calves at weaning is 13 weeks. Calves begin to use hay usually between the 1st and 2nd week of life, and a concentrated feed in 2nd week. They are held in different types of pens. Farmers specified diarrhea and respiratory diseases as the most common health problems in calves. Rearing conditions at the farms often do not meet the recommendations and the breeders do not implement all of the necessary preventive measures. Corrections in this regard could contribute to better health of calves.

Key words: calves, small farms, rearing conditions, health problems

Introduction

Cattle breeding in Serbia represent a very important branch of livestock production, which is predominantly based on small family farms. According to the

2012 census, rural households have an average of 5.1 head of cattle of all categories. Milk production is carried out on farms with an average capacity of 2.8 heads. The most numerous are farms with 1 or 2 cows and in such farms there are about 66% of the total number of dairy cows in Serbia (*Arsić et al., 2011; Popović, 2014*). Beside dairy cows, on these farms, other types of animals are usually grown in order to meet the needs of the household for milk, meat and eggs, which implies numerous possibilities for transmitting and spreading contagious diseases (*Relić, 2014*). The occurrence of disease in calves reflects on the growth and further development, but also on the production traits in adulthood, if the individual does not die. Infections can occur in the first days of life, and the most common health problems of calves are diarrhea, respiratory infections and omphalitis. Calf mortality in the first months of life ranges between 5 and 10%, and the target in calf rearing should be mortality below 5% in the mentioned period (*Miljković and Veselinović, 2005*).

Development of infectious diseases depends on the relationship between the infectious agent-host, which is affected by the environment and the breeding technology. The task of the breeder is to prevent the development of disease by applying recommended prophylactic measures, and for successful control, knowledge of as many factors as affects its occurrence is needed. For example, frequent risk factors for the appearance of diarrhea and respiratory diseases in calves are: hygiene of calving area, feeding, housing (individual or group pen), high ammonia concentration and exposure to the draft *(Samolovac, 2016)*.

First measures which should be taken are relating to adequate food and accommodation of pregnant cows. Special attention is paid to the act of calving and procedures that accompany it, and then to the colostral and milk feeding, the availability of clean water and to accommodation for calves. In order to protect against infections, especially of gastrointestinal tract, calf is kept individually in the first week of life, and later in group pen. Adequate hygiene of all surfaces, mattress and equipment, as well as sufficient light and clean air, positively affect the vitality of calves and prevent the occurrence of the most common diseases. (*Hristov, 2002*; *Romčević et al., 2007; Lorenz et al., 2011; Relić et al., 2014; Samolovac, 2016; Relić et al., 2017a*). This paper presents data on specific calf management practices and the occurrence of the most common health problems in calves on small rural farms in the Danube Region of Serbia.

Materials and Methods

Information on rearing conditions and health problems of calves, related to the period of the previous 12 months, were collected in 30 households in the Danube Region, located in the territory of the municipalities of Velika Plana and

Smederevska Palanka. The data were collected by questioning the owner of the farm, and in cooperation with the competent veterinary service, during the regular actions of the vaccination of animals.

The survey consisted of a total of 75 questions related to the following: general information on the farm, and on calving, feeding, weaning, nutrition, housing, hygiene of space for cows and calves, on calf diseases and diseases prevention measures. The collected data have been summarized and processed by the Microsoft Office Excel 2010 package. The results are displayed as integers, and due to the amount of data, except tables and charts, they are also shown in the text.

Results and Discussion

General data

According to results from the questionnaire, the dominant type of livestock production in Danube region is cattle breeding (50% farms). All of the farms are of conventional type (100%), the most often with combined production of milk and meat (58%). The most common is Simmental type of cattle (63%) and, on average, the farms own 18 heads of all categories of cattle (Table 1). This is approximately the same as data by Popović (2014), according to which family farms with up to 19 heads of cattle account for 97% of households in the Republic of Serbia.

Data shows that breeders mostly do not know the reason of calf's death. Secondly, they mentioned respiratory diseases and diarrhea as the cause of mortality in calves. As reasons for loss due to accidents, the breeders mentioned the unprofessional aiding during the difficult calving and some inadequate rearing conditions, which resulted in injuries to calves with a fatal outcome. The occurrence of death due to such reasons indicates that on some farms calves are not kept and treated according to the regulations, such as the Regulation on animal welfare conditions (*Anon., 2010*). Inappropriate care of the animals may be related to insufficient manpower, motivation and personal traits of breeders (*Samolovac, 2016*). On the examined farms is a small number of working-age population and the highest percentage of breeders have only a high school completed (Table 1). Similar to the previous, ignorance of the cause of death may be related to the lack of experience, training or interest from the breeders in the study. The situation is similar in other countries. For example, for breeders in Brazil, the world's fifth milk producer, the reason for about 38% of calves' death cases is unknown. The fact that farmers struggled to identify the cause of death highlights the lack of knowledge of signs that could aid them to tackle disease on time and apply preventive measures (*Fruscalso et al., 2017*).

Table 1. General data on cattle farms

Parameter	Answers offered in the questionary	Results
Average number of cattle	• total	18
	• cows	8
	• calves up to 6 months	7
Cases calves' death of up to 6 months of age (% of the farms)		33
Causes of calves'death (% of the farms with death cases)	• diarrhea	15
	• respiratory diseases	25
	• accidents	10
	• malformations	5
	• unknown	45
Average number of household members	• total	4
	• men	2*
	• women	1
	• underage	1
Average age of the person who manages the household (i.e. breeder)		49
Breeder's education (% of breeders)	• does not want to answer	3
	• college	7
	• not completed elementary school	7
	• elementary school	30
	• high school	53

  * Workers (men) are occasionally hired at 17% of the farms

Managing and housing of cows and calves

In 80% of the farms bed for expecting cow is cleaned (i.e. dirty straw is replaced by fresh) two or more times a day; in 10% is cleaned once a day and in 10% more times a week. For cows in the phase of transition, wich is usually defined as the period of 3 weeks before and 3 weeks after parturition, a clean and comfortable space is needed. Disturbed rest and uncomfortable resting areas in this period may result in a physiological stress response increasing the susceptibility to disease. Furthermore, during the calving, cows are agitated and often change their position, so special attention should be placed on cow comfort in the maternity i.e. calving pen, particularly to aspects of stall surface known to affect comfort when changing positions (*Huzzey et al., 2005*). However, according to the survey results, none of the farms has a pen for calving and at the calving time the cow remains tethered in place (93%) or is moved to a separate stall bed that is in the same facility (7%).

During calving, the most breeders always provide help to the cow (57%), while others help only in the case of distocia (27%), occasionally (13%), or they call a veterinarian for help (3%). Mainly farmers with a higher number of cows provide assistance from time to time, due to the lack of adequate manpower, which would enable the timely performance of all jobs on the farm.

The breeders usually separate the calf from the mother immediately after birth (67%), which is in line with the usual technology on commercial farms; in 26% farms calves stay longer with their mother, usually all the period until they suck, and in 7% they remain until mother dries them after birth. The cow should be allowed to lick the calf after birth. If the cow does not lick the calf, the newborn should be dried with clean cloths. This practice not only dries the calf but stimulates the calf's blood circulation (*Amaral-Phillips et al., 2006*).
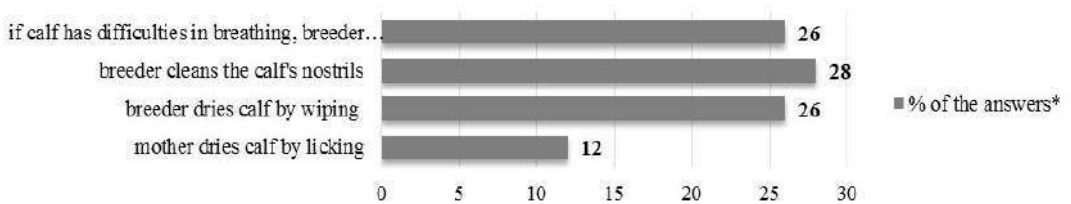
Chart 1. Providing assistance to newborn calf (*multiple answers)

Data from the Chart 1 indicate that breeders do not give enough necessary care to the newborn calves. This is also supported by the fact that 7% of breeders never wash the pen for newborn calf; 46% of them wash the pen after each calf, and 47% of breeders wash it several times a year. Regular cleaning and desinfection of all surfaces in the stall, especially the floor of the pen is significant for reduction of the infectious pressure and the occurrence of conditional diseases, as is described by *Hristov* (2002).

The calves are housed in different types of pens, whose average surface is in accordance with the recommended values (*Anon., 2010*). They are most often together with adult cattle (73%). In others, baby calves are housed separately (13%), or together with older calves (7%), or tied outside the stall (7%). According to the Regulations (*Anon., 2010*), calves should not be tied.

The straw in the calf pen is completely changed in different intervals, depending on the farm: every day (22%); every two days, with adding the fresh in the meantime (38%); and once a week (33%) or once in two weeks (7%), with adding fresh straw every day.

Ventilation in buildings is mostly natural (60% of farms) and is carried out by opening windows and doors; 17% of the farms also have a fan, while on other farms, calves are kept in semi-open structures (20%) or outside (3%).

Calf feeding and weaning

On most of the farms (60%) breeders do not let calves suck their mothers but use buckets with a nipple to feed the calves; 55% of them use one bucket for more calves, and 45% have a separate bucket for each calf. The buckets are washed after each use (50%) or at least once a day (50%), but only 15% of the breeders use the detergent when washing.

Of the remaining 40% farms where the calves suck, most breeders wash the cow's udder only if it is dirty (23%), some of them always do udder washing before the calf begins to suck (14%), and some do not wash it at all (3%). Failure to implement adequate hygiene measures before sucking and feeding calves in a large extent leads to more frequent occurrence of diseases in calves (*Samolovac*, 2016).

Hygiene measures contribute the calf is less exposed to pathogenic microorganisms from the environment. Beside that, the two most important factors in newborn calf survival are warmth and colostrum. Time after birth at which colostrums is first fed is critical to determining if the calf will acquire an adequate passive immunity and its ability to defend against disease. Calves that don't nurse within 2 to 4 hours after birth often die or become weak and unable to nurse and starve (*Relić et al., 2014*).

According to the responses, the average amount of colostrum that breeders offered to the calf at the first feed is 2 liters, which is in accordance with the capacity of abomasum in the first hours of life. More than half of them know that timely provision of colostrum is very important for calves' health, and mostly they allow calves to consume it within 2 hours of birth (Table 2).

In calves which do not sucking, the temperature of the colostrum, and later the milk (or milk replacer) should be as close as possible to the calf's body temperature, but not below 37 ℃ (*Romčević et al., 2007; Lang, 2008*). Given that quite a high percentage of breeders do not check the temperature, and of those who do it, more than two thirds use hand to determine the temperature, it is not certain that the temperature of colostrum/milk is in accordance with the recommendations. There are also a worryingly high percentage of breeders that can not provide additional heating of the milk prior to the administration, as well as those who do not carry out quality checks prior to the administration of colostrum (Table 2). On the other hand, it is positive that most breeders freeze the colostrum. Freezing and storing surplus colostrum of healthy cows allow appropriate feeding the calves even if there is insufficient quantity of fresh colostrum (Relić et al., 2014). However, thawing colostrum at the room temperature is not reccomended, as bacteria double every 20-30 minutes at room temperature (*Lang, 2008*).

Table 2. Colostral/milk feeding characteristics

Parameter	Answers offered in the questionary	Results (%)
Time of the first colostrum consumption	• immediately after birth • **up to 2 hours** • up to 6 hours	20 47 33
Breeders awareness of the importance of timely administration of colostrum in relation to the health of calves	• no impact at all • little impact • some impact • significant impact • very significant impact	10 7 20 10 53
Colostrum quality checking before administration	• no checking • only rejecting the first streams • something else	77 17 13
Storage of surplus colostrum in the freezer	• yes • no	70 30
Method of thawing colostrum	• at the room temperature • in the warm water (39 °C) • in the microwave oven	33 56 11
Colostrum/milk temperature checking	• never (calf is sucking) • never • always • occasionally	40 23 14 23
Method of colostrum/milk temperature checking	• by hand • by thermometer	73 27
Milk reheating before administration	• no • yes (mixing milk and warm water) • yes (placing milk container in hot water) • something else	44 3 20 33
Amount of powder mixture (milk replacer) that is mixed with water	• no use of milk replacer • according to the manufacturer • uses a larger amount than required	70 27 3

On 40% of farms weaning of the calves is rapidly (today they get milk, tomorrow not), and on the remaining 60% it is done gradually, within a week. The average age of calves at weaning is 13 weeks. Calves are weaned when they reach an average 159 kg and when they are able to eat 550 g of concentrate per day. These parameters indicate that full milk and/or milk replacer probably have a

significant part in the diet, given that the calves in the mentioned period usually have a lower weight (*Romčević et al., 2007; Stojanović et al., 2007*).

Diseases and preventive measures

During the day, the breeders most often monitor the calves for about 15 minutes (37%) or up to 30 minutes (37%), but also between 30 and 60 minutes (13%) or longer than 60 minutes (13%), which is usually enough to notice health problem, if any. However, answers on the prevention, appearance and treatment of some calves diseases (Table 3) indicate that breeders give insufficient attention to the health of calves or they do not know enough of the signs of these diseases or about the consequences to the animal (e.g. the weakening of resistance to other diseases, slower waight gain or death).

Table 3. Calves diseases and their control

Parameter	Answers offered in the questionary	Results (%)
performing disinfection of the navel of a newborn calf	• always • never • occasionally	23 67 10
occurrence of omphalitis (i.e. navel disease)	• never • sometimes	83 17
occurrence of joints swelling	• never • sometimes	83 17
occurrence and treatment of trichophytia (i.e. ringworm)	• never • sometimes, it is treated • sometimes, it is not treated	63 17 20
treatment against parasites*	• yes, against endoparasites • yes, against ectoparasites • no, no problems with parasites in calves • no, although calves have parasites	70 23 20 3

* multiple answers

In raising calves, succes or failure depends to a great extent on the breeder's attitude to the calves and the ability to react promptly to the calves'signals (*Moran, 2002*). The breeders'attitude can best be seen from the following data on diarrhea and respiratory diseases, as the most frequent calf diseases in our study and generally in cattle breeding (*Samolovac, 2016; Fruscalso et al., 2017*).

In the past year, at almost all of the farms (97%) have been cases of diarrhea i.e. stool that is more liquid than normal and lasts longer than two days. Almost a half of calves at the farm gets sick (46%), but less than half of them is treated (41%). This indicates that breeders do not call often a vet for calves that have diarrhea, and reasons for calling can be found in Chart 2.

Reason	%
if diarrhea occurs in other animals	2
if the diarrhea appears again in a short time	7
if the situation does not improve in a few days	51
when diarrhea is observed	24
when a high temperature is detected	7
if the calf stops eating	15
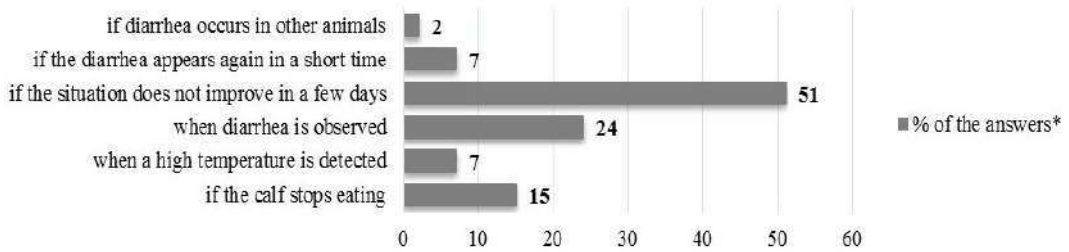
Chart 2. Reasons for calling a veterinarian for calf having diarrhea (*multiple)

Most farmers (52%) measure the temperature of sick calf if they estimate it is necessary due to poor condition of the animal, and 10% do it always (as soon as they notice the signs of illness); 38% breeders never measure the temperature.

Table 4. Procedures regarding nutrition and rehydration of calves with diarrhea

Parameter	Answers offered in the questionary	Results (%)
administration of rehydration fluids	• never • always	65 35
restrictiction of milk	• always • never • occasionally	4 55 41
restricted access to water	• calf otherwise has no access to water • no • yes	34 63 3

If a calf with diarrhea can still stand, it should continue to receive sufficient quality of milk and a mixture of electrolytes. In calves that can not stand up electrolyte must be compensated by intravenous infusion (*Smith, 2010*). Another common opinion among breeders is that deprivation of water in the calf with diarrhea can accelerate the healing (*Relić et al., 2017a*). Calves with diarrhea lose large amounts of fluid and electrolytes, with attendant dehydration and acidosis (*Groutides and Michell, 1990*). That is the reason that in calves suffering from diarrhea ad libitum availability of water is strictly required (*Wenge et al., 2014*). In Table 4 is shown that calves of 34% breeders have no permanent access to the

water. It is recommended to give calves access to water directly after birth, what also stimulates food intake and growth (*Martyn, 2017*).

Occurrence of respiratory diseases in the last year has been reported by 33% of the breeders, while the rest did not notice the signs (difficulty breathing, coughing, wheezing and/or discharge from eyes or nose). An average incidence is 26% of calves per farm. Veterinary intervention was necessary for 80% of them. The reasons for calling veterinarian (Chart 3) are in line with determined in study by *Samolovac* (2016).
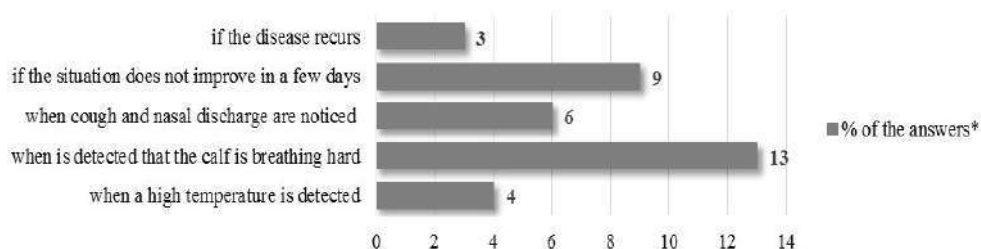
Chart 3. Reasons for calling a veterinarian for calf having respiratory disease (*multiple answers)

On 20% of the farm, the breeder never measures body temperature of the sick calves, and the remaining 80% of the farms it is measured as is needed (according to subjective assessment of the farmer).

In addition to the already mentioned measures that are directly related to the specific disease, as general biosecurity i.e. preventive measures the breeders usually carry out control of flies (35%) and rodents (28%) in the stalls. Some consider that for this purpose it is sufficient to keep only one species of animals in the stall (12%), and the least of them have a disinfection barrier at the entrance of the stall (2%). At 17% of the farms any measure aimed at the prevention of diseases is not implemented.

Successful disease prevention requires the simultaneous, regular and proper application of several hygienic-sanitary and veterinary measures (*Hristov, 2002; Relić, 2014*). According to data in this survey, the breeders implement even less preventive measures than in the similar study by *Relić et al*. (2017). On small farms where different species of animals are present and where traditional methods of breeding are applied, it is very difficult but it is not impossible to implement certain measures, such as isolation of animals and control of movement. Factors that can influence the application of preventive measures on any farm, regardless of its capacity and the species that are breeding, primarily include knowledge, awareness and economic opportunities of the breeder (*Relić, 2014*).

Conclusion

Based on these results, it can be concluded that cattle breeding is the dominant branch of animal husbandry in small rural farms of the Danube Region, with an average of 18 heads of cattle. Rearing technology at these farms is not consistent with the recommendations in many aspects. The main objections are regarding the following: ignorance of the cause of death of calves, high calves' mortality due to accidents, generally unsatisfactory care of newborn calves, practice of calves tethering, hygiene of calf's pen and buckets for milk feeding, quality and temperature control of colostrum/milk, not performing disinfection of new-born calves' navel, insufficient knowledge of signs of illness, delaying or denying veterinary assistance to sick calves, restriction of milk and water to sick calves (as well as limited access to drinking water in general) and insufficient appliance of general preventive measures. All of this could have resulted in a high incidence of diarrhea and high mortality rate due to respiratory diseases, as is found in this study. Corrections in this regard could contribute to better health of calves, and in order to achieve this, better information and a higher motivation of the producer is needed in the first place.

Uslovi gajenja i zdravstveno stanje teladi na malim seoskim farmama

Nenad Stojiljković, Nenad Mićić, Marija Gogić, Vladimir Živković, Bogdan Cekić, Jožica Ježek, Renata Relić

Rezime

Dobri uslovi gajenja su ključni faktor za očuvanje zdravlja i dobrobit teladi. Kao najčešći zdravstveni problemi teladi obično se navode prolivi, infekcije disajnih puteva i infekcije pupka. Cilj ovog istraživanja bio je sagledavanje uslova gajenja i učestalost pojave najčešćih zdravstvenih problema kod teladi na malim seoskim farmama u Podunavskom regionu Srbije. Podaci su prikupljeni sa 30 farmi anketiranjem farmera. Anketa se sastojala od 75 pitanja koja su se odnosila na: opšte informacije o farmi, teljenje, ishranu teladi, odbijanje, smeštaj, higijenu prostora za smeštaj plotkinja i teladi, bolesti koje se javljaju kod teladi i mere preventive bolesti. Prema odgovorima, prosečan broj goveda svih kategorija na farmama je 18. Telad se na 67% farmi odmah po rođenju odvaja od majki. Dobijaju kolostrum 2 sata po rođenju na 47% farmi, a na 20% farmi odmah po rođenju. Prosečna starost teladi pri odbijanju je 13 nedelja. Telad počinje da koristi

seno obično između 1. i 2. nedelje života, a koncentrat u 2. nedelji. Telad se drže u različitim tipovima bokseva. Farmeri su naveli proliv i respiratorne bolesti kao najčešće zdravstvene probleme kod teladi. Uslovi gajenja na farmama često nisu u skladu sa preporukama i odgajivači ne sprovode sve neophodne preventivne mere za sprečavanje pojave bolesti. Korekcije u ovom pogledu bi mogle doprineti boljem zdravstvenom stanju teladi.

Ključne reči: telad, male farme, uslovi gajenja, zdravstveni problemi

Acknowledgement

References

ANON. (2010): Pravilnik o uslovima za dobrobit životinja u pogledu prostora za životinje, prostorija i opreme u kojima se drže, uzgajaju i stavljaju u promet životinje u proizvodne svrhe, načinu držanja, uzgajanju i prometa pojedinih vrsta i kategorija životinja, kao i sadržini i načinu vođenja evidencije o životinjama. Sl. glasnik RS, br. 6/10

AMARAL-PHILLIPS D.A., SCHARKO P.B., JOHNS J.T., FRANKLIN S. (2006): Feeding and Managing Baby Calves from Birth to 3 Months of Age. ASC-161, University of Kentucky Cooperative Extension Service. https://afs.ca.uky.edu/files/feeding_and_managing_baby_calves_from_birth_to_3_months_of_age.pdf

ARSIĆ S., VUKOVIĆ P., KLJAJIĆ N. (2011): Stanje i perspektive proizvodnje mleka u Srbiji. Zbornik naučnih radova Instituta PKB Agroekonomik, 17, 3-4, 57-67.

FRUSCALSO V., OLMOS G., JOSÉ HÖTZEL M. (2017): A farmer survey report on calf mortality rates in southern Brazil. Proceedings of the 7th International Conference on the Assessment of Animal Welfare at Farm and Group Level, September 5-8, 2017, Ede, Netherlands, 236.

GROUTIDES C.P. AND MICHELL A.R. (1990): Changes in plasma composition in calves surviving or dying from diarrhea. British Veterinary Journal, 146, 205–210.

HRISTOV S. (2002): Zoohigijena. Univerzitet u Beogradu, Poljoprivredni fakultet.

HUZZEY J.M., VON KEYSERLINGK M.A.G., WEARY D.M. (2005): Changes in Feeding, Drinking, and Standing Behavior of Dairy Cows During the Transition Period. Journal of Dairy Science, 88: 2454–2461.

LANG B. (2008): Colostrum for the Dairy Calf. OMAFRA factsheet 411/23. http://www.omafra.gov.on.ca/english/livestock/veal/facts/08-001.htm

LORENZ I., MEE J.F., EARLEY B., MORE S.J. (2011): Calf health from birth to weaning. I. General aspects of disease prevention. Irish Veterinary Journal, 64(1): 10. doi: 10.1186/2046-0481-64-10

MARTYN R. (2017): Part one: Calf rearing – small farmers' guide to feeding and raising calves. http://farmstyle.com.au

MEE J.F. (2008): Newborn Dairy Calf Management. Veterinary Clinics of North America: Food Animal Practice, 24(1):1-17.

MORAN J. (2002): Calf Rearing: A Practical Guide. Second edition. Landlinks Press, Australia.

MILJKOVIĆ V., VESELINOVIĆ S. (2005): Porodiljstvo, sterilitet i veštačko osemenjavanje domaćih životinja. Univerzitet u Beogradu, Fakultet veterinarske medicine.

POPOVIĆ R. (2014): Stočarstvo u Republici Srbiji. Popis poljoprivrede 2012. Republički zavod za statistiku. Beograd.

RELIĆ R. (2014): Biosigurnost na malim farmama preživara. XXV Savetovanje Dezinfekcija, Dezinsekcija i Deratizacija – jedan svet jedno zdravlje, sa međunarodnim učešćem. 29. maja – 1. juna 2014., Kovačica, Srbija, 39-44.

RELIĆ R., HRISTOV S., SAMOLOVAC LJ., BOJKOVSKI J., ROGOŽARSKI D. (2014): Colostrum Management in Calves' Welfare Risk Assessment. Bulletin UASVM Veterinary Medicine 71(1): 187-192.

RELIĆ R., MIĆIĆ N., JEŽEK J. (2017): Primena higijensko-sanitarnih mera na malim seoskim farmama. Zbornik radova XXVIII Savetovanje Dezinfekcija, Dezinsekcija, Deratizacija - jedan svet jedno zdravlje, sa međunarodnim učešćem, 25. - 28. maj 2017. godine, Ečka, Srbija, 121-126.

RELIĆ R., SAVIĆ M., BECKEI ZS., KUČEVIĆ D., STARIČ J., JEŽEK J. (2017a): Watering practices at small-scale farms of ruminants. Proceedings of International Symposium on Animal Science (ISAS) 2017, 05th - 10th June 2017, Herceg Novi, Montenegro, 296-301.

ROMČEVIĆ LJ., TRIFUNOVIĆ G., LAZAREVIĆ LJ. (2007): Govedarstvo Srbije. Univerzitet u Beogradu. Poljoprivredni fakultet.

SAMOLOVAC LJ. (2016): Uticaj uslova gajenja i sezone rođenja na dobrobit teladi u prvom mesecu života. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.

SMITH G. (2010): Dealing with calf diarrhea. HoardsDairyman. https://hoards.com/article-1797-dealing-with-calf-diarrhea.html

STOJANOVIĆ B., GRUBIĆ G., ADAMOVIĆ M., ĐORĐEVIĆ N. (2007): Važnije nutritivne i fiziološke osnove ishrane teladi. 5. Simpozijum „Ishrana, reprodukcija i zaštita zdravlja goveda", Tematski zbornik radova 5. Simpozijuma „Ishrana, reprodukcija i zdravstvena zaštita goveda"-Poremećaji zdravlja krava u

puerperijumu i zdravstveni status teladi, 4. – 7- jula 2007. godine, Banja Kanjiža, 65-78.

WENGE J., STEINHÖFEL I., HEINRICH C., COENEN M., BACHMANN L. (2014): Water and concentrate intake, weight gain and duration of diarrhea in young suckling calves on different diets. Livestock Science, Vol. 159, 133–140.

ESTIMATES OF GENETIC PARAMETERS FOR GROWTH TRAITS OF LOCAL HAMRA BREED THREATENED WITH EXTINCTION IN SOUTHWESTERN ARID RANGELAND OF ALGERIA

Houari Yerou[1], Hakim OuldOuelhadj[2], Abdelrahmane Berber[3], Amine Mokadem[1], Bachir Koudad[2]

[1] Department of Agriculture, Faculty SNV, University of Mascara, BP 305- 29000. Algeria
[2]National breeding Technical institute ITELV.Saida20000, Algeria
[3]Agricultural Direction DSA, Saida, 20000, Algeria
Corresponding author email: Yerrou_houari@yahoo.fr
Original scientific paper

Abstract: The data base for body weights and growths of 411 Hamra lambs were used to estimate genetic parameters. These lambs were obtained from 31 rams and 493 ewes between 2012 and 2017. Traits analysed were weights at birth (B_W), 30 days (W_{30}), 90 days (W_{90}) of age, and average daily gains from 10 to 30 (ADG_{10-30}) and30-90 (ADG_{30-90}) days. REML estimates of variance and covariance components were obtained assuming animal models that included the fixed effects for year-type of birth (2012 single, 2012 twin, . ., 2017 single, 2017 twin), sex (male, female), and ewes age at lambing (< 18, 18-30, 30-42, 42-54, >54 months). Heritabilities were 0.12, 0.06 and 0.11 respectively for B_W, $W_{3O}$and $W_{90}$and the average daily gains were 0.05 and 0.17 for $ADG_{0-30}$and ADG_{30-90}. The estimates of genetic correlations showed no genetic antagonisms among the growth traits. The genetic correlations estimated were positive and medium to high, except those between ADG_{30-90} and ADG_{0-30} and between ADG_{30-90} and W_{30} which were slightly negative. Phenotypic correlations were positive and ranged from 0.12 to 0.85. They were high between adjacent weights and between ADG and their corresponding weights.

Keywords: Heritability, Genetic correlation, Growth Traits, Sheep

Introduction

The small ruminants contribute substantially to the livelihoods and food security of rural Mediterranean farms in dry areas of the North Africa region (*Bengoumi et al., 2013*).However, because of instability of resources and inputs,

climatic challenges, conflicts and security, livestock rearing in the marginal dry areas is challenged by low productivity and poor access to markets. On a global scale and in animal production in general, the competition looks terrible and any country which will not be able to organize itself is condemned to know serious economic and social problems. Local breeds represent an important component of farm animal diversity to be maintained and exploited. The Modern breeding animals involve genetic improvement of animals by applying the basic principles of quantitative genetics. In order to achieve this genetic improvement, it is necessary to properly select the superior parents of future generations. For all this it is necessary a good knowledge of genetic parameters heritability, correlation, covariance and variance *(Thompson et al., 2005)*.Otherwise knowledge of the genetic parameters for economically important traits of animals is necessary, is essential in order to evaluate the breeding values of individuals, made an effective plan and program breeding, and evaluate effects of selection. Algerian country is home to sheep genetic resources that renown all over with 6 principal breeds and 28 millions of heads sheep (*Yerou, 2013; MADR, 2017*).The diversity of local breeds is valuable asset for the development of agriculture and territories in Algeria and this diversity of breeds constitute a good insurance for the future. Small ruminant production throughout the region is mainly based on traditional systems where animals are managed through an agro-pastoral and extensive system with large flocks grazing in pastoral and mixed farming environments with varying degrees of livestock movement from nomadic to transhumance. However, breeding techniques currently used are generally rudimentary and limit considerably the productive capacity of this species leading to a mow productivity rate (*Yerou, 1998; Dekhili and Aggoun, 2006*) thus contributing to a lack of red meat production. Genetic diversity found in Algerian domestic breeds favored the adaptation of livestock to various environmental conditions and stresses, including disease, parasites, temperature, humidity and many other factors. Though, the programs undertaken by government for the development of this genetic resources capital were confronted to a limited knowledge of the basic genetic material and the inexistence of in situ conservation strategies, as illustrated by the case of the reduction of more than 50 % for the Hamra sheep breed (*Yerou, 1998, 2013*).The Hamra or Daghma breed is native to eastern Morocco; its extension area is between ChottEch-Chergui in the east, of Saharian Atlas in the south-east, Morocco in the west and the Tlemcen and Saida mountains in the North. Many authors agree that it has several advantages: good maternal skills, good reproductive qualities, good resistance to harsh conditions, good use of coarse foods and good walking skills (*Chellig, 1992; Benyoucef et al., 1995; Yerou, 1991*). Despite its good conformation and excellent quality meat, the effect never ceases to regress. It was estimated at 6 million in 1969s more than 2.5 million head in the 1980s, is at present only about 0.5 million head. The downsizing of Hamra puts this breed in a situation of breed at risk (*IANO, 2007*; *Yerou, 2013*). The work done on the Hamra

breed is rare; the existing ones have been limited in numbers and for short periods. However, there is no report of genetic parameters for traits in this breed, calculated using animal model methodology and accounted for maternal genetic effect.The risk of disappearance of this population among breeders of steppe and pre-saharian rangeland is a priority in research programs because they are animals well adapted to environmental conditions that take a particular interest in the context of global warming. It is a national genetic heritage which must be able to manage and maintain. There is no published research on genetic traits of Hamra sheep, to date. Thus, this paper analyzed data from ITELV (StationNational breeding technicalinstitute), and estimated genetic parameters of growth traits of lamb weights and daily gains from birth to weaning, providing a scientific evidence for breed selection in futureat the station.

Materials and methods

Animals and management

The data used in this study to estimate genetic parameters of growth traits were obtained from flocks in ITELV of Ain El Hadjar. An initial database of 441Hamralamb's progeny of 493 ewes, collected during six years (from 2012 to 2017), were used. The station is situated 11 km in west-South of Saida at 1015 m average altitude, cordoned 34-35 of Latitude and 0-8^{E}longitude. Climate is arid; the maximum temperature recorded is approximately 39°C in summer (august), while the temperature drops to -2°C in January. The mean annual rainfall is around 437 mm. Ewes were managed in extensive lambing rhythm to lamb in autumn (September, October, and November). The lambs had complete records for all traits from birth to weaning. The environmental, nutritional, and management conditions were the same for all of the animals. The rams were 2-5 years of age and kept separated from ewes, generally. The mating season start from 10 April to 20 May. The lambs were weighed, early after birth, the date; sex and type of birth were recorded and at average age of 90 days the lambs were weaned. Supplemental feeding was offered during mating and late pregnancy. During lambing and suckling periods, the experimental herd receives sheep concentrate and ground barley and oat forage. An annual program of vaccination, deworming and dipping was carried out for all animals. Lambs were weighed individually every 20 days, until weaning at around 90days of age. Lambs birth weight (B_W), age-standardized weights at 30 days (W_{30}), and 90 days (W_{90}) of age and average daily gains from 10 to 30 (ADG_{10-30}) and 30 to 90 days (ADG_{30-90}) were analysed.

Statistical analysis

Recorded data were statistically analysed using, fixed linear models with the proc GLM for General Linear Model in SAS software (SAS Institute, 2000).

Fixed model included effects for year-type of birth (2012 single, 2012 twin, .., 2017 single, 2017 twin), sex (male, female), and ewes age at lambing (< 18, 18-30, 30-42, 42-54, >54 months) for B_W, W_{30} and ADG_{10-30}, and the same fixed effects, except year-type of birth effect was replaced by year-type of rearing for W_{90} and ADG_{30-90}. To estimate the genetic parameters, the same fixed effects were integrated in mixed model together with the random animal effects. The estimation of covariance components was obtained using the software MTDFREML (*Boldmanand et al., 1993*). Heritabilities were initially estimated using single-trait analyses. Then, genetic correlations between all traits were estimated using two-trait analyses which accounts for only one random genetic effect, direct genetic effect, in the model. The standard errors of genetic correlations were calculated using the approximate formula given by *Falconer and Mackay (1996).*

Results and discussion

Non genetics factors effects

Average weights and daily gains, standard deviations, and the proportion of variation explained by the models (R^2) are given in Table 1. The analyses of variance showed that the fixed models explained 35to 51% of the phenotypic variances in all traits, and that effects of year-type of birth, the sex were important environmental sources of variation for all growth traits ($p<0.01$) but the age of ewe was only significant for traits B_W and W_{30} of age, Table 2.

Table1. Variation of Average weights (Kg) and Average daily gains (g) of lambs

Traits	B_w	W_{30}	W_{90}	ADG_{10-30}	ADG_{30-90}
μ	3.4	7.8	15.1	132	141
SD	0.48	1.2	1.8	32	29
R^2 of the model (%)	41	49	51	35	48

μMean, (SD) standard deviations, (R^2, %) of total variance explained by model

Table 2.Significance of model for weights and Average daily gains of lambs

Effects	B_w(kg)	W_{30}(kg)	W_{90}(kg)	ADG_{10-30}(g)	ADG_{30-90} (g)
Sex	**	**	**	**	**
Ewe age	**	**	NS	**	NS
Year-birth type	**	**	**	**	**

B_W: Birth weight, W_{30}, W_{90}: weights at 30, 90 days age, ADG_{10-30}, ADG_{30-90}: average daily gain.

These effects of non-genetic factors are in accord with those signalled in other south Mediterranean studies on growth traits in Ouled Djellal (*Allaoui* and al, 2013; *Dekhili and Aggoun, 2006a, b*), Beniguil *(Boujenane and Mharchi, 1992)*,Timahdit *(EL Kihal, 1990,Tijani and Boujenane, 1993)* breeds. As a result,

growth records should be adjusted for these non-genetic factors in subsequent analyses.

Genetics factors effects

Heritability parameter

The estimated values of heritability's for growth traits using the variance and regression components methods are presented in Table 3.In general estimated values were generally low to medium. As regards, the standard errors related to h^2werewider. Heritabilities of B_W, W_{30}, at W_{90}, average daily gain ADG_{0-30} and ADG_{30-90} are respectively equal to 0.12 and 0.06, 0.11 and 0.05, 0.17. The set of heritability values estimated in our study coincide with those indicated by *Fogarty et al. (1985)*; *Khaldi et al. (1987)*; *Nacir (1987); Abdulkhauqa et al. (1989)*; *El kihal (1990)*; *Boujenane* and *Mharchi (1992)*.The low h^2 estimates for B_W fall within the range of values reported for Mediterranean breeds in Beniguil lambs ($h^2= 0.15$) *Boujenane and Mharchi (1992)*, and in D'man ($h^2= 0.34$) *Boujenane et al.* (2013).

Table 3. Estimated values of heritability's (h^2)and genetic correlations (r)of growth traits

Traits	$h^2 \pm$ SD.	r_{WB}	r_{w30}	r_{w90}	$r_{ADG\,0-30}$	$r_{ADG30-90}$
B_W(Kg)	0.12±0.08	-	0.39	0.37	0.12	0.11
W_{30}(Kg)	0.06±0.09	0.69±0.19	-	0.67	0.85	0.24
W_{90}(Kg)	0.11±0.08	0.36±0.33	0.28±0.31	-	0.57	0.73
ADG_{0-30} (g)	0.05±0.06	0.22±0.35	0.72±0.07	0.12±0.32	-	0.28
ADG_{30-90}(g)	0.17±0.09	0.10±0.31	-0.13±0.35	0.83±0.08	-0.18±0.23	-

B_W:Birth weight ,W_{30},W_{90}: weights at 30, 90 days age, ADG_{10-30} and ADG_{30-90}: average daily gain.

On the other hand, heritabilities of daily average weights and gains, estimated increase with age. These estimates highlight the existence of a mother effect on growth performance and show that this effect decreases with the advancing age of the lamb. The value of the heritability obtained by the variance components gives an idea about the sum of direct and maternal effects. Otherwise in sheep, *Burfening and Kress (1993)* found important h^2, ranging from 0.30 to 0.65, depending on the model applied, for B_W in Rambouillet, Targee and Columbian breeds. *Poivey et al. (1994)* reported medium h^2 estimate of 0.30 for W30 in Ile de France lambs. Our estimates of h^2 were higher than those reported by (*Maria et al., 1993; Tosh and Kemp, 1994*) for B_W and $W_{90}$of Romanov lambs respectively 0.22, 0.01, and of 0.13 and 0.02. These differences are probably due to

the conditions of the breeding environment, the genetic potential of each breed and the size of the experimental sample and the estimation model. For average daily gains, the h^2 estimates were lower than those reported for other breeds *Tijani and Boujenane (1993)* for Timahdit lambs, and *Boujenane and Mharchi,(1992*) for Beniguil lambs.Our h^2 estimates for ADG30$_{-90}$ were higher than those observed by *Khaldi and Boichard, (1989)* and *Maria et al. (1993)*, who reported low estimates of maternal heritability ranged from 0.01 to 0.06 for growth traits in Barbarine and Romanov sheep.

Genetic correlations

The values of genetic correlations obtained in this estimation for Hamra breed are generally positive and average. Otherwise under breeding systems conditions similar to our country the results of genetics correlation for local breeds obtained for the Moroccan breeds (*Boujenane and Mharchi, 1992; Djemali et al., 1994*) were ranged from 0.59 to 0.92 between W_{30} and the first month of suckling, and from 0.79 to 0.98 between W_{90} and ADG$_{30-90}$.The highest genetic correlation (0.83) was recorded between W_{90} andADG$_{30-90}$ day, while the lowest was found between W_{90} and ADG$_{0-30}$ (0.12). Two negative correlations were obtained between W_{30} and ADG$_{30-90}$ (-0.13) and between ADG$_{0-30}$ and ADG$_{30-90}$ daily gain (-0.18).The genetic correlation between (W_{30}, ADG$_{0-30}$) was higher (0.85) and those between (B_W and ADG$_{0-30}$) was lowest value (0.12). The values of the genetic correlations between (ADG$_{30-90}$ W_{30}) and (ADG$_{30-90}$,ADG$_{0-30}$) which are negative, the other estimated values are positive and indicate that selection on a character will guide to the improvement of other characters. The negative correlation value gives an idea of the lamb's earliness, and the value of ADG$_{0-30}$, used as a decisive factor for assessing the dairy value, the maternal comportment and aptitude of suckling of the ewe, shows that the improvement of one of the two characters without deteriorating the other requires the selection of lambs on the basis of a selection index combining both characters at a time. Positive genetic correlations are in the range of the values indicated by the authors cited above. The correlations between the different characters are between 0.11 and 0.85. They are higher between the close weights and between the weights and the corresponding gains.

Conclusions

All over the world, local small ruminant breeds such as sheep are playing key roles in the lives of farming systems in arid area; no serious consideration is given to sheep genetic assets management policies in Algeria. The current investigation was conducted to evaluate the genetic parameters for growth traits in the endangered local Hamra breed. The results reveal the presence effects of some

non-genetic factors on growth traits. In the light of the results obtained, it can be conclude that the growth performance achieved by the lambs at ITELV station is satisfactory but remain below the potentialities of the breed cited in literature. The heritability estimates obtained in this study indicated that it would be possible to improve growth traits through genetic selection at all ages. The estimates of genetic correlations were high and showed no genetic antagonisms among the growth traits. However, estimates of genetic correlations were high and negative but the accuracy of such estimates is low due to the small data set in the present study. In addition the growth performance of Hamra breed is largely influenced by the age of ewe, sex and mode of birth. Thus any breeding program aimed at improving these performances must eliminate the effect of these factors through the use of appropriate adjustment factors

Acknowledgements

Procene genetskih parametara za osobine porasta kod ugrožene lokalne rase hamra u jugozapadnoj pustinji Alžira

Houari Yerou, Hakim Ould Ouelhadj,Abdelrahmane Berber, Amine Mokadem, Bachir Koudad

Rezime

Baza podataka za težinu tela i porast 411 jagnjadi rase hamra korišćena je za procenu genetskih parametara. Ova jagnjad su dobijena od 31 ovna i 493 ovce u periodu između 2012. i 2017. godine. Analizirani su sledeće osobine: težina na rođenju (B_w), uzrastu od 30 dana (W_{30}), 90 dana (W_{90}) starosti i prosečni dnevni prirasti od 10 do 30 (ADG_{10-30}) i 30-90 (ADG_{30-90}) dana. REML procene komponenti varijanse i kovarijanse dobijene su pod pretpostavkom da su životinjski modeli uključivali fiksne efekte za godinu-tip rođenja (2012 jedinaca, 2012 blizanac, ... 2017 jedinaca, 2017 blizanaca), pol (muški, ženski) i uzrast ovce na jagnjenju (<18, 18-30, 30-42, 42-54,> 54 meseci). Heritabiliteti su bili 0,12; 0,06 i 0,11 za B_w, W_{30} i W_{90}, a prosečni dnevni prirasti su bili 0,05 i 0,17 za ADG_{10-30}i ADG_{30-90}. Procene genetskih korelacija nisu pokazale genetske antagonizme između osobina porasta. Procenjene genetske korelacije su bile pozitivne i srednje do visoke, osim onih između ADG_{30-90} i ADG_{0-30} i između ADG_{30-90} i W_{30} koje su bile negativne. Fenotipske korelacije su bile pozitivne i

kretale su se od 0,12 do 0,85. Bile su visoke između susednih težina i između ADG i njihovih odgovarajućih težina.

Ključne reči: heritabilnost, genetička korelacija, osobine porasta, ovce

References

ABDULKHAUQA A.M., HARVEY W.R., PARKER C.F. (1989): Genetic parameters for ewe productivity traits in the co1umhia, Suffolk and Targhee. Animal Science, 67, 3250-3257.

ALLAOUI A., SAFSAF B., DJAALIB I., LAGHROUR W., TLIDJANE M: (2013): Efficiency of ovine artificial insemination in Ouled Djellal breed. Option Mediterranean, A: n 108.61-65.

BENGOUMI M., AMEZIANE EL HASANI T. (2013): Evolution and efficiency of transfer of technology in small ruminant production systems in North Africa. Option Mediterranean, A; n 108.15-24.

BENYOUCEF M. T., ZAHAF A., BOUTEBILA, S. (1995): Aspects organisationnels et techniques d'un programme d'étude génétique de la race ovine Hamra dans la région de l'ouest (Algérie). Ciheam-Iamz, 11, 215-224.

BOLDMAN K.G., KRIESE A., VAN VLECK L.D., KACHMAN S.D. (1993): A manual for use of MTDFREML.USDA, ARS, Washington, DC, 114 pp.

BOUJENANE I., MHARCHI A. (1992): Estimation des paramètres génétiques et phénotypiques des performances de croissance et de viabilité des agneaux de race Beniguil. Actes Institut. Agronomique. Veterinaire, 12 (4), 15-22.

BOUJENANE I., CHIKHI A., SYLLA M., IBNELBACHYR M. (2013): Estimation of genetic parameters and genetic gains for reproductive traits and body weight of D'man ewes. Small Ruminant Research, 113: 40-46.

BURFENING P.J., KRESS D.D. (1993): Direct and maternal effects on birth and weaning weight in sheep. Small Ruminant Research, 10, 153-163.

CHELLIG R. (1992): Les races ovines algériennes.Office des Publications Universitaires. Ben-Aknoun, Alger.

DEKHILI M., AGGOUN A. (2006a): Paramètres génétiques de la productivité numérique des brebis Ouled-Djellal. 13es Rencontres autour des Recherches des Ruminants. INRA, 13: 221.

DJEMALI M., ALOULOU, R., BENSASSI, M. (1994): Adjustment factors and genetic and phenotypic parameters for growth traits of Barbarine lambs in Tunisia. Small Ruminant Research, 13, 41-47.

EL KIHAL (1990) : Analyse des performances de croissance et de reproduction chez la raceTimahdit à l'UREO de Sidi Aissa. Mémoire de 3ème Cycle Agronomie, Institut. Agro -Veterinaire. Hassan II, Rabat.

FALCONER D.S., MACKAY T.F.C. (1996): Introduction to quantitative genetics, 4th Edition. Longman, London, 464 pp.

FOGARTY N.M., DICKERSON G.E., YOUNG L.D. (1985): Lamb production and its components in pure breeds and composite lines. III. Genetic parameters. Journal. Animal Science, 66: 40-57.

IANO (2007): Le standard de la race Hamra. Institut Algérienne de Normalisation n°2 édition: Date 2007. 6p Cinfo@ianor.org.

KHALDI G., BOICHARD D. (1989): Effets directs et maternels sur les caractères de croissance en race ovine Barbarine. INRATARIANA (Tunisie) 62 (2), 3-20.

KHALDI G., BOICHARD D,. TCHAMITCILLAN L. (1987): Facteurs de variation des paramètres de croissance des agneaux la race barbarine annales de 'INRA. Tunisie, 60, 5-18.

KUCHTÍK J., DOBEŠ I. (2006): Effect of some factors on growth of lambs from crossing between the Improved Wallachian and East Friesian. Czech Journal of Animal Science, 51, 2, 54–60.

MADR. (2017): Statistique du ministère de l'agriculture et du développement rural.

MARIA G.A., BOLDMAN K.G., VAN VLECK, L.D. (1993): Estimates of variances due to direct and maternal effects for growth traits of Romanov sheep. Journal of Animal Science, 71, 845-849.

NACIR O. (1987): Estimation des paramètres génétiques des performances de reproduction et de croissance chez les races d'man et sardi mémoire 3ème cycle agronomie, I.A.V. Hassan II, rabat. Maroc

POIVEY J.P., JULLIEN E., BIBE B. (1994): Utilisation du modèle animal chez les ovins allaitants. In: Foulley, J.L., Molenat, M. (Eds.), Séminaire modèle animal, 26-29 septembre 1994.

SAS. (2000). User's Guide, Release 6.12 Ed., SAS Institute, Cary, NC.

THOMPSON R., BROTHERSTONE S., WHITE I. M. S. (2005): Estimation of quantitative genetic parameters. Philosophical Royal Society B, 360, 1469–1477.

TIJAN A., BOUJENANE I. (1993): Estimates of genetic and phenotypic parameters for growth traits of Timahdit lambs in Morocco. The 44th Annual Meeting of the European Association for Animal Production (1), 176-177.

TOSH J.J., KEMP R.A. (1994): Estimation of variance components for lamb weights in three sheep populations. Journal of Animal Science, 72, 1184-1190.

YEROU H. (1991): Contribution a l'étude des performances laitières de la race Hamra élevée a ITEBO- Ain El Hadjar. Mémoire Ingénieur Institut national Agronomique-Alger.

YEROU H. (1998): Caractérisation des systèmes d'élevage ovin dans la steppique de la commune de Maamora Wilaya de Saida Thèse de Magister 110p Institut national Agronomique-Alger.

YEROU H. (2013): Dynamique des systèmes d'élevage ovin et leurs impacts sur l'écosystème steppique cas de la Wilaya de Naama.Thèse doctorat Es Science 107p. Université de Tlemcen. Algérie.

EFFECT OF SINGEING METHODS ON CARCASS QUALITIES AND SENSORY PROPERTIES OF RED SOKOTO BUCK MUSCLE

Ani Ugochukwu[1], Elisha Zhiri Jiya[1], Ocheme[2] Boniface Ocheme

[1]Department of Animal Production, Federal University of Technology Minna
[2]Department of Food Science and Technology, Federal University of Technology Minna
Original scientific paper

Abstract: An experiment was conducted using twenty four red Sokoto bucks to evaluate effect of singeing methods on the carcass quality and sensory properties of red Sokoto buck (Chevon) meat. The study was carried out at the Animal Production Teaching and Research laboratory, Federal University of Technology Minna. The buck carcasses were randomly allocated to four singeing methods of hot water, firewood, rubber tyre and kerosene singeing after slaughter. Data were collected on the sensory parameters, mineral composition, physical and chemical properties and proximate composition. Singeing methods significantly ($P<0.05$) affects the crude protein and fat contents of the meat samples with crude protein significantly ($P<0.05$) higher in rubber tyre (31.53%) singed carcasses. While the fat content were significantly ($P<0.05$) higher in hot water (5.80%) singed carcasses. The mineral contents differs significantly ($P<0.05$) among singeing methods. The pH and thermal shortening were significantly ($P<0.05$) higher in buck carcasses singed with kerosene (6.75 and 35.35 respectively). Methods of singeing had significant effect on the carcass quality and sensory properties of meat samples. It was therefore concluded that hot water be used to remove hairs in slaughtered goat carcasses, as this will reduce to practicable level cross contamination of meat during processing. The use of firewood, tyre and kerosene should be total discouraged in meat processing and the public should be educated about the health implication of consuming animal carcasses singed with these methods.

Key words: singeing methods, carcass qualities, sensory properties, buck, muscle

Introduction

Goat meat is the world's most popular meat, as about 75 % of the world populations eat goat meat (*Suzanne, 2012*). Goat meat is lower in fat than chicken,

but higher in protein than beef. It is preferred to beef, mutton and pork due to its lower calories, total fat content, saturated fat and cholesterol (*Anaeto et al., 2010*). These low levels of saturated fat and cholesterol, combined with its high iron and protein content, make goat meat a good choice for anyone looking for a healthy red meat. It is a leaner, healthier choice when compared to equal serving sizes of chicken, beef and pork (*Suzanne, 2012*). *Anaeto et al. (2010)* reported that goat meat is easier to digest as a result of its molecular structure, thus it presents a healthier alternative compared to other types of red meat.

        The quantity and quality of meat produced are increasingly becoming important. This situation arose from the increased consciousness and demand of consumers who desired meat characterized by special dietetic and health properties (*Brunso et al., 2005*). Meat quality is a generic term used to describe properties and perception of meat which includes attributes such as carcass composition and configuration, eating property of meat, health issues associated with meat, as well as animal welfare and environmental impact (*Maltin et al., 2003*). Meat quality is very important for consumers when it comes to making purchasing decisions. Many factors (both pre and post mortem) can affect carcass quality from producer to consumer. Paramount among the post mortem activities that affect nutritional and eating qualities of meat are processing methods especially flames from singeing that generates phenolic substances, which are of considerable importance to the organoleptic properties of roasted meat products and show antimicrobial as well as antioxidative properties (*Wolfgang et al., 2013*), and also the biochemical changes that occur in meat during post- slaughter storage and distributions (*Keith et al., 2002*). Most consumers especially in Africa eat chevon meat together with the skin processed by singeing with either hot water, open fire using either tyre, fire wood and or kerosene (*Omojola and Adesehinwa, 2006; Obiri-Danso et al., 2008*). Singeing maintains the carcass hide for consumption and also evokes flavours in the meat which are acceptable to the consumer (*FAO, 1985*). The aim of this study was to evaluate the effect of singeing methods on carcass quality of red Sokoto buck muscle**.**

Materials and methods

Experimental animals

        Twenty four red Sokoto bucks, seven (7) to eight (8) months old, with body weight between 10 to 12 Kg were used for this study. The animals were purchased from Beji Livestock Market in Bosso Local Government area of Niger State, Nigeria and allowed to acclimatize to the same diet conditions for two weeks. They were dewormed, dipped and vaccinated against known parasites and diseases. The goats picked were kept off feed for 12 hours and slaughtered by severing the jugular vein at the neck region and allowed to bleed for 5 minutes after which the carcass were weighed. Their carcasses were randomly assigned to

four singeing methods namely: hot water singeing, firewood, rubber tyre and kerosene singeing. Each method was replicated thrice with two goats per replicate. Hot water was used to process the goat meat according to the method described by (*Monin et al., 1995*). Hot water of 75 to 90^{0}C was poured on each slaughtered buck carcass to soften the hairs which was then scrapped with razor blade and afterward washed with sponge and clean water. The group singed with fire wood was carried out as described by *Okubanjo (1997).* Each slaughtered goat carcass was roasted on firewood (230-250^{0}C) derived from shea wood until the hairs are carefully burnt off with minimal damage to the skin. Fresh clean water with sponge was used to wash off the charred surface as much as possible, thus to reduce the level of contamination on the carcass. Group three were roasted using car rubber tyres. The hairs from the slaughtered goat carcasses were carefully burnt off with minimal damage to the skins. After which fresh clean water with sponge was used to wash off the charred surface in order to reduce the level of contamination on the carcass. The fourth group was carried out using kerosene. Kerosene was rubbed all over the slaughtered goat carcass and then set ablaze until the hairs were carefully burnt off, with minimal damage to the skin. Fresh clean water with sponge was used to wash off the charred surface as much as possible to reduce the level of contamination on the carcass.

Data were collected on proximate and mineral compositions of buck meat samples using the procedures of (*AOAC, 2000*). The pH of the meat was determined. 10g of meat samples were homogenized for two minutes with 90 mls of distilled water using a laboratory blender (plate 5mm) model 242, Nakal Japan, the meat suspension was filtered and the pH of the meat was measured using a digital pH meter model H18424 micro-computer, Havanna Instruments Romania. The water holding capacity was determined using the procedures described by *Kauffman et al. (1992).* Cooking loss, thermal shortening and cooking yield were determined using the procedures described by *Kauffman et al. (1992*).

The sensory evaluations of the meat samples were carried out as described *by Iwe, (2002*). The (*Musculus semimembranosus*) from leg cut was cut into bites sizes. There were boiled in water without any form of seasoning for 30 minutes and allowed to cool. The boiled meat samples were served in plates to a twenty (20) member semi-trained panelists to assess the colour, tenderness, juiciness, flavour and overall acceptability using a 9 point hedonic scale (where 1= dislike extremely and 9=like extremely).

Data Analysis

The data collected from this study was subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS Version 9.0). The variations in means were separated using Student-Newman-Keuls (SNK) test at 5% level of probability.

Results and discussion

The effect of singeing methods on the proximate composition of meat samples obtained from red Sokoto buck carcasses is shown in Table 1. The results showed that moisture content, ash, crude fibre and nitrogen free extract were not significantly (P>0.05) affected by the singeing method. However, crude protein and fat contents were significantly (P<0.05) affected by the singeing method. Hot water singed carcasses (27.70) were significantly (P<0.05) lower in protein than tyre (31.53) but not lower than others. The significantly (P<0.05) higher amount of protein observed in buck carcasses singed with tyre, kerosene and firewood over that of hot water mighty be due to lesser amount of fat contents observed in buck carcasses singed using these methods, thus leading to an increase in the amount of protein. This result is in agreement with the reports of *Akwetey et al. (2013)* and *Ijeoma et al. (2015).* The authors reported high protein content in carcasses singed with tyre and firewood and lower fat content with the same carcasses.

Fat content were significantly (P<0.05) higher in hot water (5.80) singed carcasses than the others. This significantly (p<0.05) higher amount of fat content observed in carcasses singed with hot water over those singed with rubber tyre, kerosene and firewood may be due to the buck carcass processed with hot water did not undergo intensive heat treatment, therefore the subcutaneous fat content were not altered by the intensity of the heat treatment, while the variations in temperature of the thermal treatments applied may be responsible for fat losses observed in the other methods due to the degradation of the fatty acid by heat treatment. This finding disagreed with the findings of (*Apata, 2014*) that reported lower fat content in meat samples prepared with hot water over those singed with rubber tyre and firewood. Meat composition, especially its fat content, combined with specific heat treatment methods is among the factors that mostly affect the final quality of meat products (*Alflaia et al., 2010*). Thermal treatments can cause undesirable changes, such as loss of essential fatty acids (FA), mainly due to lipid oxidation, reducing the nutritive value of meat.

Table 1. Proximate composition of meat samples from red Sokoto buck singed using different methods

Parameters %	HW	FW	TYR	KERO	SEM	LS
Moisture	24.50	23.29	24.68	23.96	0.674	NS
Crude protein	27.70[b]	29.33[ab]	31.53[a]	30.21[ab]	0.504	*
Crude fibre	0.67	0.26	0.56	0.27	0.126	NS
Fat	5.80[a]	2.67[b]	2.87[b]	2.30[b]	0.538	*
Ash	3.53	2.16	2.43	1.80	0.321	NS
NFE	37.81	42.30	37.94	41.46	0.966	NS

a, b Means in the same row not followed by the same superscript are significantly different (P<0.05). HW = Hot water, FW = Firewood, TYR = Rubber tyre, KERO = Kerosene, NFE = Nitrogen Free Extract, LS= Level of significant, *= Significantly different at (P<0.05), NS = Non significantly different.

The result on the effect of singeing methods on the mineral contents of meat samples from red Sokoto buck (Table 2) revealed that singeing methods had significant (P<0.05) effects on some of the mineral composition tested. Meat samples singed with tyre (12.89) were significantly (P<0.05) higher in calcium and the least values were recorded in hot water (12.31) singed carcasses, while those carcasses singed with firewood and kerosene were statistically similar. Magnesium and potassium were shown to be significantly (P<0.05) higher in carcasses singed with tyre (19.86 and 296.13 respectively) than other meat samples. These significant (P<0.05) differences obtained in calcium, magnesium and potassium among carcass singed with tyre, firewood as well as kerosene over those singed with hot water may be attributed to the more moisture losses observed with those carcasses leading to the concentration of these mineral contents. This result is in agreement with the report of *Lawrie and Ledward (2006)* who reported increase in mineral concentration in meat as a result of moisture loss through cooking. The results also showed that sodium (68.76) and phosphorus (176.48) were significantly (P<0.05) higher in carcasses singed with hot water and the least values were obtained with those carcasses singed with firewood (61.63 and 153.61 respectively). However the observed increase in sodium and phosphorus among carcasses prepared with hot water over other methods might be due to direct loss of mineral as a result of higher temperature observed with those methods. This is also in agreement with *Gerber et al. (2009)* the authors reported loss of minerals in meat due to heat treatment. The lesser the moisture content, the more the mineral concentration, but as the intensity of the heat and temperature increases the higher the mineral losses due to leaching.

Table 2. Mineral content of meat samples from red Sokoto buck singed using different methods

Parameters (mg/100g)	HW	FW	TYR	KERO	SEM	LS
Calcium	12.31[c]	12.79[ab]	12.89[a]	12.55[bc]	0.078	*
Magnesium	19.10[c]	19.56[b]	19.86[a]	19.33[bc]	0.091b	*
Potassium	292.59[b]	292.92[b]	296.13[a]	291.55[b]	0.583	*
Sodium	68.76[a]	61.63[d]	67.10[b]	64.65[c]	0.822	*
Phosphorus	176.48[a]	153.61[d]	155.44[c]	162.05[b]	2.71	*

a, b Means in the same row not followed by the same superscript are significantly different (P<0.05). HW = Hot water, FW = Firewood, TYR = Rubber tyre, KERO = Kerosene, SEM = Standard error means, LS = Level of significant, * = Significantly different at (P<0.05), NS = Non significantly different

Table 3 shows the effect of singeing method on the physical and chemical properties of red Sokoto buck carcasses. The result showed that the singeing method had no significant (P>0.05) effect on thawing loss, cooking yield, cooking loss, as well as the water holding capacity of the singed buck carcasses. However, the pH and thermal shortening of the red Sokoto buck carcasses were significantly (P<0.05) affected by singeing methods. The carcasses singed with hot water had a

significantly (P<0.05) lower pH (6.08) than the other methods. The significantly (P<0.05) higher pH observed in buck carcasses singed with firewood, rubber tyre and kerosene may be as a result of the sources of heat materials, as well as the intensity of these heat on the rigor stage of the buck carcasses. *Dal Bosco et al. (2001)* and *Huang et al. (2011)* also found, that heat treatment of raw meat resulted in the increase in meat pH. *Huang et al. (2011)* explained that the changes in the pH values of meat during heat treatment were caused by changes in the balance of acid-base groups. According to *Istrati et al. (2011)* an increase in pH of meat means improved water holding capacity as well as improved taste and tenderness of the meat. The authors also reported that an increase in pH of meat also occurs due to the efficiency of the heat treatment leading to changes in the internal temperature of the meat.

Also buck carcasses singed with kerosene had a significantly (P<0.05) higher thermal shortening (35.35) than the samples singed with firewood (24.13) and rubber tyre (25.04). The higher thermal shortening observed in buck carcasses singed with kerosene can be attributed to the duration of the heat source on the sarcomere length of the buck carcasses. This is in agreement with the report of *Sigurgisladottir et al. (2001)* whose report stated that most sarcomere shortening occurred within the first 20 minutes of heating. This 20 minutes critical heating time also implies that short heat exposure can yield tender products, and that heating beyond this time does not improve texture as was observed in this study. According to *Bertram et al. (2004)* strong correlation between cooking losses and shrinkage (thermal shortening) of meat can be explained by the fact, that the shrinkage appearing during heat treatment causes loss of the meat liquid, which results in mass loss. The process of meat shrinkage has several phases, at approximately 40°C – myosin starts to denature and shrinkage across the muscle fibres is observed, at 55-60°C – collagen shrinkage occurs as a result of denaturation while at over 60°C which marks the beginning of the shrinkage along the muscle fibres.

The effect of singeing methods on the sensory properties of red Sokoto buck carcasses is shown in Table 4. The result shows that while singeing methods had no significant (P>0.05) effect on the juiciness of the carcasses, the colour, tenderness, flavour, as well as the overall acceptability of the meat samples were significantly (P<0.05) affected by the singeing methods. Kerosene singed carcasses had the best scores for colour (9.00), tenderness (7.55), flavour (6.90), and over all acceptability (7.75). The observed colour difference may be attributed to the material used in singeing of the carcasses and the non- enzymatic browning between the carbohydrate and protein molecules of the carcasses known as Maillard Reaction. The Maillard Reaction (also known as browning) is a type of non-enzymatic browning which involves the reaction of simple sugars (carbonyl groups) and amino acids (free amino group) that begins to occur when food samples are heated, thus creating flavours, colour changes and taste in food

(*Brunton et al., 2002*). Colour is often the first sensory quality by which foods are judged, and it may also provide an indication of chemical changes suffered by them. The improved tenderness reported in this method may be due to higher pH observed with buck carcass samples singed with kerosene that correlated to the better tenderness, according to the data reported by *Takahashi (1996*), an improvement in the tenderness of meat is proportional to the increasing pH which was also observed in this study, while the improved flavour, may be possible disposition of aromatic flavour (Maillard Reaction) by this method.

Table 3 Physical and chemical properties of meat samples from red Sokoto buck singed using different methods

Parameters	HW	FW	TYR	KERO	SEM	LS
pH	6.08[b]	7.01[a]	6.87[a]	6.75[a]	0.125	*
Thawing Loss (%)	9.70	11.43	11.37	11.43	0.651	NS
Cooking yield (%)	61.26	72.01	66.76	64.21	1.903	NS
Cooking loss (%)	38.55	27.99	33.24	35.79	1.862	NS
WHC (%)	90.82	90.02	88.77	88.22	1.350	NS
Thermal-shortening (%)	31.10[ab]	24.13[b]	25.04[b]	35.35[a]	1.803	*

a, b Means in the same row not followed by the same superscript are significantly different (P<0.05). HW = Hot water, FW = Firewood, TYR = Rubber tyre, KERO = Kerosene, WHC = Water holding capacity, LS = Level of significant, * = Significantly different at (P<0.05), NS = Non significantly different.

However the significantly (P<0.05) higher scores obtained in relation to overall acceptability with buck carcasses singed with kerosene and rubber tyre might be that the consumers are familiar with palatability of carcasses singed with kerosene, rubber tyre and firewood. This result agrees with the reports of *Omojola et al. (2012)* and *Ijeoma et al. (2015),* the authors reported significantly (P<0.05) higher scores in colour, taste, aroma and over all acceptability of meat samples singed with tyre and firewood over those prepared using hot water with razor blade. However, none of the meat samples were rejected by the panelists.

Table 4. Sensory scores of meat samples from red Sokoto buck singed using different methods

Parameter	HW	FW	TYR	KERO	SEM	LS
Colour	6.95[c]	6.10[d]	8.00[b]	9.00[a]	0.168	*
Tenderness	5.80[b]	6.80[ab]	6.50[b]	7.55[a]	0.178	*
Juiciness	5.85	5.80	6.45	6.65	0.184	NS
Flavour	5.5[c]	5.65[bc]	6.50[ab]	6.90[a]	0.182	*
Overall acceptability	6.40[b]	6.70[b]	7.40[a]	7.75[a]	0.150	*

a, b Means in the same row not followed by the same superscript are significantly different (P<0.05). HW = Hot water, FW = Firewood., TYR = Rubber tyre., KERO = Kerosene., LS= Level of significant, * = Significantly different at (P<0.05), NS= Non significantly different

Conclusion

It was concluded that hot water gave a pH of 6.08 and higher WHC numerically. Thus hot water can used to remove the hairs in slaughtered animal carcasses, as this will reduce to practicable level cross contamination of meat during processing. Goat's meat singed with kerosene gave the best sensory properties.

Uticaj različitih metoda termičkog tretmana trupova na kvalitet i senzorne osobine mišića jarčeva rase crveni sokoto

Ani Ugochukwu, Elisha Zhiri Jiya, Ocheme Boniface Ocheme

Rezime

Eksperiment je sproveden korišćenjem dvadeset četiri jarca rase crveni sokoto kako bi se procenio efekat metoda oprljivanja na kvalitet trupa i senzorna svojstva mesa jarčeva rase crveni sokoto (Chevon). Studija je sprovedena u Laboratoriji za nastavu i istraživanje životinja, Saveznog univerziteta za tehnologiju, u Mini. Trupovi jarčeva su nasumično raspoređeni na četiri metode oprljivanja -korišćenjem vruće vode, drva za ogrev, guma i kerozina posle klanja. Prikupljeni su podaci o senzornim parametrima, mineralnom sastavu, fizičkim i hemijskim svojstvima i približnom sastavu. Metode oprljivanja značajno (P <0,05) utiču na sadržaj sirovih proteina i masti u uzorcima mesa, pri čemu je sadržaj značajnim sirovih proteina (P <0,05) bio značajno viši u trupovima iz grupe sa pneumaticima (31,53%), dok je sadržaj masti bio značajno (P <0.05) veći u trupovima iz grupe tretirane toplom vodom (5.80%). Sadržaj mineralnih materija se značajno razlikuje (P <0,05) među ispitivanim metodama. Ph i termalno skraćivanje su značajno (P <0.05) veći u trupovima tretiranim kerozinom (6,75 i 35,35 - respektivno). Metode oprljivanja imale su značajan uticaj na kvalitet trupa i senzorne osobine uzoraka mesa. Zbog toga je zaključeno da se vruća voda koristi za uklanjanje dlaka na zaklanim trupovima koza, jer će to smanjiti nivo unakrsne kontaminacije mesa tokom prerade. Upotreba ogrevnog drveta, gume i kerozina treba da bude potpuno obeshrabrena u preradi mesa, a javnost treba da se obrazuje o zdravstvenim posledicama konzumiranja životinjskih trupova tretiranih ovim metodama.

Ključne reči: metode termičkog tretmana, osobine trupova, senzorne osobine, jarac, mišić

References

AKWETEY W. Y., EREMONG D.C., DONKOH A. (2013): Chemical and Nutrient Composition of Cattle Hide ("Welle") Using Different Processing Methods. Journal of Animal Science Advances, 3(4):176-180.

ALFAIA C. M. M., ALVES S. P., LOPES A. F., FERNANDES M. J. E., COSTA A. S. H., FONTES C. M. G. A., CASTRO M. L. F., BESSA R. J. B., PRATES, J. A. M. (2010): Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. Meat Science, 84, 769-777.

ANAETO M., ADEYEYE A. J., CHIOMA O. G., OLARINMOYE O. A., TAYO O. G. (2010): Goat products: Meeting the challenges of human health and nutrition. Agriculture and Biology Journal of North America, available at, http://www.scihub.org/ABJNA.

APATA E.S. (2014): Effects of post-motem processings and freezing on water holding capacity, warner bratzler value and chemical composition of chevon. American Journal of Research Communication, 2(4):100-113. Available at www.usa-journals.com

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS INC. (2000): Official methods of analysis, Gaithersburg, U.S.A

BERTRAM H. C., ENGELSEN S. B., BUSK H., KARLSSON A. H., ANDERSEN H. J. (2004): Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN-gene. Meat Science, 66, 437-446.

BRUNSO K., BREDAHL L., GRUNERT K. G., SCHOLDERER J. (2005): Consumer perception of the quality of beef resulting from various fattening regimes. Livestock Production Science, 94, 83-93.

BRUNTON N. P., CRONIN D. A., MONAHAN F. J. (2002): Volatile components associated with freshly cooked and oxidized off-flavours in turkey breast meat. Flavour. Fragrance Journal, 17: 327–334.

DAL BOSCO A., CASTELLINI 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.

FOOD AND AGRICULTURE ORGANIZATION (FAO, 1985): Animal by-products processing and utilization. FAO Animal production and health series No. 9, FAO.

GERBER N., SCHEEDER M. R. I., WENK C. (2009): The influence of cooking and fat trimming on the actual nutrient intake from meat. Meat Science, 81, 148–154.

HUANG F., HUANG M., XU X., ZHOU G. (2011): Influence of heat on protein degradation, ultra structure and eating quality indicators of pork. Journal of the Science of Food and Agriculture, 91(3), 443 – 448.

IJEOMA L. PRINCEWILL O., EFFIONG I. D. (2015): Effects of various methods of singeing on the heavy metal, proximate and sensory properties of singed cowhode (Ponmo). Archives of Applied Science Research, 7 (4): 44-50. From http://scholarsresearchlibrary.com/archive.htm

ISTRATI D., VIZIREANU C., DIMA F., DINICA R. (2011): Effect of marination with proteolytic enzymes on quality of beef muscle. Sciencetific Study and Research; Chemistry and Chemical Engineering, Biotechnology, Food Industry, 13(1), 081-089.

IWE M.O. (2002): Handbook of sensory methods and analysis. Rojoint Communication Service Ltd, Enugu, Nigeria. 1st Ed., Pages: 71

KAUFFMAN R. G., CASSENS R. G., CHERER A., MEEKER D .I. (1992): Variations in pork quality, history, definition, extent and resolution. National pork council publication, NPCP. Washington D.C.USA.

KEITH E., BELK J. A., SCANGA G.C., GRANDIN T. (2002): The relationship between good handling/ stunning and meat quality in pork and lamb. America meat institute foundation; Animal Handling and Stunning Conference, 21-22 USA. Pp1-19.

LAWRIE R. A., LEDWARD D. A. (2006): Lawrie's Meat Science, 7th Ed., Woodhead Publishing Limited and CRC Press LLC, Cambridge, U.K.

MALTIN C., BALCERZAK D., TILLEY R., DELDAY M. (2003): Determinants of meat quality: tenderness. The Proceedings of the Nutrition Society, 62, 337-347.

MONIN G., TALMANT A., AILLERY P., COLLAS G. (1995): Effects of Carcass weight and meat quality of pigs dehaired by scalding or singeing post-mortem. Meat Science, 39: 247-254.

OBIRI-DANSO K., HOGARH J. N., ANTWI- AGYEI P. (2008): Assessment of contamination of singed hides from cattle and goats by heavy metals in Ghana. African Journal of Environmental Science and Technology, 2(8), 217-221.

OKUBANJO A.O. (1997): Meat characteristics of Singed and Conventionally dressed Chevon Carcasses. Journal of Food Science Technology, 34(6): 494 –497.

OMOJOLA A. B., ADESEHINWA A. O. K. (2006): Meat characteristics of scalded, singed and conventional dressed rabbit carcasses. World Journal of Zoology, 1(1), 24- 29.

OMOJOLA A. B., APATA E. S., FAGBUARO S. S. (2012): Comparison of skinning versus scalding and singeing: Effect on temperature, pH and meat quality in goats. Journal of Animal Science Advances, 2(9): 740-748.

SAS (2002) Statistical Analysis System. SAS Stat. Version 9 SAS Institute Inc. Garry, N.C. USA.

SIGURGISLADOTTIR S., SIGURDARDOTTIR M. S., INGVARSDOTTIR H., TORRISSEN O. J., HAFSTEINSSON H. (2001): Microstructure and texture of

fresh and smoked Atlantic salmon, Salmo salar L., fillets from fish reared and slaughtered under different conditions. Aquaculture Research, 32, 1–10.

SUZANNE P. (2012): Eat goat! It is a healthy choice! (http://msue.anr.msu.edu/experts/suzanne_pish), Michigan State University Extension.

TAKAHASHI K. (1996): Structural weakening of skeletal muscle tissue during post mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. Meat Science, 43(9), 67-80.

WOLFGANG J., MARGARETE P., ALEXANDER H., FRED, S. (2013): Smoked Meat Products - Innovative Strategies for Reduction of Polycyclic Aromatic Hydrocarbons By Optimisation Of The Smoking Process. Proceedings of the International 57th Meat Industry Conference, Belgrade, Serbia, 24-32

EVALUATION OF COMPETITIVE ABILITY OF PERENNIAL RYEGRASS IN MIXTURES WITH RED CLOVER AND LUCERNE SUBJECTED TO DIFFERENT LEVELS OF N

Zorica Bijelić[1]*, Violeta Mandić[1], Dragana Ružić-Muslić[1], Vesna Krnjaja[1], Aleksandar Simić[2], Vladimir Živković[1], Violeta Caro – Petrović[1]

[1]Institute for Animal husbandry, Belgrade-Zemun, Republic of Serbia
[2]University in Belgrade, Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
*Corresponding author: zonesh@gmail.com
Original scientific paper

Abstract: Grass-legume mixtures represent complex communities of grasses and legumes that make better use of resources and have a number of positive traits in comparison to monoculture. The aim of our research was to examine competitive ability of perennial ryegrass in mixtures with lucerne and red clover (50/50, 70/30) in condition of fertilization with three different nitrogen levels (0, 50, 100 kg ha^{-1}). Dry matter yield of perennial ryegrass in mixtures with red clover was higher compared to mixtures with lucerne. Nitrogen had a very significant impact on perennial ryegrass production. Added N significantly increase DM production of grass treatment with 100 kgN ha^{-1}.

The values, of relative yield above 1 (RY >1), aggressivity index and competitive balance index, above 0 (Ag > Cbg > 0) indicated higher competitive ability of perennial ryegrass in relation to legumes. Competitive ability of ryegrass was higher in 70/30 mixtures and mixtures with lucerne. N fertilization increases competitive ability of ryegrass and decreases competitive ability of legumes.

Key words: aggressivity, competition, grass-legume mixtures, species balance, yield

Introduction

Grass-legume mixtures are a complex systems of highly productive species which contribute to better utilization of natural resources and achieving higher and stable yields.

Each species in the mixture has a special role contributing to the good functioning of the community. Species differ in their morphological and physiological characteristics, which enables them to exploit resources in different ways, i.e. to exploit the environment more efficiently than pure crops. Therefore, due to the different depth and development of the root system of grass and legumes, the utilization of water and mineral substances in the mixture is more efficient than in pure crops. Different shape and leaf exposures allow more efficient use of sunlight. As a consequence of this, there is a higher yield of mixtures relative to monoculture (*Annicchiarico and Tomasoni*, 2010). According to *Sturludottir et al.* (2014), examined mixtures were 7, 9 and 15% more productive than the most productive monoculture. In research of *Helgadottir et al.* (2018) these differences were higher and mixtures performed yields that were higher than the yields of monocultures for 36 and 39%.

Legumes are very important component of a system. Leguminous plants adopt nitrogen through nitrogen fixation from the atmosphere and enrich the soil. In this way they meet their nitrogen needs and part of the needs of non-leguminous species (*Mulder et al.,* 2002). The amount of nitrogen that legumes in the monoculture can fix is around 350 kgN ha^{-1} year^{-1} in alfalfa (*Medicago sativa* L.), 373 kgN ha^{-1} in red clover (*Trifolium pratense* L.) and 545 kgN ha^{-1} year^{-1} in white clover (*Trifolium repens* L.). Growing in the mixtures with legumes, grasses take a larger fraction of their nitrogen from N2 fixation (about 80%), depending on the management, dry matter yield and location (*Huss-Danell et al.*, 2007). Part of the fixed nitrogen is released by legumes into the root zone making it available to other non-leguminous species. *Brophy and Heichel* (1989) have found that the lucerne releases 4.5% of the total fixed nitrogen, while other species release significantly more. Thus, *Louarn et al.* (2015), by examining the mixture of alfalfa and white clover with tall fescue and perennial ryegrass, have found that the amount of transferred nitrogen to grasses ranges from 43 to 72 kgN ha^{-1} year^{-1}. From an ecological point of view it is very important, since reduces the need for the use of nitrogen mineral fertilizers.

Leguminous plants are a rich source of protein which increases the nutritional value of the mixture relative to the grass monoculture (*Sturludottir et al.*, 2014, *Brink et al.,* 2015). In addition, they increase digestion and consumption (*Peyraud et al.*, 2009).

Grass-legume mixtures as a very competitive community, does not leave enough space for weeds developmentand enhance their suppression (*Bijelić et al.,* 2017a, *Helgadottir et al.*, 2018).

Persistence of plants in swards mostly depends of species ability to cope with a complex of environmental and agro-technical conditions. According to this to maintain mixture structure and advantages it is necessary to balance between different species in the mixtures. To meet production targets producers use some agrotechnical measures like N fertilization which together with other management

practices can have depressive effect on growth and persistence of legumes in the crops. In research of *Enriquez-Hidalgo et al.* (2015), application of N fertilizer reduced the potential of white clover to compete with the associated grass. It depressed clover content for 41% and clover yield while it increased yield of accompanied grasses in the mixtures. *Leto et al.* (2008) in their study stated that fertilization with N gave 29% higher grasses DM yield, 9% higher grasses content and decreased legume contribution. Similar conclusions were made in the research by *Nyfeler et al.* (2009) and *Bijelić et al.* (2017b). *Nyfeler et al.* (2009) were summarize that for achieving maximal yield mixtures which were fertilized with 50 kgN ha^{-1} have to have the legume content of 50 – 70%. Increasing the amount of nitrogen reduces the percentage of the legumes necessary for achieving maximal yield.

Therefore, maintenance of an appropriate content of species in grass-legume mixtures for achieving the maximum yield is a great challenge hence occupies the attention of many researchers beside agronomist. Thus, breeders propose a novel framework for improving forage mixtures by breeding. They suggest that breeding programs should be based not only on agronomic traits but also on interaction traits for sustainable production (*Litrico and Violle*, 2015).

The objectives of our research were to (i) estimate forage yield of perennial ryegrass-legume mixtures sown in two different seeding ratios (50/50, 70/30) as well as dry matter yield and competitive ability of each species in the mixtures and (ii) to study the effect of N fertilization on already mentioned parameters with intention to determine the level N fertilizer at which mixtures will achieved a satisfactory yield without losing the contribution of any of the components.

Material and methods

The experiment was established at the experimental field of the Institute for Animal Husbandry in spring 2014. The design of the experiment was split-plot with forage mixtures as a main plot and the fertilizer treatments as the subplot. All plots were randomly organized in three replications. Plot size was 2.0 x 5.0 m. Three species were used for the composition of forage mixtures: red clover (cv. K-39), lucerne (cv. NS Banat) and perennial ryegrass (cv. Calibra). This species were combined in two different ratio as the % of sowing rate of pure crop: perennial ryegrass + red clover (50/50 and 70/30); perennial ryegrass + lucerne (50/50 and 70/30). Seeding rates were 25 kg ha^{-1} for perennial ryegrass and 20 kg ha^{-1} for legumes. All stands were sown at 20 cm spacing between rows. In the experiment, three nitrogen rates were tested, 0, 50 and 100 kgN ha^{-1}. Nitrogen was added in the form of ammonium nitrate in stage of stem elongation. Dry matter yield was recorded over three cuts in each two production years. Whole plot was harvested and measured fresh weight. Herbage samples of 1 kg fresh mass were taken from each plot, oven dried at 60°C for 48 h and weighed for calculation of DM yield.

The proportion of legumes, ryegrass and weeds were assessed by separation each one from $1m^2$, measuring weight proportion and calculation. The level of competitiveness in the mixture is expressed by relative yield total (RYt) and relative yield of species (RY). To estimate relative yield total and relative yield of each species in the mixtures, equations defined by *McGilchrist and Trenbath* (1971) and *De Wit and Van Den Bergh* (1965) were used:

RYT=(RYg+RYl)/2
RYg=Ygl/Ygg , for perennial ryegrass,
RYl=Ylg/Yll , for legumes,

whereYgl and Ylg represents yield of perennial ryegrass and legumes in the mixtures, respectively; Ygg and Yll are the yield of perennial ryegrass and legumes in their monocultures. If RY=1 means that tested species competing against plants of its own species as well as with other species. If RY>1 means that tested species competing better with other species then with its own species. An RY<1 means that tested species is less competitive than other species.

How aggressively species act in the mixture were quantify by aggressivity index. For calculation of perennial ryegrass aggressivity equation of *McGilchrist and Trenbath* (1971) were used:

Ag=0.5(RYg-RYl)

when Ag=0, Ag>0 and Ag<0, than perennial ryegrass are as aggressive as, more aggressive and less aggressive than legumes in the examined mixtures.

Competitive balance is an index of competitive ability of one species in the mixture. The equation of the competitive balance was first established by *Wilson* (1988) and modified by *Williams and McCarthny* (2001). It uses natural logarithm of the yield of the both species in the mixtures. The formula for Cbg calculation is as follows:

Cbg=ln(RYg/RYl)

if Cbg=0 the perennial ryegrass is competitive as legumes, if Cbg<0, perennial ryegrass is less competitive than legumes and if Cbg>0 perennial ryegrass is more competitive than legumes.

Data for the dry matter forage production, species competition and aggressivity which were obtained from four mixtures set in three replication and fertilized with three nitrogen levels in two years were analyzed by two-way ANOVA. Prior to the analysis, Shapiro-Wilk test of normality was done like as Levene's test for testing homogeneity of variances. Differences among means were

determined with LSD at the probability level of 0.05. General linear models for ANOVA was used (SPSS 2011).

Results

Significant differences were observed in total dry matter production of two mixtures. Additionally, dry matter production of their components differed significantly among examined mixtures and treatments of N fertilization for grass and seeding ratio for legumes (Table 1). The mixture of the perennial ryegrass and red clover produced about 18% higher forage yield for both species ratios than the mixture of perennial ryegrass-lucerne. The highest yield of 10.8 t ha^{-1}provided 50/50 red clover mixture while the lowest 70/30 lucerne mixture of 7.8 t ha^{-1}(Fig. 1a). Generally, the yield of the mixtures decreased with an increase of perennial ryegrass proportion in the mixtures. Although N fertilization had no significant impact, it had depressive effect on DM production, especially the level of 50 kgN ha^{-1} (Fig. 1b).

Dry matter yield of perennial ryegrass in red clover mixtures was higher by 17.6% compared to lucerne mixtures (Fig. 1c). Since grasses respond well to nitrogen fertilization this factor had a very significant impact on perennial ryegrass production. Regarding that added N let to a marked increase of grass DM production relative to N unfertilized mixtures (Fig. 1d). The response to N fertilization was the most prominent in treatment with 100 kgN ha^{-1} even by 107.3%.

The observed significant variations of DM legume production occur only as a consequence of the seeding ration. For both mixtures 50/50 ratio had higher DM legume production according to 70/30 mixtures (Fig. 1e). Although the other two factors did not have a significant impact, the yield of legumes in red clover mixtures was higher while N fertilization, conversely, reduced DM production of legumes.This reduction is particularly noticeable in the 50kgN ha^{-1} treatments. (Fig. 1f).

Table 1. Statistical significance for dry matter production of mixtures and its components (DMm, DMg, DMl), relative yield total (RYt), relative yield of grass (RYg) and legumes (RYl) in mixtures, index of grass aggressivity (Ag) and index of competing balance of grass (Cbg).

Treatments	DMm	DMg	DMl	RYt	RYg	RYl	Ag	Cbg
Mixture	p<0.05	p<0.05	ns	p<0.05	ns	ns	ns	ns
Seeding ratio	ns	ns	p<0.01	p<0.01	ns	p<0.01	ns	p<0.05
N fertilization	ns	p<0.01	ns	p<0.01	p<0.01	ns	p<0.01	p<0.01
Mixture x Seeding ratio	ns	ns	ns	ns	ns	ns	p<0.05	ns
Mixtures x N fertilization	ns	ns	ns	ns	ns	ns	ns	ns
N fertilization x Seeding ratio	ns	ns	ns	ns	ns	ns	ns	ns
Mixture x Seeding ratio x N fertilization	ns	ns	ns	ns	ns	ns	ns	ns

ns- non significant; significant at $p \leq 0.05$; significant at $p \leq 0.01$;

There were significant differences among the different level of N fertilization, seeding ratio and mixtures for the tested values of RYt, RYg, RYl, Ag and Cbg. Both mixtures and seeding ratio showed RYt <1 what means that all species in the mixtures compete for the same resources. RYt was significantly higher in red clover mixtures like in seeding ratio of 50/50. N fertilization significantly increased values of RYt. For control treatment and treatment with 50kgN ha^{-1}RYt was under 1. However, fertilization with 100 kg ha^{-1} increased values of RYt above 1.

a)   b)

c)

d)

e)

f)
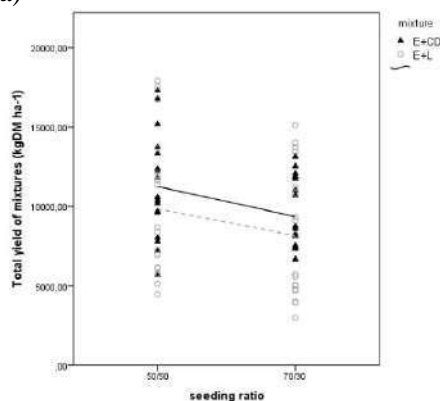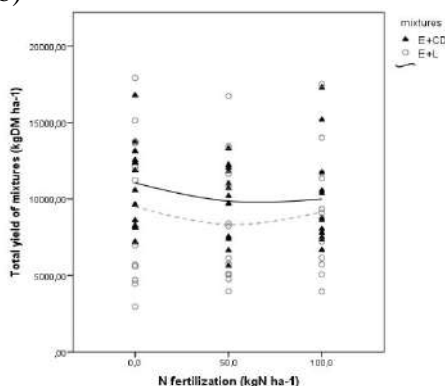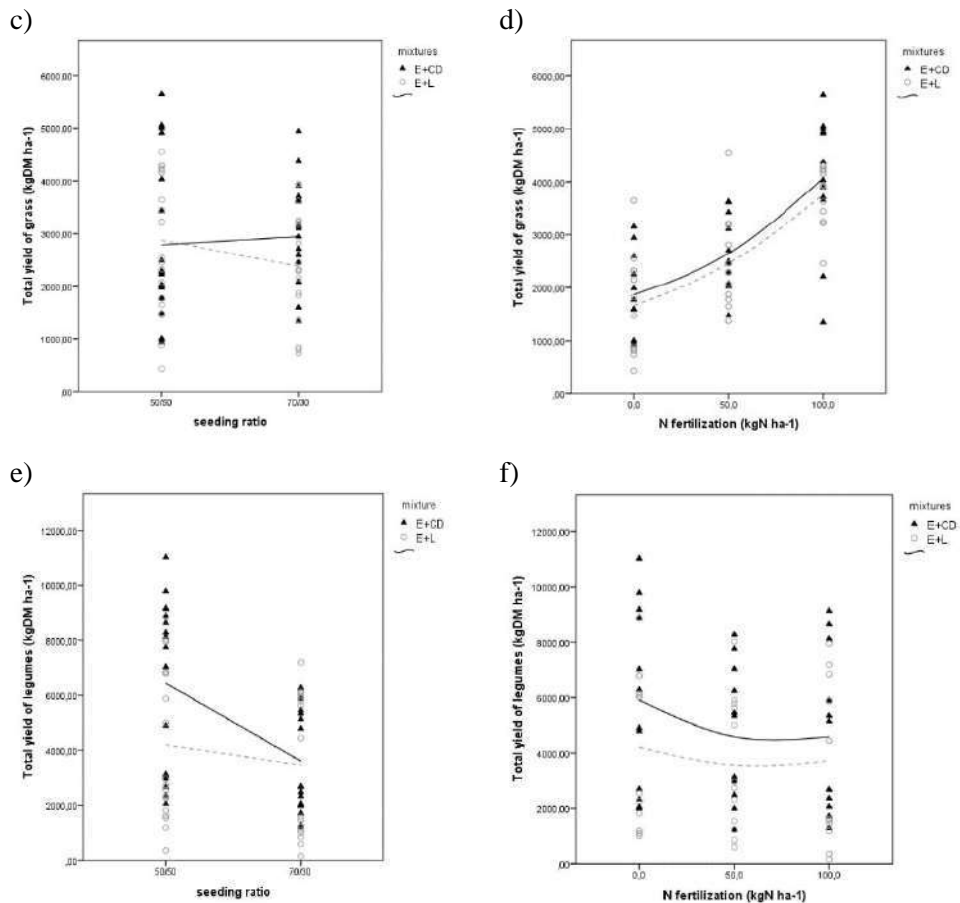
Figure 1. Impact of N fertilization and seeding rate on total dry mater yield of a mixtures (a, b), grass component (c, d), and legumes component (e, f).

As well as RYt, RYg was higher in red clover mixtures and 50:50 seeding ratio. The average RYg, across mixtures, was significantly higher for nitrogen treatments compared to control. In control values of RYg were under the 1 while in fertilized treatments indices values ranged from 1.14 – 1.62. The corresponding RY of legumes were under 1 for all examined factors. Seeding ration of 50/50 showed significant increase in RYl. Significant changes of the aggressivity index values are visible only in the fertilization treatment. N fertilization significantly increased aggressivity of perennial ryegrass in mixtures with red clover and lucerne. The treatment of 100 kgN ha^{-1} gave the highest aggressivity 0.66. For all mixtures it

showed values that were over 0 which indicates a greater aggressivity of the grass in regard to leguminous plants.

Table 2. Effect of examined factors on relative yield total (RYt), relative grass yield (RYg), relative legume yield (RYl), aggressivity index of grass (Ag) and index of competitive balance (Cbg)

Factors	RYt	RYg	RYl	Ag	Cbg
Mixture					
E+CD	0.95a	1.27	0.64	0.31	0.75
E+L	0.83b	1.10	0.55	0.27	0.73
Seeding ratio (%)					
50/50	0.98a	1.23	0.72a	0.26	0.54b
70/30	0.80b	1.13	0.47b	0.33	0.94a
N fertilization (kgN ha^{-1})					
0	0.70b	0.79c	0.66	0.09c	0.28b
50	0.83b	1.14b	0.52	0.31b	0.83a
100	1.14a	1.62a	0.60	0.48a	1.10a

E-perennial ryegrass; CD-red clover; L-lucerne; Means with the different letters are significantly different at the 0.05, according to the LSD test.

Among the tested mixtures, Cbg showed the significant highest value in 70/30 perennial ryegrass-red clover mixture. As for aggressivity, N fertilization showed positive impact on Cb. The highest value of Cb had treatments with 100 kgN ha^{-1} and the lowest treatments without fertilizer. For all treatments values were above 0 what means that ryegrass was more competitive than legumes.

Discussion

The data from the experiment indicate that dry matter production of binary perennial ryegrass-red clover mixtures was higher than that of perennial ryegrass-lucerne mixtures. In research of *Halling et al. (2004)* mixtures of perennial ryegrass and red clover yielded more compared to perennial ryegrass-lucerne mixtures in first examined year. In second year lucerne mixtures significantly outyielded other mixtures. Also, in research of *Gokkus et al. (2009)* in first experimental year mixtures of red clover and smooth bromegrass gave higher yield than lucerne-smooth bromegrass. Although the remaining two factors had no significant impact, N fertilization reduced the yield of the mixtures as well as the increase in the content of the perennial ryegrass in seeding ratio. Given that the legumes have the greatest impact on biomass production in grass-legume mixtures, this reduction of yield can be the result of reduced nitrogen fixation and the contribution of legumes in the mixture under the N fertilization (*Enriquez-Hidalgo et al.*, *2015*) and increased grass biomass, whereby causing a reduction in the space available for the growth of legumes (*Kai-yun et al., 2015*). Contrary, according to

Sengul et al. (2003) N fertilization enhance the DM yield in all mixture combination.

Evenness in grass-legume mixtures is very important for achieving high yields. In research of *Nyfeler et al.* (*2009)* equal stand mixtures yielded more than mixtures dominated by one species. Also, *Kyriazopoulos et al. (2013)* found higher yields in 50/50 mixtures than in 75/25 mixtures (*D. glomerata*: *T. subterraneum*). In our results mixtures with seeding ration 50/50 yielded more than mixtures with 70/30 species ratio.

Synchronization of N supply and demands in grass-legume mixtures is an important factor for achieving sustainable grassland production (*Lüscher et al., 2014*).

N fertilization increased significantly the herbage production of perennial ryegrass in contrast to the yield of two legumes. This result is in agreement with researches of *Youlcu et al. (2010)* and *Kia-yun et al. (2015).* Added nitrogen through the fertilization negatively affects symbiotic fixation in legumes, what encourages the grass components to occupy a wider space in the mixtures subtracting scope for legumes development. Further, this resulted in greater grass proportion and greater yield (*Soussana and Tallec*, *2010*).

The presence of legumes in mixtures had generally more positive effect on forage yield than the presences of other groups of plants (e.g. forbs, grasses) (*Lambers et al., 2004*). In research of *Nyfeler et al. (2009)* the stands were given highest yield when the clover proportion was ranged between 30 and 80% depending on the year and the N fertilizer treatment. Legumes DM yield were effected only by the seeding ratio. 50/50 mixtures had higher legumes DM yield than 70/30 mixtures. Decrease in yield from 50/50 to 70/30 ratio is greater for red clover than for lucerne. Similar results have been reported by *Kyriazopoulos et al. (2013).*

The index of relative yield total for mixtures gives indication whether between species in mixtures exists some kind of avoidance of competition, no competition or antagonism (*Williams and McCarthy, 2001*). All mixtures regardless of species and seeding ratio had RYt under 1 what means that there is strong competition for available resources. Fertilizers can change competitive relationships of a species, leading to variations in the outcome of competition (*Gao et al., 2005*). N fertilization at level of 100 kgN ha^{-1} increase that value above 1 that is provided sufficient resources for the species not to compete with each other.

The relative yield of each species corresponds to productivity of that species in the mixture relative to productivity of that species in sole crop. Values of this indicator were for grass above 1 and for legume under 1. That means that red clover and lucerne produced lower DM yield in mixture with ryegrass than in monoculture. This points to a better competitive ability of perennial ryegrass what is documented with Cbg>0 and Ag>0. Furthermore, ryegrass behaves much more competitive and aggressive in the 70/30 mixtures. Also, *Kyriazopoulos et al.*

(2013) in their research, have proven greater aggressiveness of the *Dactylis glomerata* in 75/25 mixtures with *Trifolim subterraneum*. N fertilization favoured competitive ability and aggressivity of ryegrass particularly level of 100 kgN ha^{-1}.

Conclusion

From this study we can confirmed that mixtures of red clover and ryegrass can achieve higher yields than lucerne-ryegrass mixtures in first two years of production in existing agroecological conditions. Regardless of the species, 50/50 seeding ratio mixtures revealed higher yields compared to 70/30 mixtures. Perennial ryegrass behaves like a superior competitor to red clover and lucerne under given circumstances. Nitrogen fertilization strengthens the competitive ability of the perennial ryegrass which adversely affects the yield of legumes in the mixture and the yield of the mixture themselves. Generally, we can conclude that in this conditions, cultivation of the mixture would be justified and sustainable in sowing grass to legume ratio 50/50 and without the use of N mineral fertilizers.

Ocena konkurentske sposobnosti engleskog ljulja u smešama sa crvenom detelinom i lucerkom đubrenim različitim količinama azota

Zorica Bijelić, Violeta Mandić, Dragana Ružić-Muslić, Vesna Krnjaja, Aleksandar Simić, Vladimir Živković, Violeta Caro Petrović

Rezime

Travno-leguminozne smeše predstavljaju složene zajednice trava i leguminoza koje bolje koriste prirodne resurse i imaju niz pozitivnih osobina u odnosu na monokulturu. Cilj našeg istraživanja je bio da se ispita konkurentna sposobnost engleskog ljulja u smešama sa lucerkom i crvenom detelinom (50/50, 70/30) u uslovima đubrenja sa tri različita nivoa azota (0, 50, 100 kg ha-1). Prinos suve materije engleskog ljulja u smeši sa crvenom detelinom bio je veći u poređenju sa smešom sa lucerkom. Azot je imao značajan uticaj na prinos engleskog ljulja. Dodati azot je značajno povećao prinos engleskog ljulja, naročito doze od 100 kgN ha-1. Vrednosti relativnog prinosa iznad 1 (RI> 1), indeksa agresivnosti i indeksa konkurentske ravnoteže iznad 0 (Ag> Cbg> 0) ukazuju na veću konkurentsku sposobnost engleskog ljulja u odnosu na mahunarke. Konkurentna sposobnost engleskog ljulja bila je veća kod 70/30 smeša i smeša sa

lucerkom. N djubrenje povećava konkurentsku sposobnost ljulja i smanjuje konkurentsku sposobnost leguminoza.

Acknowledgement

References

ANNICCHIARICO P., TOMASONI C. (2010): Optimizing legume content and forage yield of mown white clover–Italian ryegrass mixtures through nitrogen fertilization and grass row spacing. Grass and Forage Science, 65 (2), 220–226. doi: 10.1111/j.1365-2494.2010.00740.x

BIJELIĆ Z., KRNJAJA V., MANDIĆ V., RUŽIĆ-MUSLIĆ D., MIĆIĆ N., ŽIVKOVIĆ Ž., LAZAREVIĆ M. (2017a): Weed abundance in alfalfa crop and alfalfa-grass mixtures under nitrogen fertilization. Journal of Mountain Agriculture on the Balkans, 20 (2), 211–219.

BIJELIĆ Z., MANDIĆ V., SIMIĆ A., KRNJAJA V., RUŽIĆ-MUSLIĆ D., CEKIĆ B., MIĆIĆ N. (2017B): Benefits of mixing grasses and legumes for forage yield and impact of different levels of nitrogen fertilization. Proceedings of the 11th International Symposium „Modern Trends in Livestock Production", Belgrade, Serbia: 713–723. http://istocar.bg.ac.rs/wp-content/uploads/2017/10/Proceedings-2017.pdf

BRINK E.G., SANDERSON A.M., CASLER D.M. (2015): Grass and legume effects on nutritive value of complex forage mixtures. Crop Science, 55, 1329–1337. doi: 10.2135/cropsci2014.09.0666

BROPHY S.L., HEICHEL H.G. (1989): Nitrogen release from roots of alfalfa and soybean grown in sand culture. Plant and Soil, 116, 77–84.

DE WIT C.T., VAN DEN BERGH J.P. (1965): Competition between herbage plants. Netherlands Journal of Agricultural Science, 13, 212–221.

GAO Y.Z., WANG S.P., HAN X.G., PATTON B.D., NYREN P.E. (2005): Competition between Artemisia frigida and Cleistogenes squarrosa under different clipping intensities in replacement series mixtures at different nitrogen levels. Grass and Forage Science, 60, 119–127. https://doi.org/10.1111/j.1365-2494.2005.00458.x

GOKKUS A.¸ KOC A., SERIN Y., COMAKLI B., TAN M., KANTAR F. (2009): Hay yield and nitrogen harvest in smooth bromegrass mixtures with alfalfa and red

clover in relation to nitrogen application. European Journal of Agronomy, 10, 145–151.

ENRIQUEZ-HIDALGO D., GILLILANDT.J., HENNESSY D. (2016): Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. Grass and Forage Science, 71 (4), 559–574.doi: 10.1111/gfs.12201

HALLING M.A., TOPP C.F.E., DOYLE C.J. (2004): Aspects of the productivity of forage legumes in Northern Europe. Grass and Forage Science, 59, 331–344. https://doi.org/10.1111/j.1365-2494.2004.00435.x

HELGADOTTIR A., SUTER M., GYLFADOTTIR T., KRISTJANSDOTTIR T., LUSCHER A. (2018): Grass-legume mixtures sustain strong yield advantage over monocultures under cool maritime growing conditions over a period of 5 years. Annals of Botany. https://doi.org/10.1093/aob/mcy074.

HUSS-DANELL K., CHAIA E., CARLSSON G. (2007): N2 fixation and nitrogen allocation to above and below ground plant parts in red clover-grasslands. Plant and Soil, 299, 215–226. DOI 10.1007/s11104-007-9376-4

IBM Corp. Released (2011): IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

KAI-YUNX., XIANG-LINL., FENGH., YING-JUNZ., LI-QIANGW., HANNAWAY B.D., DONGW., YANQ., FADULM.A.G. (2015): Effect of nitrogen fertilization on yield, N content, and nitrogen fixation of alfalfa and smooth bromegrass grown alone or in mixture in greenhouse pots. Journal of Integrative Agriculture,14 (9), 1864–1876. doi:10.1016/S2095-3119(15)61150-9

KYRIAZOPOULOS A.P., ABRAHAM E.M., PARISSI Z.M., KOUKOURA Z., NASTIS A.S. (2013): Forage production and nutritive value of *Dactylis glomerata* and *Trifolium subterraneum* mixtures under different shading treatments. Grass and forage Science, 68, 72–82. doi: 10.1111/j.1365-2494.2012.00870.x

LAMBERS, J.H.R., HARPOLE, W.S., TILMAN, D., KNOPS, J., REICH, P.B. (2004): Mechanisms responsible for the positive diversity-productivity relationship in Minnesota grasslands. Ecology Letters, 7, 661–668. https://doi.org/10.1111/j.1461-0248.2004.00623.x

LETO J., KNEŽEVIĆ M., BOŠNJAK K., VRANIĆ M., GUNJAČA G. (2008): Changes in grassland yield and botanical –870 composition under contrasting managements. VII. Alps-Adria Scientific Workshop, Stara Lesna, Slovakia, 867. https://bib.irb.hr/datoteka/358976.LetoAA08.pdf

LITRICO I., VIOLLE C. (2015): Diversity in plant breeding: A new conceptual framework. Trends in Plant Science 20, https://doi.org/10.1016/j.tplants.2015.07.007

LOUARN G., FRAK E., ZAKA S., PRIETO J., LEBON E. (2015): An empirical model that uses light attenuation and plant nitrogen status to predict within-canopy nitrogen distribution and upscale photosynthesis from leaf to whole canopy. AoB PLANTS, 7 (1), 1–16. https://doi.org/10.1093/aobpla/plv116

LÜSCHER A., MUELLER-HARVEY I., SOUSSANA J.F., REES R.M., PEYRAUD J.L. (2014): Potential of legume-based grassland–livestock systems in Europe: a review. Grass and Forage Science, 69 (2), 206–228. DOI: 10.1111/gfs.12124

MCGILCHRIST C.A., TRENBATH B.R. (1971): A revised analysis of plant competition experiments. Biometrics, 27, 659–671. https://www.jstor.org/stable/2528603

MULDER C.P.H., JUMPPONEN A., HOGBERG P., HUSS-DANELL K. (2002): How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. Oecologia, 133, 412-421. https://www.jstor.org/stable/4223435

NYFELER D., OLIVIER 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. https://doi.org/10.1111/j.1365-2664.2009.01653.x

PEYRAUD J.L., LE GALL A., LÜSCHER A. (2009): Potential food production from forage legume-based-systems in Europe: an overview. Irish Journal of Agricultural and Food Research, 48 (2), 115–135. https://www.jstor.org/stable/20720364

SENGUL S. (2003): Performance of some forage grasses or legumes and their mixtures under dry land conditions.European Journal of Agronomy, 19, 401–409. DOI:10.1016/S1161-0301(02)00132-6

SOUSSANA J.F., TALLEC T. (2010): Can we understand and predict the regulation of biological N2 fixation in grassland ecosystems? Nutrient Cycling in Agroecosystems, 88, 197–213. http://springerlink.metapress.com/lin...

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. https://doi.org/10.1111/gfs.12037

WILLIAMS C., MCCARTHY C.B. (2001): A new index of interspecific competition for replacement and additive designs. Ecological Research,16, 29–40. https://doi.org/10.1046/j.1440-1703.2001.00368.x

YOLCU H., SERIN Y., TAN M. (2010): The effects of seeding patterns, nitrogen and phosphorus fertilizations on production and botanical composition in lucerne-smooth bromegrass mixtures. Bulgarian Journal of Agricultural Science, 16 (6), 719–727. https://www.agrojournal.org/16/06-09-10.pdf

IMPACTS OF CLIMATIC CONDITIONS ON AFLATOXIN B_1 AND FUMONISINS CONTAMINATION OF MAIZE KERNELS AND THEIR CO-OCCURRENCE

Ana Obradović[1], Vesna Krnjaja[2], Milica Nikolić[1], Goran Delibašić[3], Milomir Filipović[1], Goran Stanković[1], Slavica Stanković[1]

[1]Maize Research Institute, Zemun Polje, Slobodana Bajića 1, 11185 Belgrade, Serbia
[2]Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
Corresponding author: Ana Obradović, aobradovic@mrizp.rs
Original scientific paper

Abstract: Agroecological and climatic conditions in Serbia greatly affected the development of toxigenic fungi and occurrence of mycotoxins in the maize. The presence of fungal toxigenic *Aspergillus* and *Fusarium* species and levels of aflatoxin B_1 (AFB_1) and sum of fumonisins B_1, B_2 and B_3 (FBs) were established in 127 maize kernel samples harvested during 2012 (37 samples) and 2013 (90 samples). The periods of silking and grain filling of the maize in 2012 in comparison to 2013 were characterised with extremely dry spells, with high temperatures and low precipitation sums. The mean incidences of *A. flavus* and *F. verticillioides* were 50.4 and 11.7% in 2012 and 18.9 and 33.4% in 2013, respectively. According to the regulations of the World Health Organisation, unacceptable levels of AFB_1 (>20 µg kg^{-1}) and FBs (>2000 µg kg^{-1}) were established in the 30.6 and 24.1% samples of 2012 and 16.7 and 40% maize kernel samples of 2013, respectively.

It can be concluded that high temperatures and low precipitation sums in 2012 favoured the development of *A. flavus* affecting the high level of AFB_1 , in comparison with *F. verticillioides* and the production of FBs. There was no positive correlation between the incidences of *A. flavus* and *F. verticillioides*, while a statistically significant positive correlation has been found between AFB_1 and FBs levels, in both investigated years (2012-2013). This indicates that the mycotoxin production depended more on weather conditions than on the distribution of corresponding toxigenic fungal species.

Key words: toxigenic fungi, *A. flavus*, *F. verticillioides*, aflatoxin B_1, fumonisins

Introduction

Recently, the variability of climatic conditions together with global climate changes contributes to higher biosynthesis of mycotoxins in maize, which causes economic losses in the production and risk for human and animal health. It is well known that aflatoxins and fumonisins are threat to human health, and in some cases, directly cause a disease and even death. Aflatoxins are hepatotoxins, teratogens, mutagens and carcinogens. Fumonisins cause more frequently diseases in animals, such as leukoencephalomalacia in horses and pulmonary oedema in swine and they are also tumour promoters in rats (*Abbas et al., 2002; Fandohan et al., 2004*). Aflatoxins are secondary metabolites of fungi *Aspergillus flavus* and *Aspergillus parasiticus*, while fumonisins are produced by species *Fusarium verticillioides* and *Fusarium proliferatum* (*Abbas et al.,* 2002).

Increased temperatures and drought stress are the principal factors causing high levels of aflatoxins in maize. High temperatures, especially night-time temperatures above $20^{o}C$, and drought favour the development, sporulation and distribution of *A. flavus* in the period from plant silking to grain filling. It is considered that night-time temperatures are more important for the growth of mycotoxin producing fungi, because the energy balance of the plant is negative during the night and its ability to defend itself is lower (*Abbas et al., 2002, 2006*). Likewise, drought, extremely high temperatures, insect damages and water activity (a_w) are the main factors causing the risk of the *Fusarium* infection and later fumonisin contamination in maize kernels. Furthermore, maize contamination with fumonisin-producing *Fusarium* species depends on the geographical area and agroecological conditions (*Medina et al., 2015*).

Maize contamination with aflatoxins is of a great concern, since this crop is a major source of food and feed worldwide. Until recently, aflatoxins have not been signalled as a matter of concern for the primary production in Europe. Under agroecological conditions of many European countries, such as Germany (*Curtui et al., 2004*), Hungary (*Varga et al., 2004*), Belgium (*Chandelier et al., 2004*), Poland (*Perkowski et al., 2004*), Austria (*Öhlinger et al., 2004*), *Aspergillus* cereal infection and aflatoxin contamination in fields are rare and usually with low mycotoxin levels. However, years 2003 and 2012 have to be mentioned, for Italy and south Europe, because of the alarming aflatoxin contamination in maize (*Battilani et al., 2016*). During 2013, in Italy, the intensive occurrence of *A. flavus* and aflatoxins in maize was observed as a result of extremely high temperatures during the growing season, as well as of conditions of insufficient moisture/drought stress in the period from May to September (*Moretti et al., 2004*). In south-eastern Romania, *Tabuc et al. (2009)* recorded approximately 30% maize samples, collected between 2002 and 2004, contaminated with aflatoxin B_1 (AFB_1). *Aspergillus* species are considered poor maize pathogens under average climatic

conditions of Serbia. However, due to extreme high temperatures and low precipitation sums in Serbia in the 2012 summer, the incidence of *A. flavus* species was high in many plant species (soya bean, maize, sunflower, barely, wheat), which resulted in a high aflatoxins level. The intensity of *Aspergillus* spp. infestation in 2012 was up to 95.3% in maize kernels (*Lević et al., 2013*) with a high AFB_1 level (>40 µg/kg) in 55.17% of analysed maize samples (*Krnjaja et al., 2013*).

Agroecological conditions in Serbia are suitable for the intensive development of pathogenic and toxigenic species of the genus *Fusarium* (*Lević et al., 2004*). It is considered that in the past few years there has been an increasing occurrence of species of the section *Liseola* (*F. verticillioides, F. proliferatum* and *F. subglutinans*), and in particular *F. proliferatum* in cereal grain (*Stanković et al., 2008*). These species produce fumonisin B, of which FB_1 is mostly distributed under natural conditions (*Nelson et al., 1993*). It has been established that these fungi synthesised FB_1 more in maize kernels than in wheat and barley kernels (*Visconti and Doko, 1994*). In Serbia, there is less data on the presence of fumonisins in cereals in comparison with other toxins (*Stepanić et al., 2011*).

There are different results on the correlation between occurrences of aflatoxins and fumonisins, as well as their producers in maize kernels. Results obtained by *Abbas et al. (2006)* indicated that the natural infection with *Fusarium* spp. did not affect the occurrence of *Aspergillus* spp. nor aflatoxin production (*Widstrom et al., 1994*). Some authors reported a negative correlation between *Aspergillus* spp. and *Fusarium* spp. infections in maize kernels (*Marin et al., 1998*), so it could be expected that the levels of aflatoxins and fumonisins would be negatively correlated. However, results obtained by *Abbas et al. (2006)* showed a positive correlation between these two mycotoxins. Moreover, *Scudamore (1997)* determined that maize samples were predominantly contaminated with several mycotoxins. Aflatoxins and fumonisins were the most common combination (28%) in total of 60% of maize samples positive for more than one mycotoxin.

Taking into account that agroecological conditions in Serbia are favourable for the development of toxigenic species of the genus *Fusarium*, and in some years even species of the genus *Aspergillus*, the aim of this study was to determine the impact of climatic factors on the incidence of fungal toxigenic *Aspergillus* and *Fusarium* species and the level of their mycotoxins, AFB_1 and FBs. The correlation between maize grain contamination with AFB_1 and FBs was observed in particular and the frequency of their co-occurrence was established.

Materials and Methods

A total of 127 kernel samples were collected during the maize harvests in 2012 (37 samples) and 2013 (90 samples) in the production plots in Zemun Polje

(in the vicinity of Belgrade, Serbia). Each sample contained 1 kg of the maize kernels, out of which subsamples of 200 g were drawn and used in the further mycological and mycotoxicological analyses.

In mycological analyses, the maize kernels was disinfected with the solution of sodium hypochlorite (NaOCl) and water (1:3) for 3 minutes, rinsed with distilled water and dried on sterile filter paper. Then, 100 kernels of each sample were plated on potato dextrose agar (PDA) in Petri dishes (five kernels per Petri dish). The kernels were incubated in the dark during 7 days at 25°C. According to the fungal keys of *Singh et al. (1991)* and *Burgess et al. (1994)* the identification of toxigenic species, *A. flavus* and *F. verticillioides*, was performed by morphological criteria. The incidence was calculated by percentage of fungal species in each maize kernel sample.

A total of 100 g of maize kernels from each sample were dried at 50°C for 72h. After drying, the samples were ground to the fine powder using the basic analytical mill (A11, IKA, Germany). The samples were analysed for the presence of AFB_1 and FBs (sum of FB_1, FB_2 and FB_3) using ELISA (Enzyme linked immunosorbent assay). The mycotoxin analysis was performed according to the manufacturer's instructions (Tecna S.r.l., Italy, Celer AFLA B_1 Test Kit and Celer Fumo Test Kit). The quantitative determination of mycotoxins was performed at 450 nm wavelength using an ELISA reader (BioTek EL x 800^{TM}). The detection limit values for AFB_1 and FBs were 1 µg kg^{-1} and 750 µg kg^{-1}, respectively.

The obtained results were processed in the software package Statistica 12.0. The Pearson's coefficient of correlation (Pearson Product-Moment Correlation) was used to establish the relationship between the species of *A. flavus* and *F. verticillioides*, as well as, between the concentrations of AFB_1 and FBs. The t-test was used to determine the significance level of the coefficient of correlation.

Results

Meteorological data for the Zemun Polje area have been provided by the meteorological station of the Maize Research Institute, Zemun Polje. In 2012, high mean daily temperatures in July and August (>26°C), very low precipitations (4 mm) and a very low precipitation factor (0.15) in August, caused an extreme drought in comparison to the corresponding period in 2013. At the end of the growing season, the higher recorded precipitations and the precipitation factor were, recordedin 2013 than in 2012 (Table 1).

In the mycological analyses, the incidence of toxigenic species belonging to the genera *Aspergillus* and *Fusarium* varied depending on the year of investigation. In 2012, *A. flavus* and *F. verticillioides* were isolated in the range of 6.6-95.5% (mean 50.4%), and 0-44.4% (mean 11.7%), respectively. The statistically very significant negative correlation (r=-0.55**) was established

between distributions of these two species. In 2013, the incidence of the species *A. flavus* was lower (18.9%) and of *F. verticilioides* was higher (33.4%) in comparison with incidences recorded in 2012, while the correlation between distributions of these two species was statistically insignificantly negative (r=-0.08) (Table 2).

Table 1. Total monthly precipitation, mean daily temperature and precipitation factors per month from May to September in the Zemun Polje area (in the vicinity of Belgrade) in 2012 and 2013

Month	Year					
	2012			2013		
	Rainfall (mm)	Temperature (°C)	Pf*	Rainfall (mm)	Temperature (°C)	Pf*
May	127.5	17.9	7.12^{SH}	93.9	19.68	4.77^{SA}
June	13.9	24.56	0.57^A	37.8	21.89	1.73^A
July	39.0	27.08	1.45^A	16.0	23.78	0.67^A
August	4.0	26.21	0.15^A	12.7	23.72	0.53^A
September	31.4	22.14	1.42^A	70.1	16.87	4.15^{SA}

*Pf = precipitation / average monthly temperature: A- arid climate (Pf <3.3), SA- semi-arid climate (Pf 3.4-5.0), SH- semi-humid climate (Pf >5) (Gračanin, 1950, cit. Lević, 1987)

Table 2. Incidence (%) of toxigenic species *A. flavus* and *F. verticillioides* in maize kernel samples collected in 2012 and 2013 and their correlation coefficient (r)

Year	Number of total samples	*A. flavus*	*F. verticillioides*	*A. flavus*	*F. verticillioidies*	Correlation coefficient
		Range		Mean		
2012	37	6.6 –95.5	0 – 44.4	50.4	11.7	-0.55**
2013	90	0 – 79	7 – 88	18.9	33.4	-0.08^{ns}

**significant at the 0.01 level of probability; *significant at the 0.05 level of probability; ns, not statistically significant.

Table 3. Sample size (number of positive samples/number of total samples), incidence rate and levels of AFB$_1$ (µg kg^{-1}) and FBs (µg kg^{-1}) in tested maize samples in 2012 and 2013

Year	2012		2013	
Items	AFB$_1$	FBs	AFB$_1$	FBs
Sample size	36/37	29/37	12/90	30/90
Incidence (%)	97.3	78.4	18.9	33.3
Range (µg kg^{-1})	0 – 491.7	0 – 10790	0 – 27.9	0 – 10860
Mean (µg kg^{-1})	60.3	1300	1.3	2800
Correlation coefficient (r)	0,40*		0,61**	

**significant at the 0.01 level of probability; *significant at the 0.05 level of probability; ns, not statistically significant.

Table 4. Distribution of AFB$_1$ and FBs levels in investigated maize kernel samples harvested in 2012 and 2013

	Year 2012	Year 2013		Year 2012	Year 2013
AFB$_1$ (µg kg^{-1})	% of total samples		FBs (µg/kg)	% of total samples	
< 20	69.4	83.3	< 2000	75.9	60.0
> 20	30.6	16.7	> 2000	24.1	40.0

In the mycotoxicological analyses, AFB$_1$ was detected in 97.3% (2012) and 18.9% (2013) of maize kernel samples, and FBs was recorded in 78.4% (2012) and 33.3% (2013) of maize kernel samples. In 2012, levels of AFB$_1$ ranged from 0 to 491.7 µg kg^{-1} (mean 60.3 µg kg^{-1}), while FBs ranged from 0 to 10790 µg kg^{-1} (mean 1300 µg kg^{-1}). The corresponding values in 2013 amounted to 0-27.9 µg kg^{-1} (mean 1.3 µg kg^{-1}) and 0-10806 µg kg^{-1} (mean 2800 µg kg^{-1}), respectively. Statistically significantly positive (r = 0.40*) and statistically very significant (r = 0.61**) correlations were established between AFB$_1$ and FBs in 2012 and 2013, respectively (Table 3).

According to the regulation of World Health Organisation (WHO) unacceptable levels of AFB$_1$ (>20 µg/kg) and FBs (>2000 µg/kg) were detected in 30.6 and 24.1% maize kernel samples in 2012 and 16.7 and 40% maize kernel samples in 2013, respectively (Table 4).

Discussion

The incidence of toxigenic species, *A. flavus* and *F. verticillioides*, and production of AFB$_1$ and FBs in investigated maize harvested during the growing seasons of 2012 and 2013 had significantly depended of climate factors (temperatures and precipitation). It was determined that high temperatures, low precipitations and a low precipitation factor during the summer in 2012 favoured the intensive development of toxigenic species *A. flavus* and a high AFB$_1$ production in maize kernels. However, high mean daily temperatures in July and August (>20°C) favoured the development of the species *F. verticillioides* in both years of investigation, while increased precipitations prior to harvest resulted in a greater FBs production in 2013. Similarly, *Magan et al. (2011)* were established that temperatures at different levels of water availability (a$_w$) affected the scope of the development of toxigenic fungi and the mycotoxin concentration. According to the results of *Miraglia et al. (2009)* the ability of fungi to produce mycotoxins was most dependent on temperatures, relative air humidity, insect damages and stress in host plants. *Abbas et al. (2006)* also observed that climatic conditions in 1998 favoured high levels of both, aflatoxins and fumonisins, while in 2001, a high level of fumonisins and a low level of aflatoxins in maize kernels were favoured. These authors concluded that climatic conditions, high temperature stress during the kernel development period, especially night-time temperatures above 20°C, were

significant for the explanation of the stated differences. High risk for maize contamination with fumonisins is caused by high temperatures, drought and abundant precipitation during the growing season (*Miller, 2008*).

The species *A. flavus*, i.e. *F. verticillioides* was dominant in 2012, i.e. 2013, respectively. Although both species were present in both years of investigation the coefficient of their co-occurrence was negative (Table 2). However, the correlation between AFB_1 and FBs levels was positive and statistically significant in both years of investigation (Table 3). Similar to our study, *Sun et al. (2011)* were established 100% AFB_1 positive and 85.3-100% FB_1 positive maize samples with the co-occurrence of AFB_1 and FB_1 in 90.3-100% maize samples. Likewise, *Abbas et al. (2006)* established a positive correlation between the levels of aflatoxins and fumonisins, emphasising that the natural infection of maize with *Fusarium* spp. did not provide protection against the aflatoxin production. In addition, the greater incidence of *F. verticillioides* compared to *A. flavus* in maize kernel samples drawn in the 1999 and 2000 harvests was explained by *Bush et al. (2003)* by the competitive relationship between these two species. Contrary, *Sreenivasa et al. (2011)* isolated a high percentage of species, *F. verticillioides* (86%) and *A. flavus* (84.9%), from 86 maize kernel samples. According to *Zorzete et al. (2008)* in analysed maize samples the species *F. verticillioides* was isolated in a greater percentage (34.6%) than the species *A. flavus* (0.5%) and the correlation coefficient between these two species was negative. *Rocha et al. (2009)* established a statisticaly significant negative correlation (r=-0.61) between isolated *Fusarium* and *Aspergillus* genera. *Marin et al. (1998)* observed that activities of the species *F. verticillioides* and *F. proliferatum* reduced the presence of the species *A. flavus*, *A. niger* and *A. ohraceus*, particularly under conditions of high a_w and temperatures of 15°C. Species *F. verticillioides* and *F. proliferatum* tend to increase the production of fumonisins in the presence of *A. niger* and *A. flavus* under certain environmental conditions.

In this study, unacceptable AFB_1 and FBs levels were established in 30.6 and 16.7% and 24.1 and 40% maize samples in 2012 and 2013, respectively. In China, according to the results of *Sun et al. (2011)* the unacceptable levels of AFB_1 and FB_1 were recorded in 35% and in 58% of the maize samples, respectively. These authors concluded that the climatic conditions in the location of Huaian favoured the development of toxigenic fungi and a greater accumulation of mycotoxins in observed maize samples. Similarly, in Malaysia, *Reddy and Salleh (2011)* observed 80 maize samples and detected AFB_1 and FB_1 in 81.2% and 100% of analysed samples, respectively. Eighteen (22.5%) samples had an AFB_1 level above the internationally prescribed limit (20 µg/kg) ranging from 20.6 to 135 µg/kg.

In conclusion, the present study confirmed an exceptionally significant effect of climatic factors on the presence and incidence of toxigenic fungi of

Aspergillus and *Fusarium* genera as well as on AFB_1 and FBs levels. In order to recommend preventive measures in the management risks of the mycotoxin presence in maize and other cereals, it is necessary to provide constant and relevant information on the impact of abiotic and biotic factors on cereal contamination with toxigenic fungal species and on the production of their mycotoxins. This information should contain data on health safety of cereals in the field and later during their storage. Considering the differences in the incidence of toxigenic species established in this study during the two growing seasons, it can be concluded that the meteorological, weather conditions have a crucial role in the development of toxigenic fungi in the maize. It is therefore important to improve the monitoring of weather conditions under conditions of global climate changes that can radically alter the distribution and the spectrum of toxigenic fungal species in maize worldwide. Maize is used as a staple food in many countries around the world. Animal husbandry as an economic branch is also largely dependent on maize as a main source of feed. The mycotoxin accumulation in cereal crops in fields and later during storages is a risk to human and animal health. Setting appropriate tolerance values for mycotoxins in cereals will be challenges for current and future researchers. These tolerances should have a significant impact on the improvement of public health, especially children's health. Further research should be focussed on studying the effects of major factors affecting the intensive occurrence of mycotoxins and, based on their results, on the development and application of integrated measures that will contribute to better management practices for these harmful agents and on the provision of food safety.

Uticaj klimatskih uslova na kontaminaciju zrna kukuruza sa aflatoksinom B_1 i fumonizinima i njihova združena pojava

Ana Obradović, Vesna Krnjaja, Milica Nikolić, Goran Delibašić, Milomir Filipović, Goran Stanković, Slavica Stanković

Rezime

Agroekološki i klimatski uslovi u Srbiji veoma su pogodni za razvoj toksigenih gljiva i njihovih mikotoksina u kukuruzu. Prisustvo toksigenih vrsta gljiva iz rodova *Aspergillus* i *Fusarium,* kao i nivoi aflatoksina B_1 (AFB_1) i ukupnih fumonizina B_1, B_2 i B_3 (FBs) utvrđeni su u 127 uzoraka zrna kukuruza iz berbe tokom 2012 (37 uzoraka) i 2013. godine (90 uzoraka). U fenofazama svilanja i nalivanja zrna kukuruza u 2012. u odnosu na 2013. godinu zapaženi su ekstremno sušni periodi sa visokim temperaturama i niskim količinama padavina. Prosečne vrednosti za učestalost vrsta *A. flavus* i *F. verticillioides* bile su 50,4 i 11,7% u

2012., odnosno 18,9 i 33,4% u 2013. godini. Prema pravilniku Svetske zdravstvene organizacije (WHO) nedozvoljeni nivoi AFB_1 (>20 µg/kg) i FBs (>2000 µg/kg) utvrđeni su u 30,6% i 24,1% uzoraka u 2012., odnosno u 16,7% i 40% uzoraka u 2013. godini.

Na osnovu dobijenih rezultata zaključeno je da su visoke temperature i niske količine padavina u 2012. godini uslovile značajno veću učestalost *A. flavus* i visoku produkciju AFB_1 u poredjenju sa vrstom *F. verticillioides* i produkcijom FBs. U obe ispitivane godine (2012-2013), između učestalosti *A. flavus* i *F. verticillioides* nije ustanovljena pozitivna korelacija, dok je statistički značajna pozitivna korelacija ustanovljena između nivoa AFB_1 i FBs. Ovo ukazuje da produkcija mikotoksina je više zavisna od vremenskih uslova u odnosu na distribuciju toksigenih vrsta gljiva.

Ključne reči: toksigene gljive, *A. flavus*, *F. verticillioides*, aflatoksin B_1, fumonizini

Acknowledgment

References

ABBAS H.K., WILLIAMS W.P., WINDHAM G.L., PRINGLE H.C., XI W., SHIER W.T. (2002): Aflatoxin and fumonisin contamination of commercial corn (Zea mays) hibrids in Mississippi. Journal of Agricultural and Food Chemistry, 50, 5246-5254.

ABBAS H.K., CARTWRIGHT R.D., XIE W., SHIER T.W. (2006): Aflatoxin and fumonisin contamination of corn (maize, Zea mays) hybrids in Arkansas. Crop Protection, 25, 1, 1-9.

BATTILANI P., TOSCANO P., VAN DER FELS-KLERX H.J., MORETTI A., CAMARDO LEGGIERI M., BRERA C., RORTAIS A., GOUMPERIS T., ROBINSON T. (2016): Aflatoxin B_1 contamination in maize in Europe increases due to climate change. Scientific Reports, 1-7.

BURGESS L.W., SUMMERELL B.A., BULLOCK S., GOTT K.P., BACKHOUSE D. (1994): Laboratory manual for *Fusarium* research. Third edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney, 133 pp.

BUSH B.J., CARSON M.L., CUBETA M.A., HAGLER W.M., PAYNE G.A. (2003): Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology, 94, 88-93.

CHANDELIER A., MICHELT J.Z., TANGNI E.K., BAERT K., MOONS E., VINKX C. (2004): Mycotoxins survey in Belgium and toxic Fusarium in Belgian wheat. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 11-32.

CURTUI V., SEIDLER C., SCHNEIDER E., USLEBER E. (2004): Mycotoxins in food in Germany. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 109-121.

FANDOHAN P., ZOUMENOU D., HOUNHOUIGAN D.J., MARASAS W.F.O., WINGFIELD M.J., HELL K. (2004): Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. International Journal of Food Microbiology, 98, 249-259.

KRNJAJA V.S., LEVIĆ J.T., STANKOVIĆ S.Ž., PETROVIĆ T.S., LUKIĆ M.D. (2013): Molds and mycotoxins in freshly harvested maize. Matica Srpska Journal of Natural Sciences, 124, 111-119.

LEVIĆ J. (1987): Inheritance of maize leaf resistance to *Kabatiella zeae* Narita et Hiratsuka and screening for source of resistance (Summary in English). Journal for Scientific Agricultural Research, 48, 170, 173-203.

LEVIĆ J., STANKOVIĆ S., BOČAROV-STANČIĆ A., ŠKRINJAR M., MAŠIĆ Z. (2004): The overview on toxigenic fungi and mycotoxins in Serbia and Montenegro. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 201-218.

LEVIĆ J., GOŠIĆ- DONDO S., IVANOVIĆ D., STANKOVIĆ S., KRNJAJA V., BOČAROV-STANČIĆ A., STEPANIĆ A. (2013): An Outbreak of Aspergillus Species in Response to Enviromental Conditions in Serbia. Pesticides and Phytomedicine, 28, 3, 167-179.

MAGAN N., MEDINA A., ALDRED D. (2011): Possible climate-change effects on mycotoxin contamination of food crops pre- and postharvest. Plant Pathology, 60, 150-163.

MARIN S., SANCHIS V., ARNAU F. (1998): Colonisation and competiveness of *Aspergillus* and *Penicillium* species on maize grain in the presence of *Fusarium moniliforme* and *Fusarium proliferatum*. International Journal of Food Microbiology, 45, 107-117.

MEDINA Á., RODRÍQUEZ A., SULTAN Y., MAGAN N. (2015): Climate change factors and *Aspergillus flavus*: effects on gene expression, growth and aflatoxin production. World Mycotoxin Journal, 8, 2, 171-179.

MILLER J.D. (2008): Mycotoxins in small grains and maize: old problems, new challenges. Food Additives and Contaminants Part A, 25, 219-230.

MIRAGLIA M., MARVIN H.J.P., KLETER G.A., BATTILANI P., BRERA C., CONI E., CUBADDA F., CROCI L., DE SANTIS B., DEKKERS S., FILIPPI L., HUTJES R.W.A., NOORDAM M.Y., PISANTE M., PIVA G., PRANDINI A., TOTI L., VAN DEN BORN G.J., VESPERMANN A. (2009): Climate change and food safety: an emerging issue with special focus on Europe. Food and Chemical Toxicology, 47, 1009-1021.

MORETTI A., LOGRIECO A., VISCONTI A., BOTTALICO A. (2004): An overwiew of mycotoxins and toxigenic fungi in Italy In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 141-160.

Nelson P., Desjardins AE, Plattner RD, 1993. Fumonisins, mycotoxins produced by *Fusarium* species: Biology, chemistry, and significance. Annual Review of Phytopathology, 31: 233-252.

ÖHLINGER R., ADLER A., KRÄUTLER O., LEW H. (2004): Occurrence of toxigenic fungi and related mycotoxins in cereals, feeds and foods in Austria. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 1-10.

PERKOWSKI J., CHEŁKOWSKI J., GOLIŃSKI P. (2004): Occurrence of mycotoxins in cereals, plants, foods and feeds in Poland. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 161-172.

REDDY K.R.N., SALLEH B. (2011): Co-occurrance of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. Journal of Animal and Veterinary Advances, 10, 5, 668-673.

ROCHA L.O., NAKAI V.K., BRAGHINI R., REIS T.A., KOBASHIGAWA E., CORREA B. (2009): Mycoflora and co-occurrence of fumonisins and aflatoxins in freshly harvested corn in different regions of Brazil. International Journal of Molecular Science, 10, 5090-5103.

SINGH K., FRISVAD J.C., THRANE U., MATHUR S.B. (1991): An illustraded manual on identification of some seed-borne *Aspergilli*, *Fusaria*, *Penicillia* and their mycotoxins. 1st ed., Danish Government Institute of Seed Pathology for Developing Countries, Ryvangs Alle 78 DK 2900 Hellerup, Denmark, 133 pp.

SREENIVASA M.Y., SHARMILA DASS R., CHARITH RAJ A.P., JANARDHANA G.R. (2011): Mycological evaluation of maize grains produced in Karnataka (India) for the post harvest fungal contamination. World Applied Sciences Journal, 13, 4, 688-692.

STANKOVIĆ S., LEVIĆ J., PETROVIĆ T., KRNJAJA V. (2008): Toxicological profile of F. proliferatum isolated from maize seed, root and stalk. Cereal Research Communications, 36, suppl. B, 397-398.

STEPANIĆ A., STANKOVIĆ S., LEVIĆ J., IVANOVIĆ M., KRNJAJA V. (2011): Fusariotoxins in Wheat Grain in Serbia. Pesticides and Phytomedicine, 26, 317-323.

SUN G., WANG S., HU X., SU J., ZHANG H., XIE Y., ZHANG H., TANG L., WANG J.S. (2011): Co-contamination of aflatoxin B_1 and fumonisin B_1 in food and human dietary exposure in three areas of China. Food Additives and Contaminants, 28, 4, 461-470.

TABUC C., MARIN D., GUERRE P., SESAN T., BAILLY J.D. (2009): Molds and mycotoxin content of cereals in southeastern Romania. Journal of Food Protection, 72, 662-665.

VARGA J., TÓTH B., MESTERHÁZY Á., TÉREN J., FAZEKAS B. (2004): Mycotoxigenic fungi and mycotoxins in foods and feeds in Hungary. In: Logrieco, A. and Visconti, A. (eds.) An Overview on Toxigenic Fungi and Mycotoxins in Europe, Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 123-139.

VISCONTI A., DOKO M.B. (1994): Survey of fumonisin production by Fusarium isolated from cereals in Europe. Journal of AOAC International, 77, 546-550.

WIDSTROM N.W., MCMILLIAN W.W., WILSON D.M., RICHARD J.L., ZUMMO N., BEAVER R.W. (1994): Preharvest aflatoxin contamination of maize inoculated with Aspergillus flavus and Fusarium moniliforme. Mycopathologia, 128, 119–123.

ZORZETE P., CASTRO R.S., POZZI C.R., ISRAEL A.L.M., FONSECA H., YANAQUIBASHI G., CORRÊA B. (2008): Relative populations and toxin production by *Aspergillus flavus* and *Fusarium verticillioides* in artificially inoculated corn at various stages of development under field conditions. Journal of the Science of Food and Agriculture, 88, 48-55.

Manuscript submission

By submitting a manuscript authors warrant that their contribution to the Journal is their original work, that it has not been published before, that it is not under consideration for publication elsewhere, and that its publication has been approved by all co-authors, if any, and tacitly or explicitly by the responsible authorities at the institution where the work was carried out.

Authors are exclusively responsible for the contents of their submissions, the validity of the experimental results and must make sure that they have permission from all involved parties to make the data public.

Authors wishing to include figures or text passages that have already been published elsewhere are required to obtain permission from the copyright holder(s) and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Authors must make sure that all only contributors who have significantly contributed to the submission are listed as authors and, conversely, that all contributors who have significantly contributed to the submission are listed as authors.

The manuscripts should be submitted in English (with a summary in English or Serbian language – translation of Summaries into Serbian language for non-domestic authors will be performed by the Editor's office) by email to: biotechnology.izs@gmail.com

Manuscripts are be pre-evaluated at the Editorial Office in order to check whether they meet the basic publishing requirements and quality standards. They are also screened for plagiarism.

Authors will be notified by email upon receiving their submission. Only those contributions which conform to the following instructions can be accepted for peer-review. Otherwise, the manuscripts shall be returned to the authors with observations, comments and annotations.

Manuscript preparation

Authors must follow the instructions for authors strictly, failing which the manuscripts would be rejected without review.

The manuscript should be prepared in Microsoft Word for Windows, maximum 8 pages of typed text using, Paper size: Custom size, Width 17 cm, Height 24 cm; format (Portrait), normal spacing (Single Space). Margins: Top 2.0 cm, 2.0 cm Left, Bottom 2.0 cm, 2.0 cm Right, no pagination.

Use font Times New Roman, size 11 (except where it is stated otherwise), single space, justify

Title of the paper should be Times New Roman, font size 14, bold, capital letters, justify

Authors – Times New Roman, font size 12, bold, specify the full names of all authors on the paper. Use 1,2, … numbers in suffix to refer to addresses of authors, only in the case of different affiliations (institution)

Affiliations of authors – Times New Roman, font size 9, normal, under affiliations of authors should be mentioned e-mail of corresponding author and after that category of paper.

Example 1

POTENTIALS OF SERBIAN LIVESTOCK PRODUCTION – OUTLOOK AND FUTURE

Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[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: Milan M.Petrović, **e-mail address**
Review paper

Example 2

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

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
Original scientific paper

Original scientific paper should contain following paragraphs with single spacing (title of paragraphs should be in Times New Roman 14 **bold**, except for **Abstract** and **Key words** where font size is 11 **bold**):

Abstract: up to 250 words, Times New Roman, font size 11, justify. Abstract should contain a brief overview of the methods and the most important results of the work without giving reference. Abstract submitted in English language.

Key words: not more than 6. The selection carried out by relying on widely accepted international source such as a list of keywords Web of Science.

Introduction – present the review of previous research and objective of the paper.

Materials and Methods – state methods applied in the paper; experimental research design. Use SI system of measurement units.

Results and Discussion – present investigation results separately from discussion or together in one paragraph. Presentation of the results should be precise and without repetitions, and include the evaluation of significant differences and other parameters.

Text and titles of tables, figures and graphs, Times New Roman, font size 9, **bold**, in the following form:

Table 1. Least square means for the reproductive traits of cows

Tables and figures should be numbered and with adequate title and legend, width and height not exceeding 12 cm and 17 cm, respectively. Tables should be prepared according to instruction for forming of tables in Office Word. Each column in table must have heading and, when necessary, abbreviations should be explained in the legend/footnote.

Conclusion – containing the most important issues of the paper

After Conclusion the title of the paper in Serbian in Times New Roman 14 **bold**, is stated, followed by authors in Times New Roman 11 *italic*, example:

Potencijali srpske stočarske proizvodnje – izgledi i budućnost

Milan M. Petrović, Stevica Aleksić, Milan P.Petrović, Milica Petrović, Vlada Pantelić, Željko Novaković, Dragana Ružić-Muslić

Summary – in Serbian language, 250 max. words (non-Serbian authors should provide Summary in English language that will be translated to Serbian by Editor's office)

Key words: not more than 6 (in Serbian language)

References – should be in alphabetical order. Names of the authors must be given in capital letters followed by the year of publication in brackets, titles in the language of the original. Use only the full name of the journal.

In scientific journals:

PETROVIĆ M. M., SRETENOVIĆ LJ., BOGDANOVIĆ V., PERIŠIĆ P., ALEKSIĆ S., PANTELIĆ V., PETROVIĆ D. M., NOVAKOVIĆ Ž. (2009): Quantitative analysis of genetic improvement of milk production phenotypes in Simmental cows. Biotechnology in Animal Husbandry, 25,1-2, 45-51.

ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M. (2007): Uticaj dužine tova u različitim sistemima gajenja na klanične osobine brojlerskih pilića genotipa Redbro. Biotechnology in Animal Husbandry 23, 3-4, 67-74.

WEBB E., O'NEILL H. (2008): The animal fat paradox and meat quality. Meat Science, 80, 28-36.

PhD Thesis:

RUŽIĆ-MUSLIĆ D. (2006): Uticaj različitih izvora proteina u obroku na proizvodne rezultate jagnjadi u tovu. Doktorska disertacija. Univerzitet u Beogradu, Poljoprivredni fakultet.

CAETANO A.R. (1999): Comparative mapping of the horse (*Equss caballus*) genome by synteny assignment of type-I genes with a horse-mouse somatic cell hybrid panel. Ph.D. Dissertation, University of California, Davis.

In Scientific Books:

PETROVIĆ P.M (2000): Genetika i oplemenjivanje ovaca. Naučna knjiga, Beograd, pp365.

FITZGERALD M. (1994): Neurobiology of Fetal and Neonatal Pain. In: Textbook of Pain. 3rd edition. Eds Wall P. and Melzack R. Churchchill Livingstone, London, UK, 153-163.

At Scientific Meetings:

ŠKRBIĆ Z., LUKIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ S., RAKONJAC S., PETRIČEVIĆ V., DOSKOVIĆ V., STANOJKOVIĆ A. (2015): Importance of farm management in reducing broilers skin lesions. Proceedings of the 4th International Congress "New Perspectives and Challenges of Sustainable Livestock Production", October 7 – 9, Belgrade, 145-158.

Citations in the text are presented in italic form, examples: …results of *Petrović (2009)*; *Petrović et al. (2009)*; *Webb and O'Neill (2008)....*; *(Škrbić et al., 2015); (Ružić-Muslić, 2006); (Webb and O'Neill, 2008)*

Editor's office

12th International Symposium
"Modern Trends in Livestock Production"
9th – 11th October 2019, Belgrade, Serbia

Organizer

INSTITUTE FOR ANIMAL HUSBANDRY, BELGRADE-ZEMUN

e-mail: biotechnology.izs@gmail.com
website: www.istocar.bg.ac.rs

SECOND ANNOUNCEMENT

On behalf of the International Scientific and Organizing Committee, it is our pleasure to invite you to participate at the **12th International Symposium on Modern Trends in Livestock Production**, which will be held **from 9th to 11th October 2019 in Belgrade**.

We invite you to take part with an oral or poster presentation. You also have the opportunity to present your institution or company at the Symposium.

At the Symposium, the experts from Serbia and abroad will present the results of their research in order to enable a better transfer of scientific achievements in all fields of animal husbandry and science and making them available to the scientists, researchers and practitioners in livestock production, as well as students, in the private sector and to the general public.

The aim of the scientific meeting is to establish better cooperation between researchers in the field of animal science from different institutions, and experts from the industry, trade and other related fields, as well as producers from Serbia, Western Balkans, EU and other parts of the world in the field of science, education and good livestock production practice.

MAIN TOPICS OF THE SYMPOSIUM

1. Genetics and Selection of Farm Animals
2. Breeding and Reproduction of Farm Animals
3. Physiology and Nutrition of Farm Animals
4. Organic Livestock Production
5. Livestock Feed and Ecology
6. Animal Welfare and Health Care
7. Technology and Quality of Animal Products
8. Protection of the Environment and Biodiversity in Animal Production
9. Alternative Production Methods in Livestock Production

OFFICIAL LANGUAGE

The official language of the Symposium is English.

REGISTRATION AND PAYMENTS

Registration and submission of full papers to the e-mail address: biotechnology.izs@gmail.com The authors shall submit full papers prepared acording to the **Instruction for Authors** for scientific journal "Biotechnology in Animal Husbandry" (www.istocar.bg.ac.rs). All submitted papers will be peer reviewed. Accepted papers will be published in the Proceedings.

REGISTRATION FEE	Before 30th June 2019 (Early registration)	After 30th June 2019 (Late registration)
Registration Fee, covers publishing of paper, Symposium material, participation in all sessions of the Symposium, coffee/tea break	**80 €**	**100 €**
Registration Fee, covers publishing of paper, Symposium material, participation in all sessions of the Symposium, coffee/tea break, tourist program and gala dinner	**120 €**	**150 €**

IMPORTANT DATES

Deadline for full paper submission   **Aprile, 30th 2019**

Request for Proforma invoice for Registration fee to the e-mail address:
biotechnology.izs@gmail.com

Symposium participants from Serbia can make the payment (in RSD value on the day of payment according to the exchange rate), on the following account:

Institut za stočarstvo, Beograd-Zemun
11080 Zemun, Autoput 16
Tekući račun br. 205-65958-94
Komercijalna banka

INSTRUCTION FOR EUR PAYMENTS
AIK BANKA AD BEOGRAD

Please pay as per instruction given below:

56A: Intermediary bank: **SOGEFRPP**
                                SOCIETE GENERALE
                                F-92978 PARIS
                                FRANCE

57A: Account with institution: **AIKBRS22**
                                AIK BANKA AD BEOGRAD
                                BULEVAR MIHAILA PUPINA 115Đ
                                11070 NOVI BEOGRAD
                                REPUBLIKA SRBIJA

59: Beneficiary customer: **RS35105050120000062319**
                                INSTITUT ZA STOČARSTVO ZEMUN
                                Autoput Beograd-Zagreb 16
                                Zemun
                                REPUBLIKA SRBIJA

ACCOMMODATION AND SYMPOSIUM LOCATION

The Symposium will be held in Hotel Park, Belgrade Njegoševa street 2, 11000, Belgrade, Serbia (www.hotelparkbeograd.rs)

Single room at special rate of 50 € daily per room
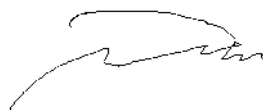Double room at special rate of 70 € daily per room
City tax is not included and is approximately 1.2 € per person daily.

Accommodation at SPECIAL RATES is possible for reservations before **August, 31st 2019.**

Hotel reservation telephone: +381114146800
Hotel reservation e-mail: reception@hotelparkbeograd.rs

On behalf of Organizing Committee

Dr. Milan P. Petrović,
Principal Research Fellow
Serbia

HOTEL RESERVATION FORM

Hotel Park Beograd welcomes the guests of
INSTITUTE FOR ANIMAL HUSBANDRY SYMPOSIUM
9-11th October 2019
We are pleased to advise the special rates and conditions available for this event:
(Please check appropriate box)

	Single room at special rate of 50 € daily per room
	Double room at special rate of 70 € daily per room

All the above mentioned rates are INCLUSIVE of full buffet breakfast in our Continental restaurant, VAT, complimentary internet access.
City tax is not included and is approximately. 1.2 € per person daily.

GUEST INFORMATION:

Family name:	
First name:	
e-mail:	
Contact phone number:	
Date of arrival:	Date of departure:

PAYMENT INFORMATION:

BY CREDIT CARD:	Visa	Master	Diners	American Express
Card Holder name:				
Card Number:			Exp. Date:	

Cancellation Policy: If you wish to cancel, please do so at least 72 hours prior to arrival. For all cancellations or no shows after this period, one night charge will be charged to your credit card.

PLEASE COMPLETE THIS FORM AND SEND TO HOTEL PARK BEOGRAD VIA FAX OR E-MAIL reception@hotelparkbeograd.rs by August 31st 2019.