UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

VOL 32, 4
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2016

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2016

Biotechnology in Animal Husbandry 32 (4), p 320-423, 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

Language editor
Olga Devečerski, grad.prof

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

Biotechnology in Animal Husbandry is covered by Agricultural Information Services (AGRIS) -Bibliographic coverage of abstracts; Electronic Journal Access Project by Colorado Altiance Research Libraries -Colorado, Denver; USA; Matica Srpska Library -Referal Center; National Library of Serbia; University Library "Svetozar Markovic", Belgrade, Serbia; EBSCO, USA; DOAJ and European Libraries
According to CEON bibliometrical analysis citation in SCI index 212, in ISI 9, impact factor (2 and 5) of journal in 2012: 0,667 and 0,467, - M51 category

Annual subscription: for individuals -500 RSD, for organizations 1200 RSD, -foreign subscriptions 20 EUR. Bank account Institut za stočarstvo, Beograd-Zemun 105-1073-11 Aik banka Niš Filijala Beograd.

Journal is published in four issues annually, circulation 100 copies.

The publication of this journal is sponsored by the Ministry of Education and Science of the Republic of Serbia.
Printed: "Mladost birošped", Novi Beograd, St. Bulevar AVNOJ-a 12, tel. 381 11 2601-506

Biotechnology in Animal Husbandry 32 (4), p 321-329 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 619:936'4
DOI: 10.2298/ BAH1604321N

THE APPLICATION OF PCR BASED METHODS IN DIAGNOSTICS OF SOME VIRAL INFECTIONS OF SWINE

Jakov Nišavić[1], Nenad Milić[1], Andrea Zorić[1], Jovan Bojkovski[1], Aleksandar Stanojković[2]

[1] University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Serbia
[2] Institute for Animal Husbandry, P.O. Box 23, 11081 Zemun, Belgrade, Serbia
Corresponding author: Jakov Nišavić, jakovmoni@vet.bg.ac.rs
Review paper

Abstract: Viral infections of swine cause significant economic losses in swine husbandry. They manifest in death of infected animals of different ages or in decreased productivity during the manufacturing process. Having that in mind, rapid and reliable diagnostics of viral infections is crucial in the prevention of disease transmission in herds of swine. Today, virological laboratories all over the world use different diagnostic methods such as isolation of virus in cell lines, ELISA, virus neutralization test, direct and indirect immunofluorescence and hemagglutination and hemagglutination inhibition tests. Virus isolation, virus neutralization test and some other standard virological methods are time consuming and rather expensive, therefore, molecular methods such as conventional PCR, RT - PCR, real-time PCR and direct sequencing methods are applied worldwide as fast and reliable. Their application is especially necessary for the detection of viruses which cannot be identified by using standard virological methods.

Key words: swine, virus, PCR, real-time PCR

Introduction

Viral infections of swine cause significant economic losses in swine husbandry. They manifest in death of infected animals of different ages or in decreased productivity during the manufacturing process. Having that in mind, rapid and reliable diagnostics of viral infections is crucial in the prevention of disease transmission in herds of swine.

Today, virological laboratories all over the world use different diagnostic methods such as isolation of virus in cell lines, ELISA, virus neutralization test, direct and indirect immunofluorescence and hemagglutination and hemagglutination inhibition tests (*Nišavić et al., 2006; Nišavić et al., 2007; Nišavić*

et al., 2008; Nišavić et al., 2013). Virus isolation, virus neutralization test and some other standard virological methods are time consuming and rather expensive, therefore, molecular methods such as conventional PCR, RT - PCR, real-time PCR and direct sequencing methods are applied worldwide as fast and reliable (*Milić et al., 2010; Nišavić et al., 2010; Veljović et al., 2013*).

Porcine circovirus 2 (PCV2) belongs to the family *Circoviridae* and genus *Cyrcovirus*. PCV2 is single-stranded with circular DNA and without outer envelope (*Segales et al., 2012*). The viral genome consists of 1767-1768 nucleotides and contains three ORF regions (*Larochelle et al., 2002*). The ORF1 region (rep gene) is responsible for the synthesis of proteins involved in the replication process, ORF2 (cap gene) region provides the synthesis of capsid proteins, whilst the third region - ORF3 encodes proteins that are likely involved in virus induced cell apoptosis. Based on the results of phylogenetic analysis of the whole genome of porcine circovirus type 2, there are four genotypes: PCV2a, PCV2b, PCV2c and PCV2d (*Liu et al., 2006; Cheung et al., 2007; Hesse et al., 2008; Wang et al., 2009*).

Depending on the localization of the pathological process and ages of pigs, PCV2 causes different kinds of disease. Today, the disease caused by PCV2 is best known as post-weaning multisystemic wasting syndrome (PMWS). This disease is manifested by the appearance of stunting, pallor of the skin, respiratory distress, and sometimes diarrhea and jaundice in weaned piglets for fattening (*Segales et al., 2005*). Infection of young animals, fattening pigs and adult pigs caused by PCV2 is also manifested as dermatitis and nephropathy syndrome or PDNS (*Rose et al., 2012*). Furthermore, porcine circovirus 2 may cause reproductive disorders and abortion (*O' Conor et al., 2001*), pneumonia (*Kim et al., 2003; Segales et al., 2004*) and enteritis (*Kim et al., 2002*).

Porcine parvovirus belongs to the family *Parvoviridae.* This is a non-enveloped single-stranded virus with linear DNA. Viral capsid consists of 32 capsomeres. The course and outcome of porcine parvovirus infection of swine depends on the immune status of infected animals and the stage of pregnancy at the time of infection. Infection of pregnant pigs causes fetal death and mummification (*Mengeling et al., 1975*). Fetuses infected before the 70th day of pregnancy usually die, while fetuses infected later synthesize specific antibodies against parvovirus and usually survive.

Aujeszky's disease virus (ADV) or pseudorabies virus (PrV) belongs to the family *Herpesviridae,* subfamily *Alphaherpesuirinae* and genus *Varicellovirus.* The viral genome consists of double-stranded DNA and the virus possesses an outer envelope.

Pseudorabies virus primarily causes disease in pigs, but it also occurs in cattle, sheep, goats, dogs, cats and wild boars which are thought to be the reservoir of the virus. Virus is transmitted among animals by direct and indirect contact (*Verpoest et al., 2014; Moreno et al., 2015*).

Aujeszky's disease virus causes neurological symptoms, respiratory distress and in some cases reproductive disorders in infected swine (*Pol et al., 2013; Moreno et al., 2015*). One of the most significant characteristics of pseudorabies infection in swine is the establishment of virus latency in olfactory bulb or trigeminal ganglia. The virus is reactivated as a result of stress or immunosuppressive factors leading to the appearance of clinical symptoms of the disease (*Huang et al., 2004; Steinringl et al., 2012*).

Based on available literature data, it can be concluded that molecular methods such as conventional PCR, RT-PCR, real-time PCR or direct sequencing are used worldwide in laboratory diagnostics of infections of swine caused by PCV2, PPV and PrV. Ogawa et al., 2009 used multiplex PCR and multiplex RT-PCR for the detection of PCV2, PrV, PPV, PRRS virus, Japanese encephalitis virus, porcine rotavirus, porcine epidemic diarrhea virus, and TGE virus from different kinds of samples such as feces, aborted fetuses or internal organs. The results of the investigation showed the presence of PCV2 in 32 samples and PPV and PRRS virus in 9 samples. The detection of PCV2, PPV and PRRS virus was performed by *Jiang et al. (2010)*. Seventy –six samples taken from pigs of 4 to 12 weeks of age and 27 aborted foetuses from 11 farms in Zhejiang Province in China, were examined by multiplex PCR. The presence of PCV2 was detected in 7 samples whilst PRRS virus and PPV were identified in 3 samples. Mixed infections caused by PCV2 and PPV as well as by PCV2 and PRRS virus were detected in 2 and 14 samples respectively, whilst 34 samples were negative for the presence of nucleic acids of the abovementioned viruses. *Opriessnig et al. (2014)* investigated the presence of porcine parvovirus and porcine circovirus type 2 in tissue samples from pigs originating from different parts of North America. The study included 586 blood serum samples and 164 samples of lung tissue collected from pigs in the period from 1996 to 2013. The presence of antibodies against PCV2 was found in 27.7% (162/586) samples, whilst the presence of PPV specific antibodies was found in 48.8% (286/586) of the blood sera. The presence of PCV2 was determined in 78.7% (129/164) of samples of lung tissue, while 56.7% (93/164) of lung samples were positive for the presence of porcine parvovirus. Mixed infections caused by PCV2 and PPV were discovered in 14.3% (84/586) of samples of sera and in 49.4% (81/164) of samples of lung tissue. Additionally, the prevalence of PPV DNA is significantly higher in tissues containing a large amount of PCV2 DNA in comparison to tissue samples that do not originate from animals with clinical symptoms of systemic circovirus infection. Lukač et al., 2016 used PCR for the detecion of PCV2 and PPV in swine from Republika Srpska. The presence of PCV2 was detected in 6 samples (6/80), while PPV was recovered from 5 samples (5/80). It should be noted that the pigs whose samples showed the presence of the aforementioned viruses did not express clinical signs of infection. The simultaneous detection of PCV2, PPV and PRRS virus was performed by Liu et al., 2013 using multiplex PCR. In total, fifty-eight samples of lung, tonsils,

lymph nodes and spleen and 24 samples of aborted fetuses were examined during the investigation. The presence of PRRS virus was detected in 12.19% of samples, while 21.95% of the samples were positive for PRRS and PCV2 viruses. Huang et al. 2013 used multiplex PCR method for the detection of PCV2, PCV1 and PrV. In total, 58 samples of lymph nodes, tonsils and lungs from pigs of 4 to 8 weeks of age were examined. The presence of PCV2 was observed in 30 samples, PCV in 2 samples, whilst the presence of Aujeszky's disease virus was found in only one sample. Mixed infection with PCV1 and PCV2 was detected in eight samples, whilst the presence of PCV2 and PrV was confirmed in six samples. Wilhelm et al., 2006 used the method of real - time PCR for the detection of the presence of PPV, PCV2, PrV and PRRS virus in samples of heart muscle, kidney, lungs, spleen, duodenum, jejunum, thymus and lymph nodes of swine. The obtained results confirmed the validity of real time PCR for fast and reliable routine diagnostics of parvovirus infections in swine as well as infections caused by other aforementioned viruses. Duplex real-time PCR was used by Zeng et al., 2013 for the detection of PPV and PCV2 in different samples collected from pigs. The presence of PCV2 and PPV was detected in 18 samples. Thirty-seven out of 72 samples of boar semen were positive for the presence of PPV, whilst 35 samples were positive for the presence of PCV2. *Larochelle et al. (2002)* performed the molecular characterization and phylogenetic analysis of 34 PCV2 strains identified in swine from eastern parts of Canada. The nucleotide sequences of those strains were compared with analogous sequences of 36 strains of PCV2 published in GenBank database and the results showed a high level of similarity (96% to 100%) with other PCV2 strains identified in Western Canada, the USA, Europe and Asia. The results of this investigation also demonstrated that the ORF1 region of the viral genome is highly conserved in all examined PCV2 strains. Ramos et al. 2013 analysed PCV2 strains from Uruguay. The molecular analysis of the PCV2 cap gene showed a nucleotide similarity of 99.7% among Uruguayan isolates and with two of the Brazilian isolates included in this study. Uruguayan isolates shared a nucleotide and amino acid identity of 99.1– 99.5% with Argentinean strains, which were in turn more closely related to isolates from France, Cuba, Canada and USA. Phylogenetic analysis revealed that Uruguayan PCV2 strains belong to PCV2a genotype. Molecular characterization of PCV2 strains identified in pigs in South Korea was carried out by *Chae et al, (2012)*. From a total of 21 strains of the virus, 17 belonged to the genotype PCV2b, whilst others belonged to genotypes PCV2a and PCV2c. During the extensive examination conducted by *Lukač et al. (2016)* eighty samples from non-vaccinated pigs from the territory of the Republic of Srpska were examined for the presence of PCV2 and PPV. Porcine circovirus 2 identified in this study belonged to PCV2c genotype and had a high level of similarity with some Italian, German and Chinese strains of the virus, while the identified PPV viruses were similar to viruses identified in UK, USA and China. *Cadar et al. (2012)* examined tissue samples and organs originating from 842 wild

boars that were collected in the period from 2006 to 2011 in the western regions of Romania. In addition to these samples, 120 samples collected from domestic pigs were also examined. The results showed that porcine parvovirus mostly diverged in the last 20 to 60 years and that the strains of the virus identified in wild boars have greater genetic diversity regarding to the strains of porcine parvovirus identified in domestic pigs. *Xiofen et al. (2013)* examined the evolutionary development and phylogeny of porcine parvovirus. During this investigation authors used 46 nucleotide sequences of the virus originating from the genetic database. The results showed that a common ancestor of all PPV strains existed about 250 years ago. *Xu et al. (2013)* compared the nucleotide sequences of VP2 gene of PPV - NE / 09 PPV virus and other PPV strains in China. The results showed a high level of similarity between the PPV strain NE / 09 and other PPV strains identified in China and that NE / 09 represents a mutant strain of the existing strains of porcine parvovirus, which has the highest prevalence of infection in pigs in China. *Serena et al. (2011)* compared the nucleotide sequences of Argentinean PrV isolates with the nucleotide sequences of PrV reference strains available at GenBank. A high percentage of nucleotide similarity was demonstrated between genotype I Argentinean strains (CL/7, CL/15, TL/92 and A/94) and the American strains Rice and Becker. The other genotype I Argentinean strains (CL/96, CL/98, CLP/98-10P and RC/79) had 99.7% identity with the reference strains Becker and Rice. Furthermore, the Argentinean genotype I strains showed high similarity with Brazilian genotype I strains (99.0– 99.4). *Verpoest et al. (2014)* performed the molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. The results showed that one isolate from domestic pig had a sequence identical to the Kaplan reference strain of PrV.

Conclusion

Regarding available literature data it can be concluded that the application of molecular methods based on PCR is crucial for fast and precise detection of viral infections of swine. The application of these methods is very important during the outbreaks of infection with high mortality rate when it is necessary to conduct fast and reliable diagnostics in order to prevent further dissemination of the infectious agent in animal population. Besides that, their application is especially necessary for the detection of viruses which cannot be identified by using standard virological methods.

Primena molekularnih metoda zasnovanih na lančanoj reakciji polimeraze u dijagnostici nekih infekcija svinja

Jakov Nišavić, Nenad Milić, Andrea Zorić, Jovan Bojkovski, Aleksandar Stanojković

Rezime

Virusne infekcije izazivaju značajne ekonomske gubitke u svinjarskoj proizvodnji. One se ispoljavaju kako kroz pojavu uginuća životinja, tako i kroz smanjenje produktivnosti. U cilju otkrivanja i sprečavanja širenja virusnih oboljenja svinja danas se u svetu primenjuju standardne i molekularne metode virusološke dijagnostike. Od standardnih metoda dijagnostike u upotrebi su metode izolacije virusa u kulturi ćelija, zatim ELISA, direktna i indirektna imunofluorescencija, kao i hemaglutinacija i inhibicija hemaglutinacije. Primena navedenih metoda podrazumeva duže vreme potrebno za dobijanje rezultata ispitivanja od najmanje 5 do 7 dana. Međutim, primena savremenih molekularnih metoda virusološke dijagnostike kao što su PCR, real-time PCR, odnosno metoda direktnog sekvenciranja, podrazumeva kraće vreme potrebno za dobijanje rezultata, odnosno omogućava preciznu dijagnostiku oboljenja u kraćem vremenskom periodu. Pored ovoga, značaj primene ovih metoda se ogleda i u otkrivanju virusa čije se prisustvo u uzorcima na drugi način, odnosno primenom standardnih metoda virusološke dijagnostike ne može detektovati.

Acknowledgment

The paper was financed by Ministry of Education, Science and Technological Development of the Republic of Serbia, Project TR-31008.

References

CADAR D., CSAGOLA A., LORINZ M., TOMBASZ K., SPINU M., TUBOLY T. (2012): Detection of natural inter-and intra-genotype recombination events revealed by cap gene analysis and decreasing prevalence of PCV2 in wild boars. Infection, Genetics and Evolution, 12, 2, 420-427.

CHEUNG A., LAGER K., KOHUTYUK O.I., VINCENT A.L., HENRY S.C., BAKER R.B., DUNHAM A.G. (2007): Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Archives of virology, 152, 5, 1035-1044.

HESSE R., KERRIGAN M., ROWLAND R. (2008): Evidence for recombination between PCV2a and PCV2b in the field. Virus research, 132, 1, 201-207.

HUANG C., HUNG J., WU C., CHIEN M. (2004): Multiplex PCR for rapid detection of pseudorabies virus, porcine parvovirus and porcine circoviruses. Veterinary Microbiology, 101, 209-214.
HUNG J., WU C., CHIEN M. (2004): Multiplex PCR for rapid detection of pseudorabies virus, porcine parvovirus and porcine circoviruses. Veterinary Microbiology, 101, 209-214.
JIANG Y. SHANG H., XU H., ZHU L., CHEN W., ZHAO L., FANG L. (2010): Simultaneous detection of porcine circovirus type 2, classical swine fever virus, porcine parvovirus and porcine reproductive and respiratory syndrome virus in pigs by multiplex polymerase chain reaction. The Veterinary Journal, 183, 172-175.
KIM J., CHUNG H. K., JUNG T., CHO W. S., CHOI C., CHAE C. (2002): Postweaning multisystemic wasting syndrome of pigs in Korea: prevalence, microscopic lesions and coexisting microorganisms. Journal of Veterinary Medical Science, 64, 1, 57-62.
KIM J., CHUNG H.K., CHAE C. (2003): Association of porcine circovirus 2 with porcine respiratory disease complex. The Veterinary Journal, 166, 3, 251-256.
LAROCHELLE R., MAGAR R., D ALLAIRE S. (2002): Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus research, 90, 1, 101-112.
LIU J., CHEN I., DU Q., CHUA H., KWANG J. (2006): The ORF3 protein of porcine circovirus type 2 is involved in viral pathogenesis in vivo. Journal of Virology, 80, 10, 5065-5073.
LIU J.K., WEI C.H., YANG X.Y., DAI A.L., XIAO –HUA L. (2013): Multiplex PCR for the simultaneous detection of porcine reproductive and respiratory syndrome virus, classical swine fever virus, and porcine circovirus in pigs. Molecular and Cellular Probes, 27, 149-152.
LUKAĆ B., KNEŽEVIĆ A., MILIĆ N., KRNJAIĆ D., VELJOVIĆ LJ., MILIĆEVIĆ V., ZORIĆ A., ĐURIĆ S., STANOJEVIĆ M., NIŠAVIĆ J. (2016): Molecular detection of PCV2 and PPV in pigs in Republic of Srpska, Bosnia and Herzegovina, Acta Veterinaria-Beograd, 66, 1, 51-60.
MENGELING W.L., CUTLIP R.C. (1975): Pathogenesis of in utero infection: experimental infection of five-week-old porcine fetuses with porcine parvovirus. American journal of veterinary research, 36, 8, 1173-1177.
MILIĆ, N., NIŠAVIĆ J., AŠANIN R., KNEŽEVIĆ A., AŠANIN J., VIDANOVIĆ D., ŠEKLER M. (2010): Primena lančane reakcije polimeraze (PCR) i metode Real-Time PCR u brzoj identifikaciji goveđeg herpesvirusa 1, Veterinarski Glasnik, 64, 3-4, 159-167.
MORENO A., SOZZI E., GRILLI G., GIBELLI L.R., GELMETTI D., LELLI D., CHIARI M., PRATI P., ALBORALI G.L., BONIOTTI M.B., LAVAZZA A., CORDIOLI P. (2015): Detection and molecular analysis of Pseudorabies virus strains isolated from dogs and a wild boar in Italy. Veterinary Microbiology, 177: 359-365.

NIŠAVIĆ J., MILIĆ N. (2006): Examination of the activity of glycoprotein HN and F antigens of the outer envelope of the parainfluenza virus type 3 by using fusional, hemolytic and hemagglutination tests, in vitro. Acta veterinaria, 56, 5-6, 431-36.
NIŠAVIĆ J., MILIĆ N., VELJOVIĆ Lj. (2007): Examination of the activity of glycoprotein HN and F antigens of the outer envelope of Newcastle disease virus by using fusional, hemolytic, hemagglutination and hemadsorption tests, in vitro. Acta veterinaria, 57, 1, 3-10.
NIŠAVIĆ J., KNEŽEVIĆ A., MILIĆ N. (2008): Primena lančane reakcije polimeraze (PCR) u identifikaciji izolovanih sojeva goveđeg herpesvirusa tip 1, VI Kongres medicinske mikrobiologije, Beograd, juni 2008, Zbornik kratkih sadržaja, 227-228.
NIŠAVIĆ J., MILIĆ N., KNEŽEVIĆ A., JOVANOVIĆ T. (2010): The application of polymerase chain reaction in detection of bovine herpesvirus 1 in clinical samples. Acta Veterinaria, 60, 1, 39-48.
NIŠAVIĆ J., MILIĆ N., VIDANOVIĆ D., ŠEKLER M., LAZIĆ S., PETROVIĆ T. (2013): Primena standardnih i molekularnih metoda u dijagnostici virusnih infekcija, 34. Seminar za inovaciju znanja veterinara, 8.februar, Beograd, 45-63.
O᾽CONOR B., GAUVREAU H., WEST K., BOGDAN J., AYROUD M., CLARK E., ELLIS J.A. (2001): Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. The Canadian Veterinary Journal, 42,7, 551.
OGAWA H., TAIRA O., HIRAI T., TAKEUCHI H., NAGAO A., ISHIKAWA Y., TUCHIYA K., NUNOYA T., UEDA S. (2009): Multiplex PCR and multiplex RT-PCR for inclusive detection of major swine DNA and RNA viruses in pigs with multiple infections. Journal of Virological Methods, 160, 210-214.
OPRIESSING T., XIAO C.T., GERBER P.F., HALBUR P.G. (2014): Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. Veterinary microbiology, 173, 1, 9-16.
POL F., DEBLANC C., OGER A., LE DIMNA M., SIMON G., LE POTIER M.F. (2013): Validation of a commercial real-time PCR kit for specific and sensitive detection of Pseudorabies. Journal of Virological Methods, 187: 421-423.
RAMOS N., MIRAZO S., CASTRO G., ARBIZA J. (2013): Molecular analysis of Porcine Circovirus Type 2 strains from Uruguay: Evidence for natural occurring recombination. Infection Genetics and Evolution, 19, 23-31.
ROSE N., OPRIESSNIG T., GRASLAND B., JESTIN A. (2012): Epidemiology and transmission of porcine circovirus type 2 (PCV2). Virus research, 164, 1, 78-89.
SEGALES J., ALLAN G.M., DOMINGO M. (2005): Porcine circovirus diseases. Animal Health Research Reviews, 6, 2, 119-142.

SEGALES J., KEKARAINEN T., CORTEY M. (2013): The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?.Veterinary microbiology, 165, 1, 13-20.
SERENA M., METZA G., MORTOLA E., ECHEVERNA M. (2011): Phylogenetic analysis of Suid Herpesvirus 1 isolates from Argentina. Veterinary Microbiology, 154, 78-85.
STERINRIGL A., REVILLA – FERNANDEZ S., KOLODZIEJEK J., WODAKA E., BAGO Z., NOWOTNY N., SCHMOLL F., KOFER J. (2012): Detection and molecular characterization of Suid herpesvirus type 1 in Austrian wild boar and hunting dogs. Veterinary Microbiology, 157:276-284.
VELJOVIĆ LJ., KNEŽEVIĆ A., MILIĆ N., NIŠAVIĆ J. (2013): Primena molekularnih metoda u identifikaciji izolovanih sojeva virusa parainfluence 3, 9. Kongres mikrobiologa Srbije, 30. maj – 1.jun, Beograd, 1.
VERPOEST S., CAY A., REGGE N. (2014): Molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. Veterinary Microbiology, 172, 72-77.
VERPOEST S., CAY A.B., REGGE N.D. (2014): Molecular characterization of Belgian pseudorabies virus isolates from domestic swine and wild boar. Veterinary Microbiology, 172, 72-77.
WANG F., GUO X., GE X., WANG Z., CHEN Y., CHA Z., YANG H. (2009): Genetic variation analysis of Chinese strains of porcine circovirus type 2. Virus research, 145, 1, 151-156.
WILHELM S., ZIMMERMANN P., SELBITZ H.J., TRUYEN U. (2006): Real-time PCR protocol for the detection of porcine parvovirus in field samples. Journal of Virological Methods 2006, 134: 257–260.
XIOFENG R., TAO Y., CUI J., SUO S., CONG Y., TIJSSEN P. (2013): Phylogeny and evolution of porcine parvovirus. Virus research, 178, 2, 392-397.
XU Y., WANG H., HUO G., LI S. (2013.): Characterization of the capsid protein VP2 gene of a virulent strain NE/09 of porcine parvovirus isolated in China. Research in Veterinary Science, 94, 219-224.
ZHENG L., WANG Y., LI M., CHEN H., GUOB X., GENGA J., WANG Z., WEIA Z., CUI B. (2013): Simultaneous detection of porcine parvovirus and porcine circovirus type 2 by duplex real-time PCR and amplicon melting curve analysis using SYBR Green. Journal of Virological Methods, 187, 15-19.

Received 7 October 2016; accepted for publication 20 November 2016

Biotechnology in Animal Husbandry 32 (4), p 331-339 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.082:636'4
DOI: 10.2298/ BAH1604331R

CORRELATION OF LITTER SIZE TRAITS

Čedomir Radović[1], Milica Petrović[2], Nenad Brkić[3], Nenad Parunović[4], Dragan Radojković[2], Radomir Savić[2], Marija Gogić[1]

[1] Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[2] University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
[3] Ministry of Agriculture and Environmental Protection, Belgrade, Republic of Serbia
[4] Institute of Meat Hygiene and Technology, Belgrade, Republic of Serbia
Corresponding author: cedomirradovic.izs@gmail.com
Original scientific paper

Abstract: Heredity and correlation of litter size traits were observed in 3693 litters, i.e. in 1622 daughters of two genotypes Swedish Landrace genotype - SL; and F1 ♀ SLxLW. The study included daughters (minimum ten daughters per sire) of 24 sires. Heritability estimates for the total number of piglets per litter in the first, the first two parities, and for all three parities was 0.174; 0.167 and 0.135. Heritability estimates for the number of piglets born alive were 0.181; 0.160 and 0.121, and for the weight of litter at birth 0.166; 0.174 and 0.150. On the other hand, very low heritability was determined for the number of weaned piglets, litter weight of piglets reared, individual weight of born and reared piglets, i.e. for the traits that are under greater influence of the environment (from 0.004 to 0.037). Phenotypic and genetic correlations ranged from weak to complete (r_p = 0.021 to r_p = 0.973 and r_g=0.188 to r_g=0.999, respectively). Analysis of the significance of correlations showed that the genetic correlations were statistically highly significant (P <0.01).

Keywords**:** sow, piglet, heritability, phenotypic correlations, genetic correlations

Introduction

Reproduction in pig production is necessary to achieve adequate yearly and lifetime production of piglets per sow. This can be achieved if sows have good fertility and regular farrowing and if the genetic potential of breeding pigs is used to the maximum, i.e. as long as they have vital litters with lot of piglets. Fertility of pigs is a key characteristic of this species of domestic animals, which constitutes the main importance which the production of pigs and pork have in the modern livestock production, both in terms of quantity of product and in terms of economic efficiency of production. Fertility of sows depends on the properties that can be

conditionally divided on reproductive traits and traits of the litter size. The reproductive traits include age at first puberty oestrus, age at conception and age at farrowing, period from weaning to oestrus and conception of sows and duration of exploitation of sows, while the litter size traits include the size and weight of the litter at birth and weaning. Production of pigs is expensive and very complex process in pig breeding. The litter size of primiparous sows contributes significantly to improving the economy of pig production, and is one of the most important breeding goals. The litter size and the number of piglets born alive per litter and sow per year are subject to constant research, although it is known that the selection in a short time cannot achieve significant genetic improvement, primarily due to the low heritability (h^2 = 0.02 to 0.17) (*Serenius et al., 2003; Popovac et al., 2012; Lukač et al., 2016*). Numerous authors have dedicated their research to monitoring reproductive traits which are subject to the influence of the sire, genotype, maternal effect, parity, as well as to functioning of the external factors (*Petrović et al., 2000; Varona and Noguera, 2001; John and Wähner, 2002; Popov et al., 2003; Serenius et al., 2003; Kosovac et al., 2005; Radović et al., 2006; Stančić et al., 2006; Radojković et al., 2007; Vidović et al., 2011; Lukač et al., 2014*).

Material and methods

Heredity and correlation of litter size traits were observed in 3693 litters, i.e. in 1622 daughters of two genotypes Swedish Landrace genotype - SL; and F1 ♀ SLxLW. The study included daughters (minimum ten daughters per sire) of 24 sires. The litter size traits (number of live born piglets, number of stillborn piglets, number of piglets per litter, number of piglets weaned at 28 days, litter weight at birth, litter weight at 28 days, the individual weight of piglets at birth and individual weight of piglets 28 days) were monitored in the first three parities in the period of five years and parities which took place in two seasons of fertilization (very warm - *season 1*, June, July, August and September, and cooler - *season 2*, January, February, March, April, May, October, November and December). Processing of data was done by implementation of adequate programme, i.e. use of the method of least squares (*LSMLMW and MIXMDL-Harvey, 1990*). The models for analysis are included fixed effect of genotype (G_i=1,2), fixed effect of farrowing year (Y_j=1, 2,..,5), fixed effect of fertilization season ($S_{k\ or\ l}$=1, 2), fixed effect of parity (P_k=1, 2, 3), random effect of father ($f_{l\ or\ m}$=1, 2,..., 24) and random error.

In examining the first farrowing, the following mixed model of least squares was used:

$$Y_{ijklm} = \mu+G_i+Y_j+S_k+f_l+e_{ijklm}$$

The following mixed model was used in examining thr first two parities:

$$Y_{ijklm} = \mu + G_i + Y_j + P_k + S_l + f_m + e_{ijklmn}$$

The following mixed model was used in examining the first three parities:

$$Y_{ijklm} = \mu + G_i + Y_j + P_k + S_l + f_m + e_{ijklmn}$$

Results and Discussion

Table 1 shows the heritability values of litter size traits for the first parity, first two parities, and first three parities. Based on the results presented in the table it can be seen that the heritability values are almost equal for the trait - total number of piglets per litter (0.174, 0.167 and 0.135), number of piglets born alive (0.181, 0.160 and 0.121), and the weight of the litter at birth (0.166, 0.174 and 0.150). On the other hand, we see very poor heritability for other investigated traits due to the increasing influence of the environment (from 0.004 to 0.037).

Table 1. Heritability (h^2) for litter size

Trait	first parity (n=1622)	first two parities (n=2785)	first three parities (n=3693)
Total number of piglets in litter	0.174	0.167	0.135
Number of live born piglets	0.181	0.160	0.121
Number of piglets reared 28 days	0.022	0.037	0.028
Weight of litter at birth, kg	0.166	0.174	0.150
Weight of litter at 28 days, kg	0.015	0.031	0.021
Individual weight of piglets at birth, kg	0.004	0.015	0.020
Individual weight of piglets at 28 days, kg	0.029	0.027	0.020

Based on the presented results it is obvious that the heritability was slightly higher for the total number of piglets per litter and the number of live born piglets in the first, in relation to the first two parities, as well as in relation to the first three parities. In regard to the weight of litter at birth, it is established that the degree of heritability was higher in the first two parities compared to the first litter and determined h^2 values for the first, second and third litter. The determined value of heritability in our study is in agreement with the results reached by (*Petrović et al., 2000; Popovac et al., 2012*). In regard to the share of 62.1% for the first three parities, *Wolf et al., (2008)* have found approximately the same heritability values: 0.13 for the total number of born, and 0.14 for the number of live born piglets. *Lukač et al. (2016)* have found lower values of heritability in the first two parities while for the third parity approximately the same values are determined for the

total number of live born piglets (0.18 and 0.17), as well as for the the number of reared piglets and weight of the litter of reared piglets (0.01 and 0.03). In relation to our research somewhat lower heritability values for Landrace and Large White in the first two parities have been determined by *Vidović et al. (2011).*

Table 2 shows the coefficients of phenotypic and genetic correlations (r_p and r_g) for reproductive traits in the first parity. On the basis of these phenotypic correlations, it can be seen that the total number of piglets per litter was positively correlated with the total number of piglets born alive (r_p = 0.967), while the correlation was not found between the total number of piglets per litter and the number of piglets reared at 28 days (r_p = 0.055), as well as between the number of piglets born alive and the number of piglets reared at 28 days (r_p = 0.063). While observing the genetic correlations for the same studied traits, it can be seen that the correlations are positive and stronger compared to the phenotypic correlations. In the examination of the statistical significance, it was determined that phenotypic correlations between the total number of piglets and the number of pigs reared at 28 days were not significant, as well as between the weight of litter at 28 days (P> 0.05), while the number of live born and reared piglets at 28 days was were correlated statistically significantly (P<0.05). For the other stated phenotypic correlations, as well as genetic correlations, it was established that they were highly statistically significant (P<0.01).

Table 2. The coefficients of phenotypic and genetic correlation for reproductive traits in the first parity (n=1622)

Traits			r_p	r_g
Total no. of piglets in litter	:	Number of live born piglets	0.967 **	0.999 **
Total no. of piglets in litter	:	No. of piglets reared at 28 d.	0.055 ns	0.336 **
Total no. of piglets in litter	:	Litter weight at birth, kg	0.847 **	0.997 **
Total no. of piglets in litter	:	Litter weight at 28 d., kg	0.040 ns	1.157 **
Number of live born piglets	:	No. of piglets reared at 28 d.	0.063 *	0.393 **
Number of live born piglets	:	Litter weight at birth, kg	0.877 **	0.975 **
Number of live born piglets	:	Litter weight at 28 d., kg	0.036 ns	1.172 **
No. of piglets reared at 28d.	:	Litter weight at birth, kg	0.059 ns	0.677 **
No. of piglets reared at 28d.	:	Litter weight at 28 d., kg	0.639 **	0.374 **
Litter weight at birth, kg	:	Litter weight at 28 d., kg	0.042 ns	1.343 **

-) tab $_{0.05}$ =0.062 (P<0.05); tab $_{0.01}$ =0.081 (P<0.01);

Phenotypic and genetic correlations for reproductive traits for first two parities are presented in Table 3. Significance of correlation showed that the genetic correlations were highly statistically significant (P<0.01). Also, we can see that the genetic correlation is greater than the phenotypic between the weight of the litter at

birth and litter weight at 28 days (r_p = 0.047 (P<0.05) and r_g = 0.734). Also, testing the significance of correlation showed that the genetic correlations were statistically highly significant (P<0.01).

Table 3. The coefficients of phenotypic and genetic correlation for reproductive traits for the first two parities (n=2785)

Traits			r_p	r_g
Total no. of piglets in litter	:	Number of live born piglets	0.973 **	0.998 **
Total no. of piglets in litter	:	No. of piglets reared at 28 d.	0.060 ns	0.211 **
Total no. of piglets in litter	:	Litter weight at birth, kg	0.850 **	0.988 **
Total no. of piglets in litter	:	Litter weight at 28 d., kg	0.026 ns	0.689 **
Number of live born piglets	:	No. of piglets reared at 28d.	0.068 *	0.188 **
Number of live born piglets	:	Litter weight at birth, kg	0.873 **	0.976 **
Number of live born piglets	:	Litter weight at 28 d., kg	0.021 ns	0.629 **
No. of piglets reared at 28 d.	:	Litter weight at birth, kg	0.070 *	0.294 **
No. of piglets reared at 28 d.	:	Litter weight at 28 d., kg	0.613 **	0.649 **
Litter weight at birth, kg	:	Litter weight at 28 d., kg	0.047 ns	0.734 **

-) tab $_{0.05}$ =0.062 (P<0.05); tab $_{0.01}$ =0.081 (P<0.01);

Based on the results presented in Table 4, the following phenotypic and genetic correlations for reproductive traits for the first three parities can be seen. The total number of piglets per litter was positively correlated with the total number of piglets born alive (r_p = 0.971 and r_g = 0.999), whereas no correlation was determined with the number of piglets reared at 28 days. The correlation between the total number of piglets per litter and litter weight at birth is very strong and complete (r_p = 0.848 and r_g = 0.978). There was no correlation between the total number of piglets per litter and litter weight at 28 days (r_p = 0.027; P> 0.05), while the genetic correlation was very strong (r_g = 0.755). By testing the significance values according the values in tables, it can be seen that the genetic correlations were statistically highly significant (P<0.01), while the phenotypic correlations ranged from insignificant to a highly statistically significant. Also, it can be seen that the genetic correlations were somewhat stronger than the phenotypic correlations for studied traits.

Table 4. The coefficients of phenotypic and genetic correlation for reproductive traits for the first three parities (n=3693)

Traits			r_p	r_g
Total no. of piglets in litter	:	Number of live born piglets	0.971 **	0.999 **
Total no. of piglets in litter	:	No. of piglets reared at 28d.	0.052 ns	0.207 **
Total no. of piglets in litter	:	Litter weight at birth, kg	0.848 **	0.978 **
Total no. of piglets in litter	:	Litter weight at 28 d., kg	0.027 ns	0.755 **
Number of live born piglets	:	No. of piglets reared at 28d.	0.057 ns	0.190 **
Number of live born piglets	:	Litter weight at birth, kg	0.873 **	0.967 **
Number of live born piglets	:	Litter weight at 28 d., kg	0.023 ns	0.686 **
No. of piglets reared at 28d.	:	Litter weight at birth, kg	0.073 *	0.256 **
No. of piglets reared at 28d.	:	Litter weight at 28 d., kg	0.604 **	0.643 **
Litter weight at birth, kg	:	Litter weight at 28 d., kg	0.060 ns	0.795 **

-) tab $_{0.05}$ =0.062 (P<0.05); tab $_{0.01}$ =0.081 (P<0.01);

The absence of correlation between the total number of piglets per litter and the number of piglets weaned at 28 days can be explained by equalisation of the litter which is the common procedure on the farm. On this occasion, piglets are brought under the that she has not given birth to but raised them as their own, which resulted in the presence and effect of the factors that could not be controlled. On the other hand, it can be seen that the milk yield of sows, as a form of manifestation of their maternal effect, influenced the litter weight at 28 days and individual weight of piglets at 28 days. Certainly, the results are influenced by the studied animals and their breed, as well as the housing conditions and nutrition.

Research that we conducted were consistent with the research of a number of authors (*Tolle et al., 1998; Wolf et al., 1999; Lukač et al., 2016*) who have determined the phenotypic and genetic correlations between reproductive traits ranging from weak to complete. *Roeh and Kennedy (1995)* have found also medium to strong genetic correlations for following traits: the total number of piglets between 1st and 2nd parity = 0.59, the number of live born piglets between 1st and 2nd parity = 0.49, the number of weaned piglets between 1st and 2nd parity = 0.17. Also, *Rydhmer et al. (1995)* have found a strong genetic correlation between the 1st and 2nd parities for litter size (0.67). *Popovac et al. (2012)* have found a greater correlation between the total number of piglets born and reared in comparison to our results (r_p = 0.227 and r_g = 0.619). In concordance with our research, complete phenotypic and genetic correlations between the total born and live born piglets (in the first, the first two and the first three parities) are found by *Lukač et al. (2016),* however, also weaker relation between the number of live born piglets and litter weight at weaning is determined by *Lukač et al. (2016)* as well as a stronger connection between the number of piglets born and reared, compared to our research.

Conclusion

The heritability value of traits - total number of piglets per litter and the number of live born piglets was the greatest for the first parity with respect to the first and second parity, as well as in relation to the first, second, and third parity.

This low value of heritability can be explained by the influence of the database structure, data distribution according to ordinal number of parity, as well as equalizing the litter that is normal and common processing operation on the farm. Phenotypic and genetic correlations ranged from weak to complete (r_p=0.021 to r_p=0.973 and r_g=0.188 to r_g=0.999). Significance of correlations showed that the genetic correlations were statistically highly significant (P<0.01).

Povezanost osobina veličine legla

Čedomir Radović, Milica Petrović, Nenad Brkić, Nikola Parunović, Dragan Radojković, Radomir Savić, Marija Gogić

Rezime

Naslednost i međusobna povezanost osobina veličine legla praćene su za 3693 legla, odnosno kod 1622 kćeri dva genotipa švedski landras - ŠL; i F_1 ♀ŠLxVJ. Ispitivanjem su obuhvaćene kćeri (minimalno deset kćeri po ocu) od 24 oca.

Heritabiliteti za ukupan broj prasadi u leglu u prvom, prvom i drugom i za sva tri prašenja iznosio je 0,174; 0,167 i 0,135. Za broj živorođene prasadi vrednosi heritabiliteta su iznosile 0,181; 0,160 i 0,121, dok su za masu legla pri rođenju vrednosti 0,166; 0,174 i 0,150. Sa druge strane utvrđen je jako slab heritabilitet za broj odgajene prasadi, masu legla odgajene prasadi, individualnu masu rođene i odgajene prasadi odnosno za ispitivane osobine koje su pod većim uticajem okoline (od 0,004 do 0,037). Fenotipske i genetske korelacije kretale su se od slabih do potpunih (r_p=0,021 to r_p=0,973 odnosno r_g=0,188 to r_g=0,999). Testiranje značajnosti korelacija pokazalo je da su genetske bile statistički visoko značajne (P<0,01).

Acknowledgement

Research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, Project TR 31081.

References

HARVEY R.W. (1990): User's guide for LSMLMW and MIXMDL. V. PC-2,1-91.
JOHN A., WÄHNER M. (2002): Influence of body condition during selection and insemination on reproduction performances of different pig races. Biotechnology in Animal Husbandry, 18, 1-2, 45-51.
KOSOVAC O., PETROVIĆ M., ŽIVKOVIĆ B., FABJAN M., RADOVIĆ Č. (2005): Uticaj genotipa i prašenja po redu na variranje osobina plodnosti svinja. Biotehnologija u stočarstvu, 21, 3-4, 61-68.
LUKAČ D., VIDOVIĆ V., VIŠNJIĆ V., KRNJAIĆ J., ŠEVIĆ R. (2014): The effect of parental genotype and parity number on pigs litter size. Biotechnology in Animal Husbandry, 30, 3, 415-422.
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.
PETROVIĆ M., VUKOVIĆ V., TRIVUNOVIĆ S., RADOJKOVIĆ D. (2000): Ocena fenotipske i genetske varijabilnosti veličine legla i priplodne vrednosti nerastova. Biotehnologija u stočarstvu, 17, 5-6, 17-24.
POPOV R., RADOVIĆ I., TRIVUNOVIĆ S., TEODOROVIĆ M., PETROVIĆ M. (2003): Fenotipska ispoljenost, varijabilnost i naslednost osobina plodnosti svinja. 4. Broj mrtvorođene prasadi u leglu. Savremena poljoprivreda, Novi Sad, 52, 3-4, 297-302.
POPOVAC M., RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M., GOGIĆ M., STANOJEVIĆ D., STANIŠIĆ N. (2012): Heritability and connections of sow fertility traits. Biotechnology in Animal Husbandry, 28, 3, 469-475.
RADOJKOVIĆ D., PETROVIĆ M., MIJATOVIĆ M., RADOVIĆ Č. (2007): Phenotypic variability of fertility traits of pure breed sows in first three farrowings. Biotechnology in Animal Husbandry, 23, 3-4, 41-50.
RADOVIĆ I., STANČIĆ B., POPOV RADMILA, TRIVUNOVIĆ SNEŽANA, TEODOROVIĆ M. (2006): Reproduktivna performansa prvopraskinja i krmača viših pariteta. Savremena poljoprivreda, 55, 1-2, 83-90.
ROEHE R., KENNEDY B. W. (1995): Estimation of genetic parameters for litter size in Canadian Yorkshire and Landrace swine with each parity of farrowing treated as a different trait. Journal of Animal Science, 73, 10, 2959 – 2970.
RYDHMER L., LUNDEHEIM N., JOHANSSON K. (1995): Genetic parameters for reproduction traits in sows and relations to performance test measurements. Journal of Animal Breeding & Genetics, 112, 1, 33 - 42.
SERENIUS T., SEVÓN-AIMONEN M.L, MÄNTYSAARI E.A. (2003): Effect of service sire and validity of repeatability model in litter size and farrowing interval of Finnish Landrace and Large White populations. Livestock Production Science, 81, 213-222.

STANČIĆ B., GRAFENAU P., HRENEK P., RADOVIĆ I., GAGRČIN M. (2006): Uticaj vrste inseminacionih katetera i postinseminacione stimulacije cerviksa na fertilitet krmača. Savremena poljoprivreda, 55, 1-2, 91-94.
TOLLE K. H., THOLEN E., TRAPPMANN W., STORK F. J. (1998): Possibilities of breeding value optimation for reproduction traits in pigs for a pig breeding association. Zuchtungskunde, 70, 5, 351–361.
VARONA L., NOGUERA L. J.(2001): Variance components of fertility in Spanish Landrace pigs. Livestock Production Science, 67, 217—221.
VIDOVIĆ V., ŠTRBAC LJ., LUKAČ D., STUPAR M. (2011): Influence of age and weight of landrace gilts at fertile insemination on litter size and longevity. Biotechnology in Animal Husbandry, 27, 1, 75-84.
VIDOVIĆ V, ŠTRBAC LJ., LUKAČ D, PUNOŠ D., ŠEVIĆ Š, STUPAR S, VIŠNJIĆ V, KRNJAJIĆ J. (2011): Genetic parameters of reproduction traits of prolific and conventional purebred pigs. Krmiva, 53, 5, 193-200.
WOLF J., GROENEVELD E., WOLFOVA M., JELINKOVA V. (1999): Estimation of genetic parameters for litter traits in Czech pig populations using a multitrait animal model, Czech Journal of Animal Science, 44, 193 – 199.
WOLF J., ŽÁKOVÁ E., GROENEVELD E. (2008): Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. Livestock Science, 115, 195–205.

Received 9 November 2016; accepted for publication 11 December 2016

Biotechnology in Animal Husbandry 32 (4), p 341-352 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.05'636.4
DOI: 10.2298/ BAH1604341N

SEASONAL AND YEAR DYNAMICS IN THE QUALITY CHARACTERISTICS IN PIG CARCASSES

Jivko Nakev[1], Teodora Popova[2], Maya Ignatova[2], Penka Marinova[2], Tania Nikolova[1]

[1]Agrcultural Institute, 9700 Shumen, Bulgaria
[2]Institute of Animal Science, 2232 Kostinbrod, Bulgaria
Corresponding author: Jivko Nakev, jivko_nakev@abv.bg
Original scientific paper

Abstract: The aim of our study was to assess the dynamics of the characteristics in pig carcasses as affected by the season and year of slaughter. A total of 106 027 carcasses of growing-finishing pigs of commercial production, slaughtered in the same abattoir in 2014 and 2015 were included in the study. The carcasses were classified using UltraFOM 200 device, as the characteristics controlled were back-fat thickness at two locations and the depth of *m. Longissimus dorsi*. These measurements were used to further determine the lean meat percentage. The results of the study showed significant differences in the dynamics of changes of carcass characteristics during the seasons and the years. The highest lean meat percentage was found in summer (56.48%), followed by spring (56.34%), autumn (56.29%) and winter (56.10%). On the other hand, the pigs slaughtered in winter displayed highest carcass weight and back-fat thickness at both locations.

Keywords: pigs, carcass characteristics, lean meat, back-fat, seasons, year

Introduction

The systems for classification and payments of pigs for slaughter play an important role for the intensifying of the process of improvement of the carcass characteristics. The development and implementation of SEUROP system affects considerably the qualities of the finishing pigs in the countries where it is being applied (*Kalm*, 1998; *Čandek-Potokar et al*., 2004; *Pulkrábek et al*., 2006; *Castryck*, 2007).

According to *Savescu and Laba* (2016) the accurate work of the system is a basis for a fair payment to the pig producers and in order to ensure the its efficient work, the monitoring of the results obtained as well as eliminating of the negative impacts on its accuracy are of critical importance. In this regard the influence of some factors such as the sex of the animals (*Engel and Walstra*, 1993; *Gispert and Diestre*, 1994; *Daumas and Dhorne*, 1996; *Choi and Lee*, 2001; *Radović et al.*,

2012; Knecht and Duziński, 2016), their weight (*Correa et al*., 2006; *Vítek et al*., 2012), genotype (*Vítek et al.*, 2009; *Gogić et al. 2014; Zhang et al*., 2015), carcass presentation (*Pulkrábek et al*., 2010) or season of finishing and slaughter (*Piwczyński et al*., 2013) has been thoroughly investigated.
The aim of our study was to assess the dynamics of the carcass characteristics in pigs as affected by the year and season of slaughter.

Material and methods

The study included 106 027 growing-finishing pigs of commercial production, slaughtered in the same abattoir in 2014 and 2015. The quality characteristics of the carcasses were determined according to the Regulation 15/8.05.2009. The classification was done using Ultra FOM 200 device. The traits controlled included:
X_1- thickness of back-fat with skin, measured at 7 cm from the carcass midline between 3d and 4th last lumbar vertebra (mm);
X_2- thickness of the back-fat with skin, measured at 7 cm from the carcass midline between 3d and 4th last rib (mm);
X_3- depth of *m. Longissimus dorsi* (m. LD), measured at X_2 (mm).
The weight of the carcasses was recorded up to 45 min *post mortem* 0.01 kg.
For the purposes of the study the experimental material was divided by months as follows: I - III (winter), IV-VI (spring), VII-IX (summer), and X-XII (autumn).
The carcass data were subjected to two-way ANOVA. The model included the fixed effects of year, season and their interaction. The means were further compared through Tukey post-hoc test. The statistical evaluation was performed by JMP v.13 software package.

Results and discussion

Figure 1 presents the data of the number of the studied carcasses. It can be seen that in 2014 a total of 43052 pigs were slaughtered, while in the next year their number was 63675.

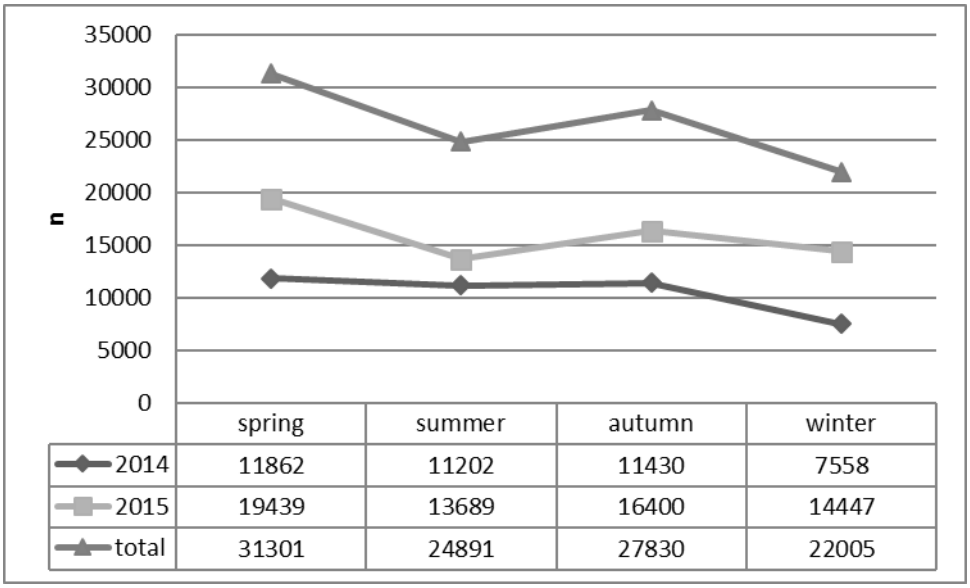


Figure 1. Number of carcasses studies in during the seasons in 2014 and 2015

The percentage of classified carcasses was the highest in spring-33 301 pigs (29.52%), followed by the autumn – 27 830 pigs (26.25%), summer – 24 891 pigs (23.48%) and winter – 22 005 pigs (20.75%). The difference in the number of the studied pigs during both years were determined by the increase of the work load of the abattoir and also by the changes of the share of meat import in the total mass of processed pork.

The average lean meat percent (LMP) was 56.34%, and the carcass weight of the pigs was 85.00 kg (Table 1).

Table 1. Mean, standard deviation (SD), coefficient of variation (CV), minimum and maximum values of the lean meat percentage, carcass weight, back-fat thickness and depth of m. LD

Item	LMP, %	Carcass weight, kg	Back-fat X_1, mm	Back-fat X_2, mm	m. LD X_3, mm
Mean	56.34	85.13	18.59	15.14	58.28
SD	1.85	8.44	3.61	3.18	8.89
CV, %	3.28	9.91	19.43	21.01	15.25
Minimum	36.6	49	7	7	7
Maximum	63.1	132.01	47	51	92

In a previous study with 100 762 pigs, carried out in 2009 (*Nakev*, 2010), the determined lean meat percentage was 56.72%, whereas the carcass weight was 76.80 kg. *Marinova et al.* (2008) reported that the carcass weight of pigs,

slaughtered in three Bulgarian districts (Razgrad, Turgovishte and Plovdiv) in the period 2006-2007 was 81.64 kg. The study included 1 865 scalded carcasses. The highest variability in the analysed traits was observed in regard to the back-fat (19.43% - 21.01%) depending on the location of the measurement. The variation coefficients of carcass weight and depth of m. LD were 9.88% and 15.25% respectively. The deviations for LMP did not exceed 3.3 %.
The results of ANOVA showed that besides the significant effect of the year and season of slaughter, both factors interacted significantly (Table 2).

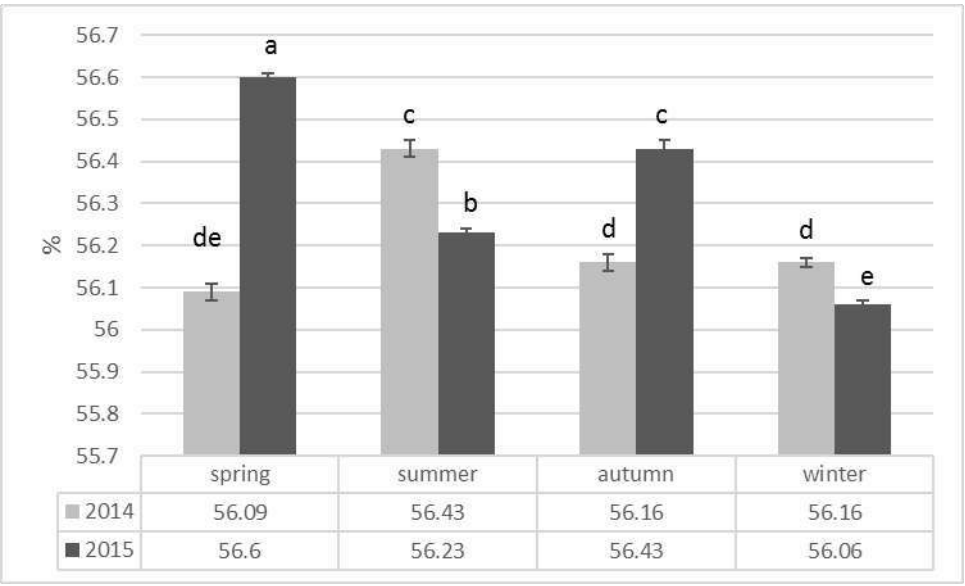
Table 2. ANOVA results for the effect of year, season and their interaction on the carcass traits

Source	LMP			
	df	MS	F	Sig.
Year	1	960.69	285.35	***
Season	3	531.45	157.45	***
Interaction	3	415.02	123.27	***
Error	106020			
	Carcass weight			
	df	MS	F	Sig.
Year	1	119323.69	1745.49	***
Season	3	23854	348.94	***
Interaction	3	17693.44	258.82	***
Error	106020			
	Back-fat X1			
	df	MS	F	Sig.
Year	1	9515.68	752.03	***
Season	3	2595.77	205.14	***
Interaction	3	5113.59	404.13	***
Error	106020			
	Back-fat X2			
	df	MS	F	Sig.
Year	1	104.18	10.39	**
Season	3	1122.95	111.45	***
Interaction	3	341.58	33.90	***
Error	106020			
	Depth of m. LD X3			
	df	MS	F	Sig.
Year	1	8786.83	113.08	***
Season	3	22757.96	292.89	***
Interaction	3	12809.82	164.86	***
Error	106020			

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

Figure 2 shows the changes of the lean meat percentage in the pig carcasses according to the year and season of slaughter. During 2014 the pigs slaughtered in

summer had the highest values of LMP, as there were significant differences between this season and the others.

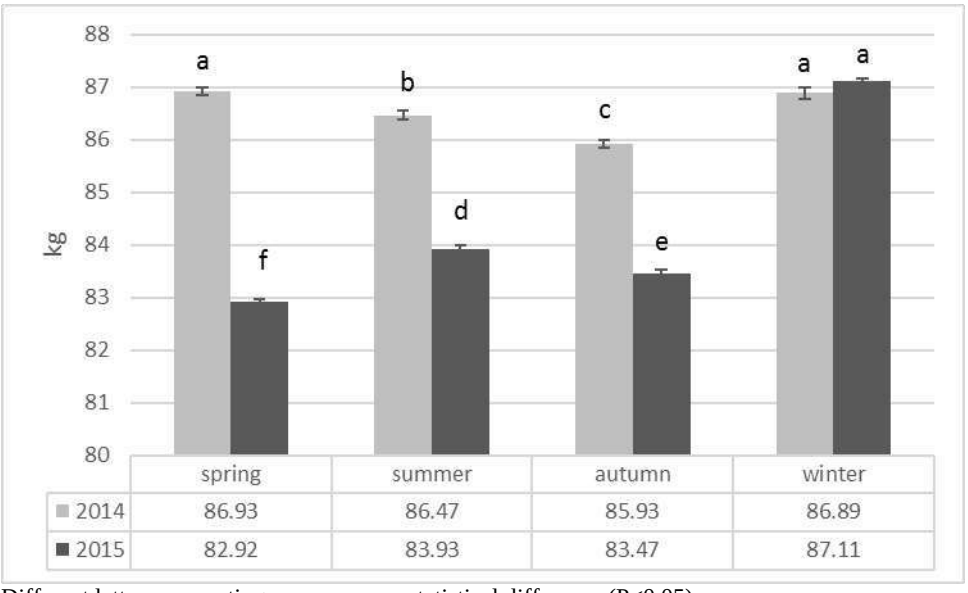


Different letters connecting seasons mean statistical difference (P<0.05)

Figure 2. Dynamics of change in the lean meat percentage (LMP) during the seasons in 2014 and 2015

For the rest three seasons there were no differences in the lean meat content and it varied in the range of 56.09-56.16%. In contrast, in 2015 the four seasons differed in regard to the lean meat percentage of the carcasses, as the highest was observed in the pigs slaughtered in spring, followed by those slaughtered in autumn. In the winter of 2015 the carcasses of the slaughtered animals displayed the lowest lean meat percent when compared to the other seasons of the year. Within the seasons, the lean meat content also differed between the years of slaughter, however the trend of change in this characteristic was opposite, which reflects the significant interaction between the factors year and season displayed in Table 2. Similar to us, Piwczyński et al. (2013) found that the lowest lean meat percentage of pig carcasses was recorded in winter (55.06%). However the authors reported also lowest percentage of the lean meat in summer (55.39%) while highest in autumn (55.01%).

The weight of the carcasses (Fig. 3) was significantly higher in the animals slaughtered in 2014, when compared to 2015 in all the seasons except in winter. In 2014 the values of this trait decreased from spring to autumn, after that increase was found in winter.

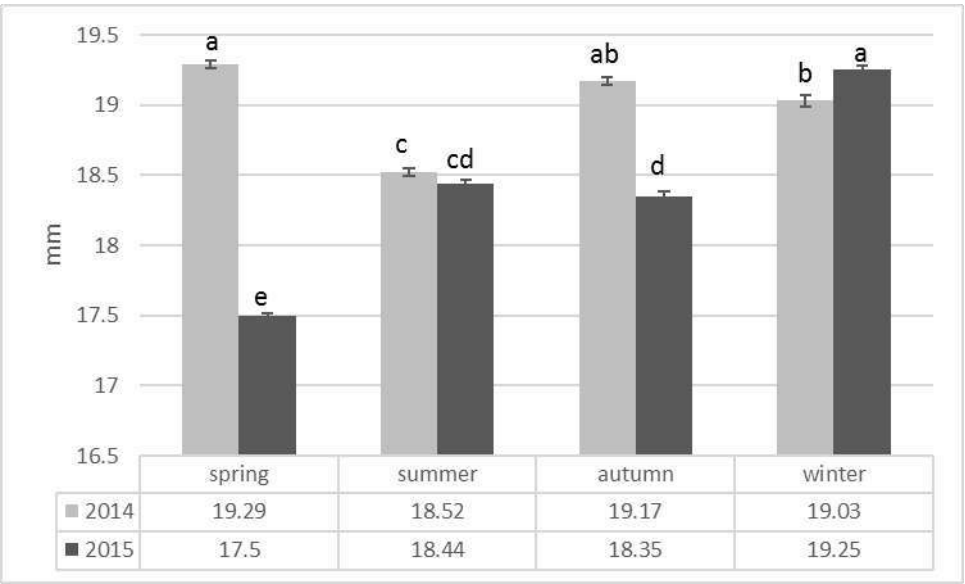


Different letters connecting seasons mean statistical difference (P<0.05)

Figure 3. Dynamics of change in the carcass weight during the seasons in 2014 and 2015

Similarly, in 2015, the pigs slaughtered in winter displayed high weight, however there was considerable difference between the winter season and the rest of the seasons in this year, as such was not observed to this extent in 2014. It could be seen that the seasonal dynamics in the carcass weight during the two years corresponded to the lean meat percentage, as the high carcass weight found in spring 2014 and the winter season of the two years corresponded to the lower lean meat percent.

The highest values of back-fat thickness X1 (Fig. 4) were measured in the pigs slaughtered in the spring of 2014 and winter of 2015.

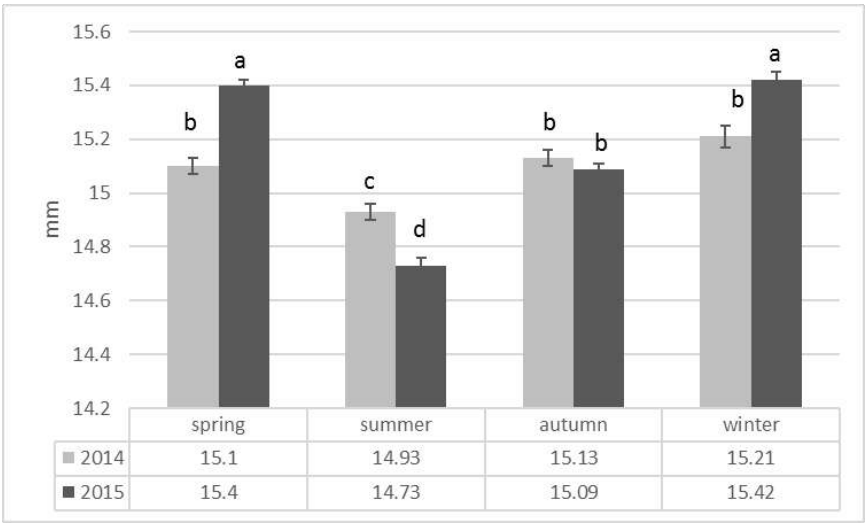


Different letters connecting seasons mean statistical difference (P<0.05)

Figure 4. Dynamics of change in the back-fat thickness (X1) during the seasons in 2014 and 2015

During 2014 the back-fat thickness decreased significantly in summer, increased in autumn and then diminished slightly in winter. Opposite changes were observed in the next year, when the pigs slaughtered in spring displayed lowest values of X1. In autumn, X1 was reduced, and then raised in winter. Except this season, as was found for the carcass weight, the values of X1 remained lower in 2015, compared to 2014, particularly in spring and autumn, when the discrepancies were statistically significant.

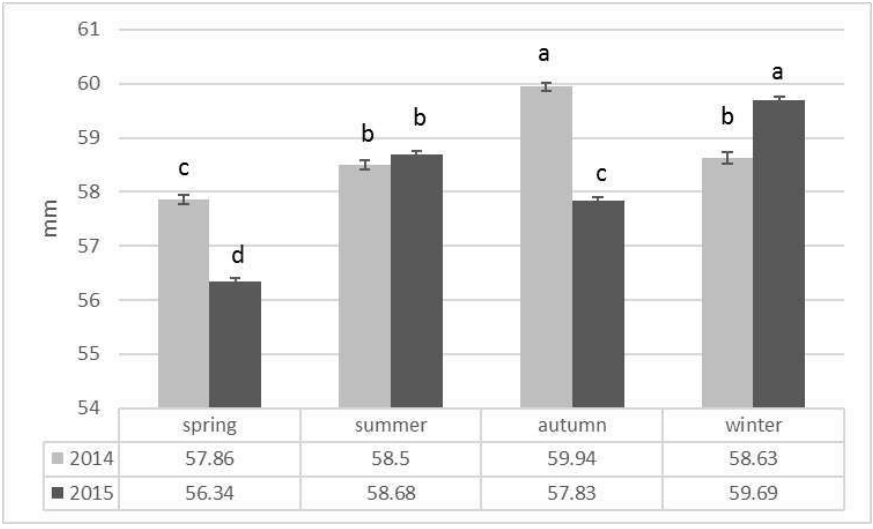
Decrease in the back-fat thickness X2 (Fig. 5) was observed in the summer of 2014, however the values of this parameter increased in autumn and winter, when they reached maximum. Similar tendency of change in X2 according to the season was recorded in 2015. In regard to the year of slaughter, significantly higher values of X2 was measured in the pigs slaughtered in spring and winter of 2015, in comparison to 2014.



Different letters connecting seasons mean statistical difference (P<0.05)

Figure 5. Dynamics of change in the back-fat thickness (X2) during the seasons in 2014 and 2015

The dynamics of change in the depth of m. LD (X3) showed constant increase in the animals slaughtered in spring, summer and autumn of 2014, when they reached maximum and consequently decreased (Fig 6).



Different letters connecting seasons mean statistical difference (P<0.05)

Figure 6. Dynamics of change in the depth of m. LD (X3) during the seasons in 2014 and 2015

In 2015, the pigs were characterized by the lowest depth of m. LD in spring and the highest in winter. In the spring and autumn, the pigs slaughtered in 2014 had higher muscle depth, while in winter the values of this parameter were significantly higher in the animals slaughtered in 2015. The analysis of data showed that the changes of X2 were opposite to those in the depth of m. LD in the same location as this was observed only in the carcasses studied in 2015. Despite the different trends of the changes in the back-fat thickness in the two years, the present study showed seasonal variations in the values of this trait in the different anatomical locations. This is agreement with results of *Salces et al*. (2006), *Škorput et al*. (2009), *Chmielowiec-Korzeniowska et al.* (2012), *Piwczyński et al.* (2013). According to *Trezona et al.* (2004), the seasonal variations in the back-fat thickness were mostly associated to the carcass weight. In our study, the carcass weight recorded in spring and winter corresponded to the back-fat thickness X1 and X2 in 2014, whereas the variation in the back-fat in the two anatomical locations in summer and autumn were opposite to the trends of changes in the carcass weight. In 2015 the changes in the carcass weight were respective to those of X1.

In addition to the carcass weight, other factors more or less related to the seasons might explain the variation in the carcass characteristics of the pigs. One of them is the ambient temperature, since it is known that the pigs are very sensitive towards this factor (*Fagundes et al.*, 2009; *Lehatoyová et al*., 2012). According to *Massabie et al*. (1997), high temperatures suppress the appetite of the pigs and hence lead to lower feed intake. This could explain the lower values of the back-fat thickness in summer, observed for X1 in 2014 and X2 in both studied years. Results of *Lefaucheur et al.* (1991) also prove that the ambient temperature might dramatically affect the characteristics of the muscle and adipose tissue and lead to protein loss which is associated with negative changes in the productive performance and carcass quality in pigs.

On the other hand, *Škorput et al*. (2009), suggested that the differences in the back-fat thickness during the seasons might be due to the production conditions as well as genotype variations. As stated by *Trezona et al.* (2004), the production conditions and particularly the stocking density and the stress might considerably contribute to the carcass quality variations. According to these authors, the above mentioned factors might lead to reduction in the back-fat thickness due to reduced feed intake, as well to inhibit protein deposition due to increased protein catabolism (*Chapple et al*., 1993).

Conclusions

The present study showed significant differences in the dynamics of changes in the carcass characteristics as affected by the season and year of slaughter. The highest lean meat percentage was recorded in the animals slaughtered in summer (56.48%),

followed by those slaughtered in spring (56.34%), autumn (56.29%) and winter (56.10%). The pigs slaughtered in winter had the highest carcass weight and back-fat thickness X_1 and X_2.

Sezonska dinamika osobina kvaliteta svinjskih trupova

Jivko Nakev, Teodora Popova, Maya Ignatova, Penka Marinova, Tania Nikolova

Rezime

Cilj našeg istraživanja je bio da se proceni dinamika osobina kvaliteta svinjskih trupova pod uticajem sezone i godine klanja. Ukupno 106.027 trupova tovnih svinja za komercijalnu proizvodnju, zaklanih u istoj klanici u toku 2014. i 2015. godine je bilo uključeno u istraživanje. Trupovi su klasifikovani upotrebom UltraFOM 200 uređaja, a osobine kontrolisane u ovom istraživanju su: debljina leđne slanine na dve lokacije i dubina *m. longissimus dorsi*. Ove mere su dodatno korišćene za određivanje mesnatosti. Rezultati istraživanja su pokazali značajne razlike u dinamici promena osobina trupa tokom godišnjih doba i godina klanja. Najveći procenat mesnatosti je pronađena u leto (56,48%), zatim proleće (56,34%), jesen (56,29%) i zimu (56,10%). S druge strane, svinje zaklane u zimskom periodu su pokazale najveće mase trupova i debljine leđne masti na obe lokacije.

References

ČANDEK-POTOKAR M., KOVAČ M., MALOVRH Š. (2004): Slovenian experience in pig carcass classification according to SEUROP during the years 1996 to 2004. Journal of Central European Agriculture, 4, 323-330.

CASTRYCK F. (2007): The Belgian pig production and health policy. EPP Congress. Ghent. Available at: httm/www.pigproducer.net/uploads/media/castytryck.pdf

CHAPPLE R.P. (1993): Effect of stocking arrangement on pig performance. In: Batterham E.S. (ed.) "Manipulating pig production IV". Australasian Pig Science Association, Victoria, 87-97.

CHMIELOWIEC-KORZENIOWSKA A., TYMCZYNA L., BABICZ M. (2012): Assessment of selected parameters of biochemistry, hematology, immunology and production of pigs fattened in different seasons. Archiv Tierzucht 55, 5, 469-479.

CHOI C. S., LEE J. G. (2001): Investigation of breed, sex and environmental factors of swine economic traits on-farm test records. Journal of Animal Science and Technology, 4, 431-444.
CORREA J. A., FAUCITANO L., LAFOREST J.P., RIVEST J., MARCOUX M., GARIÉPY C. (2006): Effects of slaughter weight on carcass composition and meat quality in pigs of two different growth rates. Meat Science,72, 1, 91-99.
DAUMAS G., DHORNE T. (1996): Historique et futur du classement objectif des carcasses de porc en France. Journées Rech Porcine en France, 28, 171-180.
ENGEL B., WALSTRA P. (1993): Accounting for subpopulations in prediction of the proportion of lean meat of pig carcasses. Animal Production, 1, 147-152.
FAGUNDES A. C. A., da SILVA R.G., GOMES J.D.F., SOUZA L.W.O., FUKUSHIMA R. S. (2009): Influence of environmental temperature, dietary energy level and sex on performance and carcass characteristics of pigs. Brazilian Journal of Veterinary Research and Animal Science, 46, 1, 32-39.
GISPERT M., DIESTRE A. (1994): Classement des carcasses de porc en Espagne; in pas vers l'harmonisation communautaire. Techniporc, 2, 29-32.
GOGIĆ M., PETROVIĆ M., RADOVIĆ Č., ŽIVKOVIĆ B., RADOJKOVIĆ D., STANIŠIĆ N., SAVIĆ R. (2014): Variation of traits of fatteners under the impact of various factors. Biotechnology in Animal Husbandry, 30, 4, 687-697.
JMP, Version 13. SAS Institute Inc., Cary, NC, 1989-2007.
KALM E. (1998): Marktgerecht Schweine produzieren heisst mit System produzieren. Landwirtsch. Bl. Weser – Ems, 19, 20-27.
KNECHT D., DUZIŃSKI K. (2016): The effect of sex, carcass mass, back fat thickness and lean meat content on pork ham and loin characteristics, Archives Animal Breeding, 59, 51-57.
LEFAUCHEUR L., LE DIVIDICH J., MOUROT J., MONIN G., ECOLAN P., KRAUSS D. (1991): Influence of environmental temperature on growth, muscle and adipose tissue metabolism, and meat quality of swine. Journal of Animal Science, 69, 2844-2854.
LEHOTAYOVÁ A., BUČKO O., PETRÁK J., MRÁZOVÁ J., DEBRECÉNI O., 2012. Effect of high ambient temperature on meat quality of pigs. Research in Pig Breeding, 6, 2, 37-40.
MARINOVA P., POPOVA T., VASILEVA V. (2008): Level of some prediction traits for determination of lean meat percentage in the carcasses of pigs reared in Bulgaria. Journal of Animal Science (BG), 3, 173-177.
MASSABIE P., GRANIER L., LE DIVIDICH J. (1997): Effects of environmental conditions on the performance of growing-finishing pigs. 5. International symposium, Bloomington, USA, In: Livestock environment V", 2, 1010-1016.
NAKEV J. (2010): Quality profile of pig carcasses. Journal of Animal Science (BG), 5, 39-42.

PIWCZYŃSKI D., WOCHNA P., KOLENDA M., CZAJKOWSKA A. (2013): The effect of slaughtering season on the carcass quality of growing finishing pigs. Polish Journal of Natural Science, 28, 4, 437–448.
PULKRABEK J., PAVLEK J., VALIŠ L., VITEK M. (2006): Pig carcass quality in relation to carcass lean meat proportion. Czech Journal of Animal Science 51, 18-23.
PULKRÁBEK J., DAVID L., VÍTEK M., VALIŠ. L. (2010): Pig carcass presentation with flare fat in Czech republic. Research in pig breeding, 4, 2, 13-16.
SALCES A. J., SEO K.S., CHO K.H., KIM S.D., LEE Y.C. (2006): Genetic parameter estimation of carcass traits of Duroc predicted using ultrasound scanning modes. Asian-Australasian Journal of Animal Science, 19, 10, 1379 – 1383.
RADOVIĆ Č., PETROVIĆ M., ŽIVKOVIĆ B., RADOJKOVIĆ D., PARUNOVIĆ N., STANIŠIĆ N., GOGIĆ M. (2012): The effect of different fixed factors on carcass quality three breed fattening pigs. Biotechnology in Animal Husbandry, 28, 4, 779-786.
REGULATION 15 of Ministry of agriculture and food from 8th of May, 2009. SG, № 37 / 19th of May, 2009.
SAVESCU R., LABA M. (2016): Multivariate regression analysis applied to the calibration of equipment used in pig meat classification in Romania. Meat Science, 116, 16–25.
ŠKORPUT D., VINCEK D., LUKOVIĆ Z. (2009): Fixed effects in models for the genetic evaluation of back-fat thickness and time on test in gilts Italian Journal of Animal Science, 8 (suppl.3), 119-121. TREZONA, M., MULLAN, B. P., D'ANTONIO M., WILSON R. H., WILLIAMS I. H. (2004): The causes of seasonal variation in back-fat thickness of pigs in Western Austria. Australian Journal of Agricultural Research, 55, 273-277.
VÍTEK M., VALIŠ L., PULKRÁBEK J., DAVID L. (2009): Carcass value and meat quality in pig final hybrids. Research in pig breeding, 3, 1, 63-66.
VÍTEK M., DAVID L., VALIŠ L., PULKRABEK J. (2012): The effect of sex, weight and lean meat content on the pig carcass realization. Research in pig breeding, 6, 2, 97-101.
ZHANG S.-H., SHEN L. –Y., LUO J., WU Z.-H., JIANG Y.-Z., TANG G.-Q., LI M.-Z., BAI L., LI X.-W., ZHU. L. (2015): Analysis of carcass and meat quality traits and nutritional values of hybrid wild boars under different crossing systems. Genetics and Molecular Research, 14, 1, 2608-2616.

Received 7 October 2016; accepted for publication 2 December 2016

Biotechnology in Animal Husbandry 32 (4), p 353-360 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.084.52'636.5
DOI: 10.2298/ BAH1604353D

EFFECT OF PROTEASE AND DURATION OF FATTENING PERIOD ON DRESSING PERCENTAGE OF BROILER CHICKENS

Vladimir Dosković[1], Snežana Bogosavljević-Bošković[1], Lidija Perić[2], Miloš Lukić[3], Zdenka Škrbić[3], Simeon Rakonjac[1], Veselin Petričević[3]

[1] University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000, Čačak, Serbia
[2] University of Novi Sad, Faculty of Agriculture, D.Obradovića 8, 21000 Novi Sad, Serbia
[3] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Serbia
Corresponding author: vladosko@kg.ac.rs
Original scientific paper

Abstract: This study evaluates the effect of different crude protein levels in broiler diets supplemented with 0.2% and 0.3% protease enzyme (Ronozyme Pro Act) on dressed carcass weight and dressing percentage during two fattening periods (49 and 63 days). The fast-growing strain Cobb 500 was used. At the end of the fattening trial i.e. at 49 and 63 days, 10 male and 10 female birds were randomly sacrificed from each experimental group to determine body weights and conventionally dressed, ready-to-roast and ready-to-grill carcass weights. The data obtained were used to calculate the dressing percentages of the differently dressed carcasses. Results indicated that carcass weights and dressing percentages were not affected by diet ($P>0.05$), but also showed that the increase in the length of the fattening period by two weeks (from 7 to 9 weeks) led to increased carcass weights, while dressing percentages decreased ($P<0.05$).

Key word: broilers, protease enzyme, length of fattening period, dressing percentage.

Introduction

The production of poultry meat in the last decades has been characterised by the increasing use of new farming practices designed to improve farming conditions and reduce environmental pollution.

Broiler chickens require high protein levels in their feeds for optimum growth and feed conversion. The main protein-containing feed ingredients for broiler diets are soybean meal and full-fat soybean groats. Problems related to the GMO contamination of these feeds demand alternatives or replacement of these feeds with some other protein sources or reduction in the proportion of these feeds

in diets through improved protein utilisation by the use of different supplements *(Meluzzi et al., 2009)*.

Recently, numerous researchers (*Hajati et al., 2009; Fidelis et al., 2010; Angel et al., 2011; Frietas et al., 2011*) have examined the effect of protease supplementation along with the reduced use of plant-based protein feeds, primarily soybean meal, in broiler diets, whereas some other authors have studied carcass and meat quality traits in broilers as affected by the length of the fattening period (*Mitrović et al., 2004; Bogosavljević-Bošković et al., 2009; 2011a,b*).

The reason underlying the implementation of new broiler farming systems to replace the existing conventional method comes from legal regulations on poultry welfare such as EU directives (VO/EWG 1538/91 and VO/EG 1804/99) which prescribe minimum standards for non-commercial and organic poultry production (*Ristić, 2003*).

The objective of this study was to compare carcass weights and dressing percentages of differently dressed Cobb 500 broilers as affected by diet (standard broiler diets and diets containing lower levels of soybean meal and supplemented with protease enzyme) and length of fattening period (49 days and 63 days).

Materials and Methods

In the experiment, 300 day-old fast-growing Cobb 500 broilers were randomly assigned to three groups, each comprising 100 birds. Feed and water were provided ad libitum, and stocking density was 10 birds/m^2.

Dietary treatments

The feeding trial was conducted over a period of 63 days through starter (the first 3 weeks), grower (22-42 days) and finisher (42-63 days) stages. The following feeding treatments were used: control – C (feed formulation adapted to hybrid producer's recommendations), experimental group E-I (crude protein levels reduced by 4% than in the control diet, 0.2% protease supplementation) and experimental group E-II (crude protein levels reduced by 6% than in the C diet, 0.6 % protease supplementation). Complete feeds in mealy form were used. Feed formulations are given in Table 1.

Table 1. Feed ingredients of experimental diets for broiler chickens[1]

Ingredient, %	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 63 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
Maize	52.49	54.92	56.26	63.15	65.28	66.34	68.62	70.60	71.59
Soybean meal	22.24	19.79	18.44	13.00	10.85	9.78	9.10	7.10	6.10
Soybean groats	18.50	18.50	18.50	17.00	17.00	17.00	15.40	15.40	15.40
Feeding yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine (78%)	0.10	0.10	0.10	0.20	0.20	0.20	0.23	0.23	0.23
DL-Methionine (99%)	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate	1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium formiate (30.5%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Captex T	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix[2]	1	1	1	1	1	1	1	1	1
Protease	0.00	0.20	0.30	0.00	0.20	0.30	0.00	0.20	0.30

[1] Treatments: C-control group, standard broiler diet, without protease; E-I- broilers fed a diet with a 4% reduction in crude protein level as compared to the control group, and 0.2% protease supplementation; E-II broilers fed a diet with a 6% reduction in crude protein level as compared to the control group, and 0.3% protease supplementation.

[2]Vitamin, mineral and additive contributions per kilogram of feed: vitamin A 14000IU; vitamin D_3 5250IU; vitamin E 83IU; vitamin B1 6.12mg; vitamin B_2 10.08mg; vitamin B_6 5.08mg; vitamin B_{12} 0.031mg; vitamin K_3 4.05mg; Ca-panthotenate 22.50mg; biotin 0.18mg; vitamin C 20.9mg; folic acid 2.04mg; niacin 85,5mg; choline chloride 600mg; Cu 28mg; Zn 100mg; Fe 48mg; Mn 100mg; Se 0.30mg, I 1mg; Co 0.30mg; antioxidant-BHT 0.12gr; coccidiostatic-Salinomycin (1 to 21day) 0.50gr; enzymes: phytase, xylanase, pectinase+β-glucanase

A protease preparation manufactured by DSM (The Netherlands) under the brand name Ronozyme ProAct (serine protease) was used for fattening at a proposed dose providing 15000 units of protease (PROT) kg^{-1} complete feed (i.e. 200 mg Ronozyme ProAct kg^{-1}). It is produced by fermentation of a sporulation-deficient Bacillus licheniformis strain which expresses a synthetic gene encoding a serine protease.

Data collection

At the end of the first experimental period i.e. at 49 days, 10 male and 10 female broilers were randomly selected from each group of birds. The same procedure was repeated at 63 days, when 10 males and 10 females were selected from among the remaining broilers. The selected chickens were individually weighed and, after slaughter, measurements of their conventionally dressed, ready-to-roast and ready-to-grill carcass weights were taken.

Statistical analysis

The data obtained were subjected to conventional statistical methods. The significance of differences for carcass quality parameters (weight at slaughter, dressed carcass weight, dressing percentage) was tested by analysis of variance i.e. in a two-factor 3x2 design (3 feeding treatments and 2 lengths of fattening period).

Carcass quality parameters were statistically evaluated using analysis of variance, F-test and Tukey's test, at a significance level of P<0.05 (*ANOVA, Microsoft STATISTICA Ver. 5.0, StatSoft Inc., 1995).*

Results and Discussion

Table 2. presents body weights at slaughter of broilers at different ages across experimental groups, and dressed carcass weights.

Table 2. Dressed carcass weights of broilers across experimental groups and lengths of fattening period

Treatment			Weight, gr			
Protease	Length of fattening period, days		at slaughter	conventionally dressed carcass	ready-to-roast carcass	ready-to-grill carcass
No	49	$\bar{x}$	3181.0[b]	2753.4[b]	2596.9[b]	2378.9[b]
		Sd	318.4	271.6	246.8	234.6
	63	$\bar{x}$	3999.5[a]	3439.1[a]	3221.5[a]	2931.3[a]
		Sd	501.4	405.2	374.4	363.4
0.2%	49	$\bar{x}$	3135.7[b]	2717.7[b]	2559.8[b]	2336.2[b]
		Sd	291.2	233.7	213.6	203.6
	63	$\bar{x}$	3986.0[a]	3415.6[a]	3201.6[a]	2929.2[a]
		Sd	498.1	407.9	383.9	363.9
0.3%	49	$\bar{x}$	3102.5[b]	2675.7[b]	2516.4[b]	2303.6[b]
		Sd	330.1	273.8	252.5	242.8
	63	$\bar{x}$	3892.0[a]	3326.0[a]	3111.7[a]	2834.8[a]
		Sd	418.1	327.5	295.2	282.5
p-value						
Source of variation						
Protease			0.573	0.412	0.353	0.388
Length of fattening period			0.001	0.001	0.001	0.001
Protease x length of fattening period			0.943	0.945	0.940	0.888

[a-b] Means within columns with different superscripts differ significantly (P<0.05)

As shown in Table 2, experimental chickens had similar body weights at the end of both fattening periods (49 or 63 days), with no significance (P>0.05) observed for the effect of experimental diets – complete feeds (with or without protease supplementation, with crude protein levels reduced). As the fattening period increased (from 49 to 63 days), live body weights of broilers expectedly increased and, hence, there was an increase in dressed carcass weights for different dressing methods (conventionally dressed, ready-to-roast and ready-to-grill (P<0.05).

Table 3. Dressing percentages of broilers across experimental groups and lengths of fattening period

Treatment			Dressing percentage, %		
Protease	Length of fattening period, days		conventionally dressed carcass	ready-to-roast carcass	ready-to-grill carcass
No	49	$\bar{x}$	86.57 ^{ab}	81.68 ^{a}	74.80 ^{a}
		Sd	0.91	1.08	0.91
	63	$\bar{x}$	86.08 ^{abc}	80.65 ^{ab}	73.32 ^{cd}
		Sd	1.31	1.71	1.44
0.2%	49	$\bar{x}$	86.73^{a}	81.71 ^{a}	74.55 ^{ab}
		Sd	1.04	1.37	1.43
	63	$\bar{x}$	85.75 ^{bc}	80.38 ^{b}	73.51 ^{bcd}
		Sd	1.03	1.41	1.41
0.3%	49	$\bar{x}$	86.28 ^{abc}	81.16 ^{ab}	74.26 ^{ab}
		Sd	0.86	1.03	0.84
	63	$\bar{x}$	85.54 ^{c}	80.06 ^{b}	72.90 ^{d}
		Sd	1.12	1.51	1.36
p-value					
Source of variation					
Protease			0.183	0.056	0.170
Length of fattening period			0.001	0.001	0.001
Protease x length of fattening period			0.598	0.930	0.723

$^{a-d}$ Means within columns with different superscripts differ significantly (P<0.05)

Similarly to dressed carcass weights, dressing percentages were not affected by diet (P>0.05). Consistently with the present results, some researchers (*Yadav and Sah, 2005)* found that dressing percentages in broilers at 48 days of age were not affected (P>0.05) by increasing protease levels and reducing crude protein concentrations. However, *Hajati et al. (2009)* observed that arabinoxylanase and β-glucanase enzyme supplementation led to an significant increase in the dressing percentage of 44-day-old broilers of the same strain (Cobb 500), with the range of values (78.10 to 80.10%) similar to those in the present study. In contrast, *Espino et al. (2000)* observed a slight increase in the dressing percentage of broilers fed diets

supplemented with protease, amylase and lipase. A slight increase in dressing percentage as induced by dietary enzyme supplementation was also reported by *Richter et al. (1991)* and *Osei and Oduro (2000)*, whereas *Hartman (1996)* obtained significantly higher values in broilers fed wheat-based diets supplemented with commercial enzymes.

The two-week prolongation of the fattening period resulted in an increase in dressed carcass weight and a concurrent decrease in dressing percentage ($P<0.05$). Dressing percentage was 85.54 - 86.73% for conventionally dressed carcass, 80.06 - 81.71% for ready-to-roast carcass, and 72.90 - 74.80% for ready-to-grill carcass. The present results on the effect of length of fattening period on dressing percentage are consistent with the findings of *Mello et al. (1996), Mitrović et al. (2004)* and *Bogosavljević-Bošković et al. (2009),* who also found that dressing percentages decreased with increasing length of fattening period. However, *Bogosavljević-Bošković et al. (2011a)* also reported a decrease in the dressing percentage of ready-to-grill carcass as the fattening period was increased from 7 to 9 weeks, but the decrease was not due to length of fattening period.

Conclusion

The results of this research indicate no differences in dressed carcass weights and dressing percentages between fast-growing Cobb 500 broilers fed complete feeds containing different crude protein levels (through reduced proportion of soybean meal in feeds) and supplemented with 0.2% and 0.3% protease (Ronozyme Pro Act), respectively ($P>0.05$). Moreover, carcass quality parameters were found to be significantly affected by the length of the fattening period, given that the prolongation of the fattening period from 49 to 63 days led to a significant increase in the weight of dressed carcass (conventionally dressed carcass, ready-to-roast carcass and ready-to-grill carcass) and a decrease in dressing percentage ($P<0.05$).

Uticaj enzima proteaze i dužine trajanja tova na randman klanja tovnih pilića

Vladimir Dosković, Snežana Bogosavljević-Bošković, Lidija Perić, Miloš Lukić, Zdenka Škrbić, Simeon Rakonjac, Veselin Petričević

Rezime

U radu su prikazani efekti različitih nivoa sirovih proteina u hrani za tovne piliće, uz dodatak enzima proteaze (Ronozyme Pro Act) u količini 0,2% i 0,3% na

masu i randmane obrađenih trupova pri različitom trajanju tova (49 i 63 dana). U ogledu je korišćen brzorastući tovni hibrid Cobb 500. Na kraju oglednih perioda, 49 i 63. dana, odabrano je slučajnim izborom po 10 muških i 10 ženskih pilića iz svake eksperimentalne grupe i izmerena je masa grla pre klanja, masa klasično obrađenog trupa, trupa spremno za pečenje i trupa spremno za roštilj. Na osnovu ovih podataka izračunat je randman različito obrađenih trupova pilića. Dobijeni rezultati ukazuju da mase i randmani trupova nisu bili pod uticajem ispitivanih obroka ($P>0,05$), ali i da su se sa produžavanjem trajanja tova za 2 nedelje (sa 7. na 9 nedelja) povećale mase trupova, uz istovremeno smanjivanje randmana obrađenih trupova ($P<0,05$).

Acknowledgments

This study is part of Project No. 31033 titled "Sustainable Conventional and Revitalized Traditional Production of Value-Added Poultry Meat and Eggs" financially supported by the Ministry of Science and Technological Development of the Republic of Serbia.

References

ANGEL C.R., SAYLOR W., VIEIRA S.L., WARD N. (2011): Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. Poultry Science, 90, 2281-2286.

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., PETROVIĆ D.M., DOSKOVIĆ V., ŠARANČIĆ D. (2009): Yield of major carcass parts of broilers as dependent on the length of fattening period and breeding system. Biotechnology in Animal Husbandry, 25 (5-6), 1039-1044.

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., MITROVIĆ S., DOSKOVIĆ V., RAKONJAC S., PETROVIĆ D.M. (2011a): Broiler meat quality: the effect of rearing systems and length of fattening period. 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production", 5-7 October 2011, Belgrade, Biotechnology in Animal Husbandry, 7 (4), 1635-1642.

BOGOSAVLJEVIC-BOSKOVIC S., PAVLOVSKI Z., PETROVIC D.M., DOSKOVIC V., RAKONJAC S. (2011b): The Effect of Rearing System and Length of Fattening Period on Selected Parameters of Broiler Meat Quality. Archiv für Geflügelkunde, 75 (3), 158-163, Ulmer, Stuttgart.

ESPINO T.M., LUIS E.S., SAPIN A.B., TAMBALO R.D., UNIDA F.B. (2000): Acid protease from Monascus sp. BIOTECH 3064 and other microbial enzymes and feed additives in broiler diets. Proceedings of the 29th Annual Convention. Philippines Society for Microbiology Inc., 121-133.

FIDELIS F.NJ., KLUENTER A.M., FISCHER M., PONTOPPIDAN K. (2010): A feed serine protease improves broiler performance and increases protein and energy digestibility. The Journal of Poultry Science, 48 (4), 239-246.
FRIETAS D.M., VIEIRA S.L., ANGEL C.R., FAVERO A., MAIORKA A. (2011): Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. Journal of Applied Poultry Research, 20, 322-334.
HAJATI H., REZAEI M., SAYYAHZADEH H. (2009): The Effects of Enzyme Supplementation on Performance, Carcass Characteristics and Some Blood Parameters of Broilers Fed on Corn-Soybean Meal-Wheat Diets. International Journal of Poultry Science, 8 (12), 1199-1205.
HARTMAN R. (1996): Wheat-based diets improved by enzymes. Journal of Applied Poultry Research, 5, 167-172.
MELO J., MALLO G., WILLAR E., MIQUEL M.C., CAPPELLETI C., FERNANDEZ P. (1996): Evaluation of two poultry commercial strains in three feeding regimes at two slaughter ages. In XX World Poultry Congress, New Delhi, India, 80.
MELUZZI A., SIRRI F., CASTELLINI C., RONCARATI A., MELOTTI P., FRANCHINI A. (2009): Infuence of genotype and feeding on chemical composition of organic chicken meat.. Italian Journal of Animal Science, 8 (Suppl. 2), 766-768.
MITROVIĆ S., OSTOJIĆ Đ., ĐERMANOVIĆ V. (2004): Uticaj trajanja tova na proizvodna svojstva brojlerskih pilića različitih genotipova. Živinarstvo, 11, 7-11.
OSEI S.A., ODURO S. (2000): Effects of dietary enzyme on broiler chickens fed diets containing wheat bran. Journal of Animal and Feed Sciences, 9, 681-686.
RICHTER G., CYRIACI-G G., SCHWARTZE J., FLACHOWSKY G., HENNING A. (EDS.). (1991): Effectiveness of enzymes in broiler diets. Vitamins und weiters zusatzstoffe bei mensch und Tier. 3. Symposium, jena, 26-27 September 1991. Friedrich-Schiller-Universitat, Jena, Germany, 384-387.
RISTIĆ M. (2003): Fleishqualität von broilerr aus der ökologischer produktion. Biotechnology in Animal Husbandry, 19 (5-6), 335-343.
STATSOFT INC. STATISTICA FOR WINDOWS (1995): Version 6.0, Computer program manual. Tulsa: StatSoft Jnc.
YADAV J.L., SAH R.A. (2005): Supplementation Of Corn-Soybean Based Broiler's Diets With Different Levels Of Acid Protease. Journal of the Institute of Agriculture and Animal Science, 26, 65-70.

Received 19 October 2016; accepted for publication 14 November 2016

Biotechnology in Animal Husbandry 32 (4), p 361-368 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.04'65
DOI: 10.2298/ BAH1604361R

CHEMICAL COMPOSITION OF MEAT OF LAYING HENS IN ALTERNATIVE REARING SYSTEMS

Simeon Rakonjac[1], Snežana Bogosavljević-Bošković[1], Zdenka Škrbić[2], Lidija Perić[3], Vladimir Dosković[1], Veselin Petričević[2], Milun D. Petrović[1]

[1]University of Kragujevac, Faculty of Agronomy Čačak, Cara Dušana 34, Čačak, Serbia
[2]Institute for Animal Husbandry, Beograd-Zemun, Autoput 16, 11 080 Zemun, Serbia.
[3]University of Novi Sad, Faculty of Agriculture, D. Obradovica 8, Novi Sad, Serbia.
Corresponding author: Simeon Rakonjac, simcepb@yahoo.com
Original scientific paper

Abstract: The aim of this paper was to estimate the effect of rearing systems (floor and organic) on the chemical composition of meat of laying hens. The tested genotypes were Isa Brown hybrid and dual purpose breed New Hampshire. Based on the results of this research can be concluded that the rearing system, generally, did not caused statistically significant differences in any of the parameters examined chemical composition of meat. On the other hand, the breast meat - white meat and thigh and drumstick - dark meat of New Hampshire genotype had significantly higher fat content compared to the Isa Brown hybrid. It must be noted that in the breast meat reported significant interaction genotype x rearing system. The other parameters of the chemical composition of meat were not significantly different between reared genotypes.

Key words: laying hens, chemical composition, white meat, dark meat.

Introduction

In recent years more and more attention is paid to the functional role of food - its characteristic to improve the state of health consumers and prevent the occurrence of diseases associated with inadequate nutrition. For this reason, consumers are increasingly deciding to buy a product that is obtained in the "natural way" and for him are willing to pay a higher price, because they believe that such products are healthier and better.

For this reason, alternative rearing systems of poultry are increasingly the focus of science and practice. In Europe, already 44% of hens are raising in some of the non-cage rearing systems, and about 20% of individuals has access the outdoor *(Committee for the Common Organisation of Agricultural Markets, 2016).* In the UK almost 50% of the birds are raising in the rearing systems with an

outdoor, while in Switzerland for many years even prohibited the rearing of poultry in cages. Situation is somewhat different in the US, where only about 5.6% of hens are rearing out of the cage.

Many researchers have studied the quality of poultry meat from different rearing systems *(Latif et al., 1996; Holcman et al., 2003, Bogosavljevic-Boskovic et al., 2006, 2011a, 2011b, Dou et al., 2009, Souza et al., 2011).* However, most of these studies related to meat quality of broilers, while about the quality of the meat of laying hens have very little information. The reason for this is because this meat represents only a by-product, which is obtained at the end of the completed cycle of egg production and represents, above all, an important raw material for the meat industry. Although the value of meat worn hens does not exceed 10% of their value at beginning egg production *(Puchala et al., 2014),* it is assumed that even between 15% and 20% of the produced poultry meat represents meat of laying hens at the end of egg production.

For this reason, the aim of this study was to investigate the chemical composition of white and dark meat of different genotypes of laying hens from alternative rearing systems at the end of the production cycle.

Material and methods

A laying hens of two genotype: commercial hybrid Isa Brown and New Hampshire breed were used in this study. These two genotypes were housed in floor and an organic rearing systems. The experiment was arranged in 2x2 factorial design with two layer genotypes and two rearing systems (30 birds per group).

Stocking density in floor rearing system was 2.5 birds/m^2. Feeding program was designed according to requirements of laying hens in conventional production system (Table 1).

Organic groups had same stocking density as floor groups in houses, but each hen have and about 5m^2 pastured outdoor. Feeding program was designed according to the regulations for organic production, without additions of synthetic amino acids, vitamins and minerals, using mostly organic produced components (Table 1). Feed and drinking water for all four experimental groups was available on an *ad libitum* basis.

After the end of the one-year production cycle, six animals per group (total 24 layers) were randomly selected. After a fasting period of 12 hours, selected animals were slaughtered. After slaughtering, samples of white (breast muscle) and dark meat (leg muscle) were collected. In these samples were conducted following examination:

- Dry matter content - standard ISO 1442/1998;
- Total ash content - standard ISO 936/1999;
- Nitrogen content - standard ISO 937/1992, and the protein

content is determined by the formula: SP (%) = N (%) x 6.25;
- Free fat content - standard ISO 1444/1998;

Analysis of the results obtained was based on the parameters of descriptive statistics and using an appropriate model analysis of variance to test the significance of differences *(Stat Soft Inc Statistica For Windows, Version 7.0. (2006): Computer program manual Tulsa).*

Table 1. Chemical composition of complete feed mixtures for feeding of laying hens

Chemical composition	Floor system	Organic system
Dry matter	88.38	89.82
Crude protein	16.79	16.82
Fat	5.15	4.31
Cellulose	4.82	4.29
Ash	12.52	12.68
BEM	49.10	51.90
Ca	3.72	3.43
Total P	0.71	0.81
Na	0.17	0.18
Lysine	0.79	0.80
Methionine + cysteine	0.68	0.48
Methabolizable energy	11.5 MJ	11.3 MJ

Results and Discussion

Tables 2 and 3 shows the results of testing the chemical composition of breast meat (white meat) and the drumsticks and thigh meat (dark meat) laying hens at the end of the production cycle. These results clearly showed that the rearing system had not significant effect ($p \geq 0.05$) of any of the parameters examined chemical composition of dark or white meat.

On the other hand, the effect of genotype was significant ($p \leq 0.05$) on the fat content of the white and dark meat. Generally speaking, New Hampshire hens had higher fat content in the muscle of the breast (0.92%) compared with Isa Brown hybrid (0.69%). However, in this case occurred and significant interaction genotype x rearing system ($p \leq 0.05$), so that the breast meat organically raised New Hampshire hens had significantly higher ($p \leq 0.05$) fat content (1.07%) of the other three experimental groups - Isa Brown organic 0.61%, Isa Brown floor 0.77%, New Hampshire floor 0.78%, which mutually were not significantly different ($p \geq 0.05$). New Hampshire birds also had significantly higher fat content in the meat of thighs and drumsticks ($p \leq 0.05$) in relation to the Isa Brown hybrid (3.61%: 2.52%). Genotype had not a statistically significant effect ($p \geq 0.05$) on the other

chemical parameters of egg quality (dry matter content, ash content, protein content).

Table 2. Chemical composition of breast meat of laying hens

		Dry matter (%)	Ash (%)	Protein (%)	Fats (%)
Rearing system					
Floor		26.85 ± 0.64	1.03 ± 0.06	25.04 ± 0.62	0.77 ± 0.21
Organic		26.61 ± 0.68	1.08 ± 0.04	24.69 ± 0.75	0.84 ± 0.28
Genotype					
Isa Brown		26.53 ± 0.61	1.04 ± 0.05	24.79 ± 0.59	0.69 ± 0.13 [b]
New Hampshire		26.93 ± 0.67	1.07 ± 0.06	24.94 ± 0.81	0.92 ± 0.28 [a]
Rearing system x Genotype					
Floor	Isa Brown	26.67 ± 0.73	1.02 ± 0.05	24.87 ± 0.68	0.77 ± 0.10 [b]
	New Hampshire	27.03 ± 0.53	1.05 ± 0.07	25.20 ± 0.56	0.78 ± 0.29 [b]
Organic	Isa Brown	26.39 ± 0.47	1.06 ± 0.04	24.71 ± 0.52	0.61 ± 0.10 [b]
	New Hampshire	26.84 ± 0.82	1.09 ± 0.05	24.68 ± 0.99	1.07 ± 0.19 [a]
ANOVA					
Rearing system		ns	ns	ns	ns
Genotype		ns	ns	ns	*
Rearing system x Genotype		ns	ns	ns	*

a-b Values within column with no common superscript are significantly different ($p \leq 0.05$). *$p \leq 0.05$

The absence of significant differences in the chemical composition of meat hens raised in different rearing systems in our study is in accordance with results that, admittedly did for broiler chickens, announced *Latif et al. (1996), Holcman et al. (2003), Dou et al. (2009), Souza et al., (2011).* Namely, these authors in their research have not determined significant differences in the chemical composition of meat of broilers reared in rearing systems with outdoor and without it.

A significant effect of genotype on the chemical composition of the meat of laying hens, in our case, on the fat content is in accordance with the conclusions expressed by the *Sirri et al. (2010),* which indicated the genotype as a major factor that affect the quality and chemical composition of poultry meat in non-industrial rearing systems. In our research, meat of New Hampshire genotype had generally a higher fat content and in the breasts and in the legs compared with Isa Brown hybrid. This is in accordance with the results published by *Puchala et al. (2014),*

who founded that this genotype has a tendency to accumulation of fat in the meat, because they found a significantly higher fat content in breast meat (1.61%), and in the meat of thighs and drumsticks (7.71%), compared to the other three genotypes: Greenleg Partridge, Rhode Island Red and Barred Rock. It must be noted that these contents of fat were significantly higher than in our experiment, what these authors and confirmed by the comments that were obtained content of fat had twice the size of their available results of other authors. On the other hand, the protein content in the meat of thighs and drumsticks New Hampshire genotype in the aforementioned study (19.56%) was lower than our results (21.66%), while the protein content of the breast meat was similar (24.89% : 24.94%). It is important to point out that in their study there were no significant differences in the content of protein in dark meat among the genotypes, while the white meat emerged statistically significant difference ($p \leq 0.05$).

Table 3. Chemical composition of drumsticks and thigh meat of laying hens

		Dry matter (%)	Ash (%)	Protein (%)	Fats (%)
Rearing system					
Floor		26.15 ±1.43	1.04 ± 0.06	22.00 ± 1.16	3.09 ± 0.85
Organic		25.74 ± 1.12	1.02 ± 0.06	21.73 ± 0.59	3.04 ± 1.01
Genotype					
Isa Brown		25.60 ± 1.47	1.02 ± 0.04	22.07 ± 1.05	2.52 ± 0.69 [b]
New Hampshire		26.28 ± 0.99	1.05 ± 0.06	21.66 ± 0.74	3.61 ± 0.78 [a]
Rearing system x Genotype					
Floor	Isa Brown	26.10 ± 1.74	1.04 ± 0.02	22.42 ± 1.32	2.64 ± 0.81 [bc]
	New Hampshire	26.19 ± 1.22	1.05 ± 0.08	21.59 ± 0.89	3.55 ± 0.66 [ab]
Organic	Isa Brown	25.10 ± 1.07	1.00 ± 0.03	21.73 ± 0.62	2.40 ± 0.61 [c]
	New Hampshire	26.37 ± 0.80	1.05 ± 0.05	21.73 ± 0.62	3.68 ± 0.95 [a]
ANOVA					
Rearing system		ns	ns	ns	ns
Genotype		ns	ns	ns	*
Rearing system x Genotype		ns	ns	ns	ns

a-b Values within column with no common superscript are significantly different ($p \leq 0.05$). * $p \leq 0.05$

Rizzi and Chiericato (2010), according to our results, founded a crucial influence genotype of laying hens on the fat content of breast and thighs. However,

in the same study, these authors reported a significant effect of genotype on the protein content of the meat, which was not confirmed in our experiment.

Conclusion

Based on the results of this research, can be concluded that the rearing system, generally speaking, did not affected statistically significant differences in any of the examined parameters chemical composition of meat. On the other hand, New Hampshire genotype had significantly higher fat content compared to the Isa Brown hybrid and in the breast meat - white meat and in thigh and drumstick - dark meat. It must be noted that in the breast meat appeared significant interaction genotype x rearing system. The other parameters of the chemical composition of meat were not significantly different between reared genotypes.

Hemijski sastav mesa kokoši nosilja iz alternativnih sistema gajenja

Simeon Rakonjac, Snežana Bogosavljević-Bošković, Zdenka Škrbić, Lidija Perić, Vladimir Dosković, Veselin Petričević, Milun D.Petrović

Rezime

Cilj ovog rada je bio da se ispita uticaj sistema gajenja na hemijski sastav mesa kokoši nosilja iz alternativnih sistema gajenja: podnog i organskog. Ispitivani genotipovi su bili linijski hibrid Isa Brown i rasa kombinovanih proizvodnih sposobnosti New Hampshire.

Na osnovu rezultata ovih istraživanja može se zaključiti da sistem gajenja, generalno posmatrano, nije uzrokovao značajne razlike ni u jednom od ispitivanih parametara hemijskog sastava mesa. Sa druge strane, i meso grudi - belo meso i meso bataka i karabataka - tamno meso, New Hampshire genotipa je imalo značajno veći sadržaj masti u odnosu na Isa Brown hibrid, s tim što se mora napomenuti da se kod mesa grudi, javila i značajna interakcija sistem gajenja x genotip. Ostali ispitivani parametri hemijskog sastava mesa nisu se značajno razlikovali između ispitivanih genotipova.

Acknowledgments

The research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR-31033.

References

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., KURĆUBIĆ V., PETROVIĆ M.D., DOSKOVIĆ V. (2006): The effect of season and rearing system on meat quality traits. Czech Journal Of Animal Science, 51, 8, 369-374.

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., MITROVIĆ S., DOSKOVIĆ V., RAKONJAC S., KURĆUBIĆ V. (2011a): Carcass composition and chemical characteristics of meat from broiler chickens reared under intensive and semi-intensive systems. Biotechnology in Animal Husbandry 27, 4, 1595-1603.

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., PAVLOVSKI Z, PETROVIĆ M.D., DOSKOVIĆ V., RAKONJAC S. (2011b): The effect of rearing system and length of fattening period on selected parameters of broiler meat quality. Archiv für Geflügelkunde, 75, 3, 158-163.

COMMITTEE FOR THE COMMON ORGANISATION OF THE AGRICULTURAL MARKETS (2016): EU Market Situation for Eggs.

DOU T.C., SHI S.R., SUN H.J., WANG K.H. (2009). Growth rate, carcass traits and meat quality of slow-growing chicken grown according to three raising systems. Animal Science Papers and Reports, 27, 4, 361-369.

HOLCMAN A., VADNJAL R., ŽLENDER B., STIBLJ V. (2003): Chemical composition of chicken meat from free range and extensive indoor rearing. Archiv für Geflügelkunde, 67, 3, 120-124.

LATIF S., DWORSCHAK E., LUGASI A., BARNA, E., GERGELY A., CZUCZU P., HOVARI J., KONTRASZTI M., NESLEZLENYI K., BODO I. (1996): Composition of characteristic components from chickens of different genotype kept in intensive and extensive farming systems. Nahrung 40, 6, 319-325.

PUCHALA M., KRAWCZYK J., CALIK J. (2014): Influence of origin of laying hens on the quality of their carcasses and meat after the first laying period. Annals of Animal Science, 14, 3, 685–696.

RIZZI C., CHIERICATO G.M. (2010): Chemical composition of meat and egg yolk of hybrid and Italian breed hens reared using an organic production system. Poultry Science, 89, 1239-1251.

SIRRI F., CASTELLINI C., RONACARTI A., FRANCHINI A., MELUZZI A. (2010): Effect of feeding and genotype on the lipid profile of organic chicken meat. European Journal of Lipid Science Technology, 112, 994-1002.

SOUZA X.R., FARIA, P.B., BRESSAN, M.C. (2011): Proximate composition and meat quality of broilers reared under different production systems. Brazilian Journal of Poultry Science 13, 1, 15-20.
STAT SOFT INC STATISTICA FOR WINDOWS, VERSION 7.0. (2006): Computer program manual Tulsa.

Received 19 October 2016; accepted for publication 14 November 2016

Biotechnology in Animal Husbandry 32 (4), p 369-381 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 619:636.32
DOI: 10.2298/ BAH1604369D

INFECTION WITH *Strongyloides papillosus* IN SHEEP: EFFECT OF PARASITIC INFECTION AND TREATMENT WITH ALBENDAZOLE ON BASIC HAEMATOLOGICAL PARAMETERS

Blagoje Dimitrijević[1], Slavoljub Jović[1], Dušica Ostojić-Andrić[2], Mila Savić[1], Žolt Bečkei[1], Vesna Davidović[3], Mirjana Joksimović-Todorović[3]

[1]University of Belgrade, Faculty of Veterinary Medicine, Bul. Oslobodjenja 18, 11000 Belgrade, Serbia
[2]Institute for Animal Husbandry, Belgrade – Zemun, Auto put 16, 11080 Zemun, Serbia
[3]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
Corresponding author: Blagoje Dimitrijević, dimitrijevic@vet.bg.ac.rs
Original scientific paper

Abstract: The aim of this study was to determine and evaluate the basic haematological parameters in conditions of natural infection of sheep with *Strongyloides papillosus*, as well as after the administration of antihelminthic albendazole (ABZ). Based on the intensity of infection with *S. papillosus* the sheep were divided into three groups: mild, moderate and high, and after that the sheep received a single dose of ABZ of 5mg/kg per body weight, per orally. Sampling of faeces and blood for parasitological and haematological assaying respectively, was performed on the 0 and the 21st day after the treatment with ABZ. The presence of parasitic infection with *S. papillosus* leads to a decrease of erythrocyte count, while the lowest values were established in the group with the highest intensity of parasitic infection ($p < 0.001$). After treatment with ABZ the decrease of erythrocyte count was more prominent, which was, based on comparison with control groups C_1 and C_2, unequivocally established to be the consequence of treatment with ABZ. Detected values of haematocrit and erythrocyte indices indicated the presence of parasitic infection: the lowest values were established in the group with the highest intensity of parasitic infection. After treatment with ABZ haematocrit levels in control group C_2 were statistically significantly lower compared to the control group C_1 ($p < 0.001$). In the presence of parasitic infection, the neutrophil and eosinophil counts increased almost linearly up to the value of 44.24±2.50% and 13.29±0.61% respectively, in the group of sheep with the highest intensity of parasitic infection ($p < 0.001$; compared to control group C_1). After treatment with ABZ the decrease of the number of these white blood cells is statistically significant ($p < 0.001$). Bearing in mind our previous research and the connection of disbalanced redox equilibrium after the treatment with ABZ with

changes, it is necessary to include antioxidative substances in the anti-parasitic treatment protocols.

Keywords: *Strongyloides papillosus*, albendazole, haematological parameters, sheep

Introduction

Parasitic form of *Strongyloides papillosus* is represented by parthenogenic females present in the sheep small intestines *(Kassai, 1999)*. The infection occurs by introduction of infectious larvae (stage L_3) per orally, through food and water (passive) and/or by percutaneous (active) invasion of L_3 larvae. There is also a possibility of galactogen infection with larvae that migrated to the udder through systemic circulation right before birth *(Šibalić and Cvetković, 1996)*. Pathogenic effect of the parasite on the host is a result of the presence of migrating larvae and/or adult forms in small intestine, which damage the host's tissues mechanically and by their secretory/excretory products. Larvae that actively penetrate the host's organism by rupturing the skin in the interdigital region enable the invasion of other etiological agents *(Abott and Lewis, 2005)*. The presence of *S. papillosus* and its developmental forms leads to the disturbance of the animal's health, not infrequently inducing a sudden death syndrome in young ruminants (lambs and calves) due to heart failure. It was also established that the degree of damage directly correlates to the intensity of parasitic infection, i.e. to the number of present parasites and/or their larval forms *(Kobayashi et al., 2009; Nakanishi et al., 1993; Ura et al., 1993; Nakamura et al., 1994)*. Disturbed gastrointestinal tract motility, which occurs during the infection with *S. papillosus*, is responsible for the occurrence of clinical symptoms (anorexia, weight loss and anaemia) and sudden death in infected animals *(Kobayashi et al., 2009)*. The same authors state that the exact mechanism that leads to the animal's death in case of infection with *S. papillosus* is still unknown.

Anaemic state in case of infection with *S. papillosus* is explained by the occurrence of erosions and ulcerations of the small intestinal mucosa and the consequential development of haemorrhagic enteritis *(Šibalić and Cvetković, 1996)*. The loss of blood and disturbances of food digestion and nutrient absorption that also occur are the reason behind the slow development of young animals, progressive weight loss in adult animals and change of haematological parameters.

On the other hand, the drugs used for treatment may also have adverse (side) effects on the treated organism. The most common drug used to treat infection with *S. papillosus* is albendazole (ABZ), benzimidazole's derivate and a broad-spectrum antihelminthic, which also effects the larvae of this parasite. The

key mechanism by which ABZ achieves its effect is a result of its interaction with eukaryotic cytoskeleton protein, tubulin, by inhibiting its polymerization into microtubules *(Rufener et al., 2009)*.

The objective of this research was to examine the effect of the intensity of infection with *S. papillosus* and treatment with ABZ on haematological parameters of sheep (erythrocyte count, haematocrit, leukocyte count, leukocyte differential count), as well as clinical significance of the resulting changes, with the purpose of possible amendments of the anti-parasitic treatment protocols used as part of the sheep health schemes. Also, we would like to emphasize that this research is a part of our previous continuous investigation *(Dimitrijević et al., 2012; Dimitrijević et al., 2015)*, of the same experimental model, but this time in course of changes of cellular components of sheep blood.

Material and methods

Experimental animals

All experiments involving animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N. L 358/1–358/6, 18, December 1986).

This study was performed in the vicinity of the city of Vranje (south-east Serbia (village Kupinince, geographic coordinates: 42°29’46.98’’ N, 21°53’50.10’’E), in a hilly-mountainous region with pastures located at altitudes between 350 and 650 m. The climate is continental, with long, cold winters and hot summers, which plays a very important role in the sterilization of pastures from infectious stages of geohelminth parasites, which also include *Strongyloides spp*. Overnight the examined sheep stayed in a shelter covered with deep litter, which represents a predisposing factor for continuous infection with *S. papillosus*. The sheep’s diet was based on daily grazing and after return to their shelter the sheep also received coarsely ground corn (cca 200 g/per day). Water and livestock salt were available *ad libitum*.

The research was carried out on Württemberg sheep (n=40), 2-3 years old, in which an infection with *S. Papillosus* was established by parasitological testing. Depending on the intensity of the infection the animals were divided into three groups (A_1 -mild; A_2 -moderate and A_3 -high intensity of infection with *S. papillosus*). After that the sheep were treated with ABZ, per orally, in single dose of 5 mg/kg and they are represented in results as groups B_1, B_2 and B_3. Control group consisted of sheep (n=10) that were negative to the presence of this parasite and after treatment with ABZ they were represented in the results as group C_2.

Sampling of faeces for parasitological examination

Samples of faeces were obtained individually from each sheep, directly from rectal ampoule, once a day for three days, packed in separate labelled plastic bags and transported in a portable refrigerator to a parasitological laboratory.
Standard keys for identification of parasites and their developmental forms based on morphological and morphometric characteristics of eggs, larvae and/or adult forms were used for detection and determination of parasites and their developmental forms. Sedimentation and flotation methods were used for coprological diagnostics *(Kassai, 1999)*. The examination of the samples was performed at magnification of 7x10 and 7x40 on Reichert microscope, Germany. The intensity of infection was established by counting the number of helminth eggs per gram of faeces using McMaster's method *(Euzeby, 1982)*.

Sampling of blood for haematological examination

The blood samples from sheep were obtained by punctuating *v. jugularis*, with restraining of animals, before dehelminthisation (day 0) and on the 21st day after dehelminthization with ABZ. The blood was sampled in plastic, sterile test tubes *(Vacuete, USA)*, using heparin as anticoagulant and it was transported in a portable refrigerator to a haematological laboratory. The blood was analysed using *Abacus Junior Vet Diatron* haemocytometer *(Mi. PLC, Hungary)*. Relative portion of individual types of white blood cells (neutrophils, eosinophils and lymphocytes) was expressed as a percentage in relationship to the total leukocyte count.

Statistical analysis

Statistical analysis of the results was performed by using computer program GraphPad Prism 5.00 (San Diego, CA, USA). Statistical significance of differences of the examined parameters was determined by means of the ANOVA test, followed by a Tukey test. The results were expressed as means ± standard error. Significance level was set at $p \leq 0.05$.

Results and Discussion

Albendazole is a drug of choice for treatment of strongyloidosis and other parasitic infections (*Kassai, 1999*). Results of the examination of the intensity of parasitic infection with *S. papillosus* before and after dehelminthisation with ABZ are shown in Table 1.

Table 1. Intensity of parasitic infection with *S. papillosus* determined based on the number of eggs/g of faeces (means ± standard error), before and after dehelminthisation with ABZ

	Intensity of infection (no. of eggs/g of faeces)					
	Before treatment with ABZ			**After treatment with ABZ**		
Sheep group	A_1 (n=10)	A_2 (n=10)	A_3 (n=10)	B_1 (n=10)	B_2 (n=10)	B_3 (n=10)
	832±34.7	1320±56.1	2918±146.5	0	0	0

Analysis of the results of coprological examination before and after dehelminthisation shows that the administered antihelminthic (ABZ) was 100% efficient (Table 1). This dehelminthisation result is interesting considering the data that can be found in scientific literature regarding the increasing number of cases of parasite resistance to ABZ (Rufener et al., 2009). This effect of ABZ in our study can be explained by the fact (obtained from anamnestic data regarding the sheep treatment) that this drug was used for the first time on treated animals, which greatly eliminated the possible presence of resistant forms of *S. papillosus* in the treated sheep population.

Table 2. Values of basic haematological parameters (means ± standard error), in sheep infected with *S. papillosus*, before (A_1 – mild; A_2 – moderate; A_3 – high intensity of parasitic infection) and after (B_1 – mild; B_2 – moderate; B_3 – high intensity of parasitic infection) treatment with ABZ; C_1 – negative control group; C_2 – negative control group treated with ABZ

Parameter	C_1 (n=10)	Before treatment with ABZ			After treatment with ABZ			C_2 (n=10)
		A_1 (n=10)	A_2 (n=10)	A_3 (n=10)	B_1 (n=10)	B_2 (n=10)	B_3 (n=10)	
Erythrocytes (x 10^{12}/L)	11.58±0.32	10.21±0.51 *	9.41±0.41 **	8.83±0.23 ***	9.27±0.35 +	8.67±0.29 ++	8.08±0.51 +	8.53±0.24 ###
Haemoglobin (g/L)	141.25±2.32	137±4.32	131±5.19	119±6.61	135±3.41	130±2.28	101±1.81	110.30±3.11
Haematocrit (%)	0.48±0.02	0.46±0.01	0.44±0.02	0.41±0.02 **	0.45±0.01	0.44±0.01	0.40±0.01	0.40±0.02 ###
MCV (fl)	45.72±0.67	43.29±0.71	42.17±0.69	39.59±0.60	43.98±0.61	43.81±0.59	44.79±0.84	44.80±0.64
MCH (pg)	11.85±0.12	10.89±0.24	10.71±0.15	8.82±0.17	9.90±0.12	9.98±0.17	8.15±0.09	10.91±0.08
MCHC (g/L)	264±9.53	269±12.61	274±15.21 *	277±18.11 **	274±15.68	276±17.14	282±18.24	273±14.76 ##
Platelets (x 10^9/L)	273±58.21	299±61.93	350±47.73 *	402±39.61 **	301±41.51	324±39.45	310±45.21 ++	261±47.21
Leukocytes (x 10^9/L)	11.05±0.21	11.52±0.30	12.28±0.29	13.37±0.19	11.31±0.35	11.94±0.28	11.99±0.31	10.65±0.29
Neutrophils (%)	36.81±2.54	38.08±2.22 *	38.51±2.89 *	44.24±2.50 ***	34.12±1.99	34.59±2.34	36.08±2.42	35.38±2.12
Eosinophils (%)	2.12±0.24	4.35±0.41 **	6.48±0.58 ***	13.29±0.61 ***	3.21±0.24 +	3.59±0.81 ++	4.09±0.31 +++	1.91±0.64
Lymphocytes (%)	61.07±8.24	57.57±11.21	55.01±8.56	42.47±9.94	62.67±8.15	61.82±8.10	59.83±10.24	62.71±7.54

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ – in relationship to control group C_1

+ $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$ – comparison between groups before and after dehelminthisation (A_1 vs B_1;A2 vs B_2; A_3 vs B_3)

$p < 0.01$; ### $p < 0.001$ – comparison of control groups C_1 vs C_2

There is a large amount of data in the literature regarding the effects of parasitic infections on certain haematological parameters *(Saleh, 2008)*. However, the data regarding the effects and possible mechanism of action of antihelminthics used for treatment of parasitic infection are scarce, therefore, the aim of this study was to determine the changes of haematological parameters and correlate them with the possible mechanism of action of antihelminthics on certain blood cells in treated animals.

In our study we established, the same as other researchers *(Nakanishi et al., 1993)*, that the number of erythrocytes decreases with the intensity of parasitic infection reaching the lowest results in the group of sheep with high intensity of infection (8.83±0.23 x 10^{12}/L), compared to the control C_1 group (11.58±0.32 x 10^{12}/L), at the statistically significant level of $p < 0.001$. After treatment with ABZ we also detected downward trend for erythrocyte count and based on the erythrocyte count in group C_2 (8.53±0.24 x 10^{12}/L), this finding was exclusively attributed to the effects of ABZ. In order to explain this finding in our study, we analysed data from literature on pharmacodynamics and pharmacokinetics of ABZ.

After peroral administration ABZ is metabolized in the sheep's organism through a two-step sulphoxidation *(Velik et al., 2004)*. Sulphoxidation is a rapid and reversible process in which the equilibrium favours formation of ABZ sulphoxide (ABZSO). ABZSO has a chiral centre and it is most likely that the formation of (+)ABZSO is influenced by flavin-containing monooxygenases (FMO). Albendazole-sulphoxide also undergoes second sulphoxidation (which occurs via cytochromes – CYP), wherein inactive metabolite albendazole-sulphone (ABZSO2) is generated *(Cristofol et al., 1998; Velik et al., 2005; Skalova et al., 2007; Capece et al., 2009)*. During the process of biotransformation of ABZ through a series of consecutive reactions on CYP and FMO (reduction, protonation, addition of oxygen, homolytic splitting of oxygen, etc.) reactive species of oxygen (ROS) and nitrogen (RNS) are generated and consequent "leakage" of these species from the biotransformation system may occur *(Guengerich, 2008)*. According to *Dubin and Goijman (1984)*, generation of ROS/RNS during redox cycling of nitroheterocyclic drugs (which also include ABZ) represents a determining factor for the intensity of peroxidative processes. Although most of the researchers in the field of pharmacokinetics of ABZ claim that practically the entire amount of this drug after peroral administration is eliminated from blood and gastrointestinal tract after 60–70 hours *(Alvarez et al., 1997; Moreno et al., 2004)*, due to the reversibility of the process ABZ↔ABZSO, the amount of ROS/RNS generated during the biotransformation of ABZ is not negligible *(Dimitrijević et al., 2012)*. Also, bearing in mind that once the peroxidative process starts it can be efficiently ended only if adequate amounts of antioxidants are present, it can be assumed that the peroxidative effect of ABZ lasts much longer than the half-life of its elimination *(Dimitrijević et al., 2012)*. In a 10-day experiment in which rats were perorally treated with ABZ *Locatelli et al.*

(2004) established that the treated animals were incapable of maintaining equilibrium between production and neutralization of ROS/RNS and avoiding adverse effect of these reactive species on the cellular homeostasis. In our previous study *(Dimitrijević et al., 2012)* we established that the level of oxidative stress in sheep was more prominent after the treatment with ABZ, compared to the level of oxidative stress determined in case of various intensities (mild, moderate, high) of parasitic infection with *S. papillosus*. On the other hand, detected decrease of erythrocyte count that correlates with the intensity of parasitic infection, as well as after the treatment with ABZ (Table 2) can be explained by the effect of ROS/RNS on erythrocytes. Erythrocytes are highly specialized cells that don't have nuclei (except in birds), which means that their antioxidative defence capacity is limited by the amount, i.e. the activity of the antioxidative enzymes that are already present in them. In other words, there is no *de novo* synthesis of antioxidative enzymes in erythrocytes, which shortens their half-life in conditions of disturbed redox equilibrium, considering that the erythrocyte's membrane, as part of the cell that is the most sensitive to the effects of the ROS/RNS, becomes fragile and brakes easily while traveling through the capillary network *(Burak, 2008)*.

Beside the fact that the increase of its value indicates the degree of dehydration, haematocrit is also a good indicator used for the detection of the presence of parasitic infection *(Amarante et al., 2004)* because its decreased value indicates possible presence of endoparasites. The results of this study are in concordance with this, given that we established the lowest haematocrit values in the group of sheep with the highest intensity of parasitic infection (0.41±0.02%), with statistical significance of $p < 0.01$, compared to control group C_1 (0.48±0.02%). Haematocrit values continue to decrease after dehelminthisation, which was in this case attributed to the effects of ABZ, given that the lowest haematocrit values were established in control group C_2 (0.40±0.02%), with statistical significance of $p < 0.01$, compared to control group C_1. Erythrocyte indices (MCV, MCH, MCHC) give information about the average cell size, the amount of haemoglobin and proportion of haemoglobin content in erythrocytes *(Radojičić, 2007)*. Slightly higher values of MCV represent a sign of cell's regenerative response, which was in our case established after dehelminthisation with ABZ, but without statistical significance ($p > 0.05$) compared to control groups C_1 and C_2 (Table 2). MCHC values are thought to be the most precise erythrocyte index and they are usually elevated in case of haemolysis, which is in concordance with our findings, given that the highest values were established in the group with the highest intensity of parasitic infection (277±18.11 g/L), with statistical significance of $p < 0.01$; compared to control group C_1 (264±9.53 g/L). After treatment with ABZ, we also detected an increase of MCHC values in all examined groups, but without statistical significance compared to the groups before dehelminthisation ($p > 0.05$). By comparing the MCHC values in control groups C_1 and C_2 ($p < 0.01$), we detected an increase of value of this parameter,

which unequivocally indicates the effect of ABZ and its side effect of leading to haemolysis by disrupting the delicate redox equilibrium *(Burak, 2008)*.

The presence of parasites in the host triggers the defence mechanisms. First, unspecific line of defence are neutrophils that synthesize reactive oxygen species, superoxide anion radical (O2•-), in their structures *(Saleh, 2008; Radfar, 2008)*. Superoxide dismutase (SOD) is an enzyme that neutralises superoxide anion radical (O2•-) and the product of this enzyme reaction is hydrogen peroxide (H_2O_2). Hydrogen peroxide, in addition to decomposition by catalase enzyme can also be homolytically decomposed in the presence of ions of transition metals such as Fe^{2+} and Cu^{+} *(Valko et al., 2006, 2007)*. In that case a hydroxyl radical, the most potent oxygen radical, is generated. Due to its extreme reactivity, it unselectively reacts with all the groups of organic compounds, which in case of reaction with DNA can result in mutagenesis and cancerogenesis *(Jomova and Valko, 2011, Kryston et al., 2011)*. The term "double nature" of ROS/RNS relates to the fact that in low concentrations these reactive species have beneficial effects, while in higher concentrations they may cause damage to all cellular structures and biomacromolecules *(Marnett, 1999; Stevanović et al., 2012)*.

The results of our analyses show that with the increase of the intensity of parasitic infection, the neutrophil counts increase as well, achieving the highest values in the group of sheep with the highest intensity of parasitic infection (44.24±2.50%) compared to control group C_1 (36.81±2.54%); $p < 0.001$. A similar trend of the count increasing with the intensity of parasitic infection was also detected for eosinophils. It is well-known that parasitic infections are followed by an increase of eosinophil count *(Radojičić, 2007)*. In our study the greatest number of eosinophils was detected in the group with the highest intensity of infection (13.29±0.61%) with statistical significance of $p < 0.001$, compared to group C_1 (2.12±0.24%). After treatment with ABZ the number of eosinophils decreased (Table 2), which is understandable, considering that the stimulus, i.e. parasitic infection, was no longer present.

Analysis of the platelet count established that the values were within the physiological reference values *(Radojičić, 2007)*. However, we detected that with the increase of the intensity of parasitic infection the platelet count increased as well (Table 2) reaching the highest values in the group with moderate ($p < 0.05$) and high ($p < 0.01$) intensity of infection compared to control group C_1. After dehelminthisation a decrease of platelet count was detected; the greatest decrease was detected in group B_3, with statistical significance of $p < 0.01$. This type of variations, even though they are within reference values, are probably a result of small haemorrhages caused by the presence and migration of *S. papillosus*, which have an incentive effect on bone marrow where the platelets are produced.

Albendazole is an antihelminthic that has been very successfully used in veterinary medicine for more than 25 years. Bearing in mind its efficiency, the aim

of this study was not to emphasize the side effects of ABZ, but on the contrary, to indicate another aspect of its mechanism of action, in addition to the one that has already been described and generally accepted *(Rufener et al., 2009)*. Our research in the past five years unequivocally showed that both the presence of parasitic infection and the treatment with ABZ induce the state of oxidative stress in the organism *(Dimitrijević et al., 2012)*. Some antihelminthics are known to achieve their effect by also interfering with the metabolic processes of the parasite thus increasing the production of ROS and RNS *(Locateli et al., 2004)*. Helminths are anaerobic or in certain stages of their development optionally aerobic organisms that live in the environments with low partial pressure of oxygen. For that reason, the majority of parasites does not possess or they have lost mechanism for neutralisation of ROS and RNS during regressive evolution, or their capacity is negligible compared to aerobic organisms that during evolution developed specialized mechanisms in order to protect themselves from the toxic effects of oxygen *(Locatelli et al., 2004; Dzik, 2005)*. On the other hand, increased exposure to ROS and RNS leads the cell to the state of oxidative stress and results in a damage of biomacromolecules (lipids, proteins and nucleic acids), which may induce programmed cell death (apoptosis) leading to development of malignant cell or uncontrolled cell death (necrosis). This mechanism is the basis for development of nearly every disease *(Lykessfeldt and Svendsen, 2007)*.

Conclusion

The results of our research indicate that the presence of infection of sheep with *S. papillosus* leads to the development of various degrees of anaemia depending on the intensity of parasitic infection, which, bearing in mind our previous research *(Dimitrijević et al., 2012; Dimitrijević et al., 2015)*, is a consequence of the development of various levels of oxidative stress depending on the intensity of parasitic infection. Dehelminthisation with ABZ further increased the degree of anaemia in all examined sheep. Bearing in mind this finding and the development of oxidative stress, phenomenon which is the basis of this condition, further research is necessary in order to define adequate treatment protocols for parasitic infections (which include administration of ABZ), first of all in terms of including substances with antioxidative properties.

Infekcija ovaca sa *Strongyloides papillosus*: Uticaj intenziteta parazitske infekcije i terapije albendazolom na vrednosti osnovnih hematoloških parametara

Blagoje Dimitrijević, Slavoljub Jović, Dušica Ostojić-Andrić, Mila Savić, Žolt Bečkei, Vesna Davidović, Mirjana Joksimović-Todorović

Rezime

Cilj ovog istraživanja bio je da se utvrde i procene osnovni hematološki parametri u uslovima prirodne infekcije ovaca sa *Strongyloides papillosus*, kao i nakon primene antihelmintika albendazola (ABZ). Na osnovu intenziteta infekcije sa *S. papillosus* ovce su podeljene u tri grupe: niski, srednji i visoki intenzitet infekcije, a zatim su ovce jednokratno dobile peroralno ABZ, u terapijskoj dozi od 5 mg/kg telesne mase. Uzorkovanje fecesa za parazitološka i za hematološka ispitivanja obavljeno je nultog i 21. dana od primene ABZ. Utvrđeno je da prisustvo parazitske infekcije sa *S. papillosus* dovodi do pada broja eritrocita, pri čemu su najniže vrednosti utvrđene u grupi sa najvećim intenzitetom parazitske infekcije ($p < 0,001$). Nakon terapije sa ABZ pad broja eritrocita je izraženiji, što je nesumnjivo nastalo kao posledica terapije ABZ (na osnovu poređenja C_1 i C_2). Utvrđene vrednosti hematokrita i eritrocitnih indeksa su ukazivali na postojanje parazitske infekcije; najniže vrednosti su utvrđene kod grupe sa najvećim intenzitetom parazitske infekcije. Nakon terapije sa ABZ vrednosti hematokrita kod C_2 bile su statistički značajno niže u odnosu na kontrolnu grupu C_1 ($p < 0,001$). U prisustvu parazitske infekcije broj neutrofila i eozinofila povećava se gotovo linearno, do vrednosti od 44,24±2,50% kod neutrofila, odnosno od 13,29±0,61% kod eozinofila u grupi ovaca sa najvećim intenzitetom parazitske infekcije ($p < 0,001$). Nakon terapije sa ABZ broj ovih ćelija bele krvne loze smanjuje se statistički značajno ($p < 0,001$). Imajući u vidu naša prethodna istraživanja i povezanost narušene redoks ravnoteže posle terapije sa ABZ sa promenama utvrđenim u ovom istraživanju, neophodno je u antiparazitske terapijske protokole uključiti antioksidativne supstance.

Acknowledgment

Research was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project N^{os} TR 31085 and OI 173034.

References

ABBOTT K.A., LEWIS C.J. (2005): Current approaches to the management of ovine footrot. Veterinary Journal, 169, 28–41.

ALVAREZ L.I., SANCHEZ S.F., LANUSSE C.E. (1997): Modified plasma and abomasal disposition of albendazole in nematode-infected sheep. Veterinary Parasitology, 69, 241–253.

AMARANTE F.T., BRICELLO A., ROCH R.A., GENNARI S.A. (2004): Resistance of santa Ines, Suffolk and Ille de France sheep to naturally acquired intestinal nematode infections. Veterinary Parasitology, 20, 91-106.

BURAK C.M.Y. (2008): Free radical metabolism in human erythrocytes. Clin Chimica Acta, 390, 1-11.

CAPECE B.P.S., AFONSO S.M.S., LAZARO R., HARUN M., GODOY C., CASTELLS G., CRISTOFOL C. (2009): Effect of age and gender in the pharmacokinetics of albendazole and albendazole sulphoxide enantiomers in goats. Research in Veterinary Science, 86, 498–502.

CRISTOFOL C., NAVARRO M., FRANQUELO C., VALLADARES J.E., ARBOIX M. (1998): Sex differences in the disposition of albendazole metabolites in sheep. Veterinary Parasitology, 78, 223–231.

DIMITRIJEVIĆ B., BOROZAN S., KATIĆ-RADIVOJEVIĆ S., STOJANOVIĆ S. (2012): Effects of infection intensity with *Strongyloides papillosus* and albendazole treatment on development of oxidative/nitrosative stress in sheep. Veterinary Parasitology, 186, 364-375.

DIMITRIJEVIĆ B., JOVIĆ S., JEZDIMIROVIĆ M., BACIĆ D., SAVIĆ M., JEZDIMIROVIĆ N., VEGARA M. (2015): Infekcija ovaca sa *Strongyloides papillosus* – Uticaj intenziteta parazitske infekcije i terapije albendazolom na određene biohemijske parametre u krvi ovaca. Veterinarski Glasnik 69, 41-61.

DUBIN M., GOIJMAN A.O.M. (1984): Effect of nitroheterocyclic drugs on lipoperoxidation and glutathione content in rat liver extracts. Biochemical Pharmacology, 33, 3419–3424.

DZIK J.M. (2005): Molecules released by helminth parasites involved in host colonization. Acta Biochimimica Polonica, 53, 33-64.

EUZEBY J. (1982): Diagnostic experimental des helminthoses animales (animaux domestiques – animaux de laboratoire – primates). Travaux Pratiques d'Helminthologie Veterinaire. Informations Techniques des Services Veterinaires, Ministere de l Agriculture.

GUENGERICH F.P. (2008): Cytochrome P450 and chemical toxicology. Chemical Research in Toxicology, 21, 70–83.

JOMOVA K., VALKO M. (2011): Advances in metal-induced oxidative stress and human disease. Toxicology, 283, 65-87.

KASSAI T. (1999): Veterinary Parasitology. Butterworth-Heinemann, Linacre House, Jordan Hill, pp 205.

KOBAYASHI I., KAJISA M., SAMIR FARID A., YAMANAKA A,. HORII Y. (2009): Paralytic ileus and subsequent death caused by enteric parasite *Strongyloides papillosus*, in Mongolian gerbils. Veterinary Parasitology, 162, 100–105.
KRYSTON T.B., GEORGIEV A.B., PISSIS P. (2011): Role of oxidative stress and DNA damage in human carcinogenesis. Mutatation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 711, 193–201.
LOCATELLI C., PEDROSA R.C., DE BEM A.F., CRECZYNSKI-PASA T.B., CORDOVA C.A., WILHELM-FILHO D. (2004): A comparative study of albendazole and mebendazole-induced, time dependent oxidative stress. Redox Reports, 9, 89–95.
LYKKESFELDT J., SVENDSEN O. (2007): Oxidants and antioxidants in disease: oxidative stress in farm animals. Veterinary Journal, 173, 502–511.
MARNETT LJ. (1999): Lipid peroxidation—DNA damage by malondialdehyde. Mutatation Research: Fundamental and Molecular Mechanisms of Mutagenesis, 424, 83–95.
MORENO L., ECHEVARRIA F., MUNOZ F., ALVAREZ L., SANCHEZ BRUNI S., LANUSSE C. (2004): Dose-dependent activity of albendazole against benzimidazole-resistant nematodes in sheep: relationship between pharmacokinetics and efficacy. Experimental Parasitology, 106, 150–157.
NAKAMURA Y., TSUJI N., TAIRA N. (1994): Wasting condition under normal cardiac rhythms in rabbits following *Strongyloides papillosus* infection. Journal of Veterinary Medical Science, 56, 1005–1007.
NAKANISHI N., NAKAMURA Y., URA S., TSUJI N., TAIRA N., TANIMURA N., KUBO M. (1993): Sudden death of calves by experimental infection with *Strongyloides papillosus*. III. Hematological, biochemical and histological examinations. Veterinary Parasitology, 47, 67–76.
RADFAR A., DIEZ A., BAUTISTA MJ. (2008): Chloroquine mediates specific proteome oxidative damage across the erythrocytic cycle of resistant *Plasmodium falciparum*. Free Radical Biology and Medicine 44, 2034–2042.
RADOJIČIĆ B. (2013): Opšta klinička dijagnostika kod domaćih papkara. Naučna KMD, Beograd, pp. 171–187.
RUFENER L., KAMINSKY R., MASER P. (2009): In vitro selection of *Haemonchus contortus* for resistance reveals a mutation at amino acid 198 of β-tubulin. Molecular Biochemistry and Parasitology 168, 120–122.
SALEH MA. (2008): Circulating oxidative stress status in desert sheep naturally infected with *Fasciola hepatica*. Veterinary Parasitology 154, 262–269.
SKALOVA L., KRIZOVA V., CVILINK V., SZOTAKOVA B., STORKANOVA L., VELIK J., LAMKA J. (2007): Mouflon (*Ovis musimon*) dicrocoeliosis: Effects of parasitosis on the activities of biotransformation enzymes and albendazole metabolism in liver. Veterinary Parasitology 146, 254–262.

STEVANOVIĆ J., BOROZAN S., JOVIĆ S., DIMITRIJEVIĆ B. (2012): Značaj slobodnih radikala u veterinarskoj medicini. Naučna KMD, Beograd, 1–181.
ŠIBALIĆ S., CVETKOVIĆ LJ. (1996): Parazitske bolesti domaćih životinja, Fakultet veterinarske medicine, Beograd, 292–295.
TSUJI N., ITABISASHI T., NAKAMURA Y., TAIRA N., KUBO M., URA S., GENNO A. (1992): Sudden cardiac death in calves with experimental heavy infection of *Strongyloides papillosus*. Journal of Veterinary Medical Science 54, 1137–1143.
URA S., TAIRA N., NAKAMURA Y., TSUJI N., HIROSE H. (1993): Sudden death of calves by experimental infection with *Strongyloides papillosus*. IV. Electrocardiographic and pneumographic observations at critical moments of the disease. Veterinary Parasitology 47, 343–347.
VALKO M., RHODES C.J., MONCOL J., IZAKOVIC M., MAZUR M. (2006): Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biologocal Interactions 160, 1–40.
VALKO M., LEIBFRITZ D., MONCOL J., CRONIN M.T.D., MAZUR M., TELSER J. (2007): Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology 39, 44–84.
VELIK J., BALIHAROVA V., FINK-GREMMELS J., BULL S., LAMKA J., SKALOVA L. (2004): Benzimidazole drugs and modulation of biotransformation enzymes. Research in Veterinary Science 76, 95–108.
VELIK J., BALIHAROVA V., SKALOVA L., SZOTAKOVA B., WSOL V., LAMKA J. (2005): Liver microsomal biotransformation of albendazole in deer, cattle, sheep and pig and some related wild breeds. Journal of Veterinary Pharmacology and Therapy 28: 374–377.

Received 28 November 2016; accepted for publication 24 December 2016

Biotechnology in Animal Husbandry 32 (4), p 383-391 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.05:637.52'64
DOI: 10.2298/ BAH1604383L

EFFECT OF SODIUM CHLORIDE REDUCTION IN DRY PORK ON SENSORY QUALITY PARAMETERS AND INSTRUMENTALLY MEASURED COLOUR

Slobodan Lilić[1], Jelena Babić[1], Branka Borović[1], Ljiljana Spalević[2], Danka Maslić-Strižak[2], Miloš Pavlović[3], Milan Milijašević[1]

[1]Institute of meat hygiene and technology, Kaćanskog 13, 11000 Belgrade, Serbia
[2]Scientific institute of veterinary medicine of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia
[3]Faculty of veterinary medicine, Belgrade University, Bulevar oslobođenja 18, 11000 Belgrade, Serbia
Corresponding author: Slobodan Lilić, slobodan.lilic@inmes.rs
Original scientific paper

Abstract: The aim of this paper was to examine sensory properties and instrumentally measured colour of dry pork produced with less amount of sodium chloride. Trial was consisted from five batches, two control and three experimental. Pork from control group was cured with 6% nitrite curing salt (C1 group) and with 3% nitrite curing salt (C2 group), respectively. Pork from 1st experimental group was produced with 2% nitrite curing salt and 1% potassium chloride; pork from 2nd experimental group was produced with 1,5% nitrite curing salt and 1,5% potassium chloride; dry meat from 3rd experimental group was produced with 2% nitrite curing salt and 1% ammonium chloride. Curing process lasted for 7 days; smoking, drying and ripening for 21 days. In final products, water activity, moisture, protein and fat content was determined. Taste was best evaluated in dry pork from C2 group and worst in dry pork from 2nd group. The most expressed saltiness was determined in dry pork from the first control group (C1) that corresponded to the largest amount of added salt. Due to most expressed bitter taste, the evaluation for overall acceptability for dry meat from the second experimental group was the lowest. The evaluation for overall acceptability of dry pork from the first control group was significantly lower in the comparison with the evaluations for dry meat from the second control group (C2) and the first experimental group ($P \leq 0.05$).

Keywords: dry pork, sodium chloride reduction, saltiness, sensory evaluation

Introduction

Due to several negative health influences of excessive dietary sodium intake, it is necessary to reduce salt/sodium content in food. Excessive sodium

intake may cause hypertension that is one of the major risk for prevalence for cardio-vascular diseases and can lead to direct risk of heart attack (*Perry and Beevers, 1992*), hypertrophy of the left heart chamber (*Schmieder and Messerli, 2000*), sodium retention in extracellular fluid (*MacGregor and de Wardener, 1997*), greater possibility of infection by *Helicobacter pylori* and risk of gastric cancer (*Tsugane et al., 2004*), increase of urinary excretion of calcium and risk of forming of kidney calculi (*Cappuccio et al., 2000*), risk of reduced bone density (*Devine et al., 1995*), exacerbations of asthmatic seizures (*Mickleborough et al., 2005*) and increase of HOMA (homeostasis model assessment) insulin resistance in patients with essential hypertension (*Kuroda et al., 1999*).

Dietary sodium intake in many cases exceeds requirements recommended by World Health Organization. Sodium chloride (salt) content can be reduced in meat products in different ways but most common is partial replacement of sodium chloride with potassium chloride (*Terell, 1983; Guàrdia et al., 2006*). Besides potassium chloride, other chloride salts, mainly salts of magnesium and calcium and ascorbates can be used as replacers (*Ruusunen and Puolanne, 2005*). The main problem in this case is the occurrence of a bitter taste of product, because only sodium chloride has a clearly salty taste.

According to some literature data, the sodium content could be reduced in dry-cured pork loin down to 50% by using a mixture of potassium-chloride, magnesium-chloride and calcium-chloride without significantly affecting either the sensory and/or safety quality of the final product (*Aliño et al., 2009; Armenteros et al., 2009*). Sodium content could also be reduced in dry-cured ham by about 40% by similar mixture of chloride salts keeping similar physicochemical properties and low microbiological development (*Blesa et al. 2008; Aliño et al., 2010*).

Partial substitution of sodium-chloride with other chloride salts, *Blesa et al. (2008)* did not found out significant changes in microorganism count at different formulations of salts.

The aim of this paper was to examine the changes in sensory quality of dry pork caused by replacing of sodium chloride with potassium-chloride and ammonium chloride as well as instrumentally measured colour.

Material and Methods

Dry pork production

Five groups of dry meat were produced. Pork (*m. longissimus dorsi*) was cured with nitrite curing salt in the different amount and with the mixtures of sodium-chloride and other chloride salts according to the Table 1. After curing for 7 days, smoking and drying lasted for 21 days in the smoking house under the environmentalconditions.

Table 1. **Added salts and additive, g/kg**

Group	Sodium chloride, g/kg	Potassium chloride, g/kg	Ammonium chloride, g/kg	Sodium nitrite, mg/kg
C1	60.00	-	-	150
C2	30.00	-	-	75
1st	20.00	10.00	-	50
2nd	15.00	15.00	-	37.5
3rd	20.00	-	10.00	50

Instrumental colour determination

Colour of dry pork was evaluated using colorimeter (Minolta Chroma Meter RC-400). The CIE system color profile of lightness (L*), redness (a*) and yellowness (b*) was measured by reflectance colorimeter using illuminant source D65, 8-mm aperture and 10° observation angle (*CIE, 1976*).. The colorimeter was calibrated throughout the experiment using a standard white ceramic tile (Y = 87.2; x = 0.3173; y = 0.3348). Color was measured on three cut surface of dry pork at room temperature of 22°C, at samples temperature of 10°C and on each surface three measurements were carried out.

Sensory evaluation

Surface and cut colour, consistency, odour, taste and overall acceptability were assessed by a sensory panel. Numeric-descriptive scales with 5 points were used, whereas 5 is the best evaluation and 1 is the worst. Saltiness, hardness and bitter taste evaluated by 5 points system, whereas the 5 is the most expressed attribute and 1 is at least expressed attribute. Sensory evaluation was carried out by 6 trained assessors under the same conditions.

Data analysis

The results are presented as mean ± SD. Statistical differences between averages were significant at the levels $P \leq 0.05$ and $P \leq 0.01$ by Student's t-test.

Results and discussion

The results of the instrumental determination of cut surface colour of products are presented in Table 2.

Table 2. Results of the instrumental determination of cut surface colour of dry pork, CIE Lab system

Group	L* - lightness	a* - redness	b*- yellowness
C1	33.10 ± 3.24^{x}	8.10 ± 0.87^{x}	6.81±0.80
C2	34.44 ± 0.10^{x}	9.47 ± 0.80^{y}	8.47±1.42
1	37.97 ± 2.18^{y}	8.44 ± 1.10^{x}	8.68±0.58
2	34.52 ± 1.93^{x}	8.64 ± 0.40^{x}	8.80±1.31
3	32.44 ± 2.41^{x}	9.25 ± 0.37^{y}	7.50±1.71

^{x,y} Numbers with different superscript letters are significantly different ($P \leq 0.01$)

In this study, lightness of samples of the first experimental group (37.97±2.18) was significantly higher ($P \leq 0.01$) compared to lightness of dry meat from the first control group (33.10±3.24), from the second control group (34.44±0.10) and products from the second and third experimental group (34.52±1.93 and 32.44±2.41, respectively). Highly significant ($P \leq 0.01$) differences was determined between redness of dry meat from the first control group (8.10±0.87) and from the second control group (9.47±0.80) as well as samples of third experimental group (9.25±0.37). No significant differences ($p > 0.05$) were determined between yellowness for all examined group.

Results of sensory evaluation of dry pork are shown in tables 3, 4 and 5.

Regarding surface colour, cut colour and odour all groups recieved similar grades. The lowest grades for consistency received dry pork from the first control group (C1), significantly different from all other groups ($P \leq 0.05$). It can be explained with the largest amount of salt used for the production of dry pork production in this group. Regarding to smaller amount of salt used in dry meat from other groups, consistency was evaluated as better and more desirable. Sensory evaluation for consistency was in relation with the hardness which was significantly higher ($P \leq 0.01$) in dry pork from the first control group (C1) compare with dry meat from other groups.

The higher grade for taste received dry pork from the second control group (C2) and it was significantly different ($P \leq 0.05$) in the relation to dry meat from the first control group (C1) and from the first and third group of dry meat. The lowest grade received product from the second experimental group and it was statistically different from dry meat from other groups ($P \leq 0.01$). The lowest grade

for taste for dry pork from this group is the result of bitter taste originated from potassium chloride that highly evaluated in the comparison with products from other groups ($P \leq 0.01$). Bitternes was expressed in the highest level in dry pork from the first and from the third group and it was significantly different from the products from the first and the second control group ($P \leq 0.01$). It was result of reducing sodium chloride content and adding of potassium chloride and ammonium chloride.

The most expressed saltiness was determined in dry pork from the first control group (C1) that corresponded to the largest amount of added salt (6%). It was statistically different from grades for dry meat from other groups ($P \leq 0.01$). According to this finding, the evaluation for overall acceptability of dry pork from the first control group was significantly lower in the comparison with the evaluations for dry meat from the second control group (C2) and the first experimental group ($P \leq 0.05$). Due to most expressed bitter taste, the evaluation for overall acceptability for dry meat from the second experimental group was statistically different from others ($P \leq 0.01$).

Obtained results are not completely in accordance with findings of some other authors which reduced sodium chloride content replacing with other chloride salts. *Aliño et al. (2009)* and *Armenteros et al. (2009)* found out that sodium content can be reduced in dry cured pork loin down to 50% by using a mixture of potassium chloride, magnesium chloride and calcium-chloride without significant affecting either the sensory quality of the final products. Also *Aliño et al. (2010)* claim that sodium content can be reduced in dry-cured ham production about 40% by replacing with mixture of chloride salts keeping similar physicochemical properties.

Table 3. Sensory evaluation of surface and cut colour and consistency of dry pork, n = 6

Group	Surface colour	Cut colour	Consistency
C1	4.50±0.41	4.25±0.38	3.83 ± 0.37^{a}
C2	4.83±0.24	4.67±0.47	4.50 ± 0.41^{b}
1	4.83±0.24	4.92±0.19	4.50 ± 0.76^{b}
2	4.92±0.19	4.50±0.29	4.50 ± 0.41^{b}
3	4.92±0.19	4.42±0.45	4.50 ± 0.41^{b}

^{a,b} Numbers with different superscript letters are significantly different ($P \leq 0.05$)

Table 4. Sensory evaluation of odour, taste and overall acceptability of dry pork, n = 6

Group	Odour	Taste	Overall acceptability
C1	4.67±0.47	$4.25\pm0.48^{a,x}$	$4.08\pm0.19^{a,x}$
C2	4.58±0.45	$4.92\pm0.19^{b,x}$	$4.58\pm0.34^{b,x}$
1	4.67±0.47	$4.25\pm0.48^{a,x}$	$4.33\pm0.55^{b,x}$
2	4.33±0.47	$3.50\pm0.58^{b,y}$	3.50 ± 0.50^{y}
3	4.67±0.47	$4.25\pm0.48^{a,x}$	$4.17\pm0.90^{a,x}$

^{a,b} Numbers with different superscript letters are significantly different ($P \leq 0.05$)
^{x,y} Numbers with different superscript letters are significantly different ($P \leq 0.01$)

Table 5. Sensory evaluation of saltiness, hardness and bitter taste of dry pork, n = 6

Group	Saltiness	Hardness	Bitter taste
C1	4.67 ± 0.47^{x}	4.67 ± 0.47^{x}	1.33 ± 0.75^{x}
C2	3.67 ± 0.75^{y}	4.17 ± 0.90^{y}	1.50 ± 0.76^{x}
1	3.67 ± 0.69^{y}	4.17 ± 0.90^{y}	$2.33\pm1.37^{a,y,z}$
2	4.00 ± 0.58^{y}	4.25 ± 0.25^{y}	$3.50\pm0.76^{y,q}$
3	4.17 ± 0.69^{y}	3.92 ± 0.45^{y}	$2.83\pm0.37^{a,y}$

^{a,b} Numbers with different superscript letters are significantly different ($P \leq 0.05$)
^{x,y,z,q} Numbers with different superscript letters are significantly different ($P \leq 0.01$)

Conclusion

Moderate reduction of sodium-chloride by replacing with potassium-chloride and ammonium-chloride had no influence on sensory perception of colour of dry pork, as the surface colour, so as cut colour.

No differences were obtained in instrumentally measured yellowness that was similar in dry pork from all groups. Higher level of lightness determined in the product in which sodium-chloride was replaced with one third of potassium chloride, but redness was lower in the dry meat in which sodium-chloride replaced with one third and one half of potassium chloride.

Dry meat produced with the largest amount of sodium-chloride had higher grade of hardness that influenced lower grade for consistency.

Perception of saltiness was lower in products with smaller amount of added sodium-chloride and in dry meat with in which sodium-chloride was replaced with potassium-chloride and ammonium-chloride. Also these products

had better overall acceptability except dry meat in which sodium-chloride was replaced with potassium chloride in the amount of one half.

Uticaj smanjenja sadržaja natrijum-hlorida u suvom svinjskom mesu na parameter senzornog kvaliteta i instrumentalno merenu boju

Slobodan Lilić, Jelena Babić, Branka Borović, Ljiljana Spalević, Danka Maslić-Strižak, Miloš Pavlović, Milan Milijašević

Rezime

Cilj ovog rada je bio da se ispitaju senzorske karakteristike i instrumentalno merena boja suvog svinjskog mesa proizvedenog sa smanjenim sadržajem natrijum-hlorida. Za potrebe eksperimenta, proizvedeno je pet grupa suvog mesa, od kojih su dve kontrolne. Meso iz prve kontrolne grupe salamureno je sa 6% nitritne soli za salamurenje (C1 grupa), a meso iz druge kontrolne grupe (C2) sa 3%. Meso iz prve ogledne grupe (1) proizvedeno je uz dodatak 2% nitritne soli za salamurenje i 1% kalijum-hlorida; meso iz druge ogledne grupe (2) uz dodatak 1,5% nitritne soli za salamurenje i 1,5% kalijum-hlorida, dok je meso iz treće ogledne grupe (3) proizvedeno sa 2% nitritne soli za salamurenje i 1% amonijum hlorida. Proces salamurenja trajao je sedam dana, a dimljenje, sušenje i zrenje 21 dan. U gotovim proizvodima određivani su aktivnost vode, sadržaj vlage, proteina i masti. Suvo svinjsko meso iz druge kontrolne grupe dobilo je najbolju ocenu za ukus, dok je najnižu dobilo meso iz druge ogledne grupe. Slanost je bila najizraženija u suvom mesu iz prve kontrolne grupe, što je u saglasnosti sa najvećom količinom upotrebljenog natrijum-hlorida. Usled najviše izraženog gorkog ukusa, ocena za ukupnu prihvatljivost suvog mesa iz druge ogledne grupe bila je najniža. Ocena za ukupnu prihvatljivost suvog mesa iz prve kontrolne grupe bila je statistički značajno niža u poređenju sa ocenama za suvo meso iz druge kontrolne grupe (C2) i prve ogledne grupe ($P \leq 0,05$).

Acknowledgements

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31083 and III 46009.

References

ALIÑO M., GRAU R., BAIGTS D., BARAT J.M. (2009): Influence of sodium replacement on pork loin salting kinetic. Journal of Food Engineering 95 (4), 551–557.

ALIÑO M., GRAU R., BAIGTS D., BARAT J.M. (2010): Influence of low-sodium mixtures of salts on the post-salting stage of dry-cured ham process. Journal of Food Engineering, 99, 198-205.

ARMENTEROS M., ARISTOY M., BARAT J., TOLDRÁ F. (2009): Biochemical changes in dry-cured loins salted with partial replacements of NaCl by KCl. Food Chemistry, 117, 627-633.

BLESA E., ALIÑO M., BARAT J. M., GRAU R., TOLDRÁ F., PAGÁN M. J. (2008): Microbiology and physico-chemical changes of dry-cured ham during the post-salting stage as affected by partial replacement of NaCl by other salts. Meat Science, 78, 135-142.

PRÄNDL O. (1988): Verarbeitung des Fleisches, Grundlagen der Haltbarmachung, Fleisch: Technologie und Hygiene der Gewinnung und Verarbeitung, Stuttgart: Ulmer, 234–372.

CAPPUCCIO F. P., KALAITZIDIS R., DUNECLIFT S., EASTWOOD J. B. (2000): Unravelling the links between calcium excretion, salt intake, hypertension, kidney stones and bone metabolism. Journal of Nephrology, 13, 169–177.

CIE: Colorimetry: Official Recommendations of the International Commission on Illumination, 1976. Paris: Comisión Internationale de l'Èclairage [International Commission on Illumination] (CIE No. 15 (E-1.3.1)

DEVINE A., CRIDDLE R. A., DICK I. M., KERR D. A., PRINCE R. L. (1995): A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. American Journal of Clinical Nutrition, 62, 740–745.

GUÀRDIA M. D., GUERRERO L., GELABERT J., GOU P., ARNAU J. (2006): Consumer attitude towards sodium reduction in meat products and acceptability of fermented sausages with reduced sodium content. Meat Science 73, 484−490.

KURODA S., UZU T., FUJII T., NISHIMURA M., NAKAMURA S., INENAGA T, KIMURA G. (1999): Role of insulin resistance in the genesis of sodium sensitivity in essential hypertension. Journal of Human Hypertension, 13, 257−262.

MACGREGOR G. A., DE WARDENER H. E. (1997): Idiopathic edema. In: Schrier, R.W., Gottschalk CW, eds. Diseases of the Kidney. Boston, MA: Little Brown and Company, 2343–2352.

MICKLEBOROUGH T. D., LINDLEY M. R., RAY S. (2005): Dietary salt, airway inflammation, and diffusion capacity in exercise-induced asthma. Medicine and Science Sports Exercise, 37, 904–914.

PERRY I. J., BEEVERS D. G. (1992): Salt intake and stroke: a possible direct effect. Journal of Human Hypertension, 6, 23–5.

RUUSUNEN M., PUOLANNE E. (2005): Reducing sodium intake from meat products. Meat Science, Volume 70, 3, 531−541.
SCHMIEDER R. E., MESSERLI F. H. (2000): Hypertension and the heart. Journal of Human Hypertension, 14, 597–604.
TERELL R. N. (1983): Reducing the sodium content of processed meats. Food Technology, 37, 7, 66−71.
TSUGANE S., SASAZUKI S., KOBAYASHI M., SASAKI S. (2004): Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. British Journal of Cancer, 90, 128–134.

Received 31 October 2016; accepted for publication 4 December 2016

Biotechnology in Animal Husbandry 32 (4), p 393-402 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.354
DOI: 10.2298/ BAH1604393P

PRODUCTION, COMPOSITION AND CHARACTERISTICS OF ORGANIC HARD CHEESE

Anka Popović-Vranješ[1], Snežana Paskaš[1], Anka Kasalica[2], Marija Jevtić[3], Milka Popović[3], Branislava Belić[4]

[1]Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Serbia
[2]JPS Dairy Institute, Belgrade, Serbia
[3]Faculty of medicine, University of Novi Sad, Serbia
[4]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia
Corresponding author: AnkaPopović-Vranješ, anka.popovic@gmail.com
Original scientific paper

Abstract: Organic cheeses are value added products that provide small dairy farmers with a viable source of income and has the potential to revitalize farms, provide new jobs, and develop new cheese varieties with unique flavours for consumers to experience. Production of hard organic cheese must comply with organic standards and regulations of organic production. Whole organic milk that does not contain residues of pesticides, hormones and antibiotics represents a quality raw material for hard organic cheese with added value. Together with the existing, producers develop and create new technologies and new branded products which are more original and recognizable. The goal of any technology is obtaining technologically reproducible protocol and constant uniform quality of the cheese with desired properties. In this paper some variables which influence quality of organic hard cheese were investigated. Tested samples of hard organic cheese from different production time showed consistent quality and obtained parameters followed the standards of full-fat hard cheeses.

Key words: the organic production, hard-type cheese, technology, quality

Introduction

Organic farming is an agricultural system which is identified as production of a high value where standards and methods of organic production should enable producers to get certified to produce high quality and safety product.

Organic cheeses should be recognized on the market and represent the best part of organic production. The demand for organically produced cheese is increasing and if we want this to continue than organic cheeses should meet the quality that justifies an additional price in relation to the conventionally produced

cheese (*Nielsen et al., 2001).* Consumers showed a willingness to pay organic cheese despite its higher price. The information about organic farming could be a major determinant of cheese liking and consumer willingness to pay, thus providing a potential tool for product differentiation, particularly for small scale and traditional farms (*Napolitano and Braghieri, 2010).*

Cheese is the most complex of the dairy products, involving chemical, biochemical and microbiological processes. Hard cheeses represent a large group of cheese that has a distinctive taste and smell (a pleasant, sharp, spicy, never mild), color of dough is golden-yellow, firm texture and plastic, with (2-6 mm) or without holes (*Popović-Vranješ, 2015*). Both artisanal and mass production cheese making methods use the same basic steps. The manufacturing different cheeses does not require widely various procedures but rather the same steps with variations during each step, special applications, or different ripening practices. Organic cheese is produced following the same manufacture methods as with conventionally cheese, the only differences are in some substances, which are not allowed in organic cheeses. Codex Alimentarius Commission in the regulations for organic products allows microorganisms and enzymes derived from microorganisms that are normally used in food production, except genetically obtained/modified microorganisms or enzymes originating from genetic engineering. Also, some additives used in conventional production as colorants, flavor enhancers or preservatives are not allowed (*CODEX, 1999).*

The manufacturing process plays a crucial role, especially with artisan cheeses, like raw, PDO and regional type traditional cheeses, because cheese making methods affect the composition of the original milk differently (*Lucey and Fox, 1993).* For raw milk cheese, milk production is the first critical control point (CCP) in the cheese maker's Hazard Analysis and Critical Control Point (HACCP) plan (*Marler, 2009).* In the research done by *Coppa et al., (2011)* the cheese making technology seemed to be critical, but also the microbiological and chemical composition of the milk. The quality of cheese is influenced by many aspects of milk quality: milk composition, microbiology, somatic cell count (SCC), enzymatic activity, and chemical residues (*Law and Tamime, 2010*). Pasteurization destroys most useful microorganisms, inactivates the enzymes and destroys some substances that are specific for organic milk. Cheeses obtained from raw milk have more complex aroma and taste.

Organic cheese minimizes exposure to the toxins and pesticides often associated with conventional farming practices. For many people this is an important consideration for buying organic milk and cheese. There are different feeding strategies and feeds like pasture, conserved forages (hay, silages, etc.) and concentrates which are used in animal diets influence on the quality of milk and cheese (*Tsiplakou et al., 2010).* Organic cows are fed large amount of silages, especially in winter. Between organic samples is also possible to distinguish cheeses obtained from milk produced in different months. Cheeses obtained in

spring and summer seasons (from April to August) are clearly set apart from those produced during winter months and this latter are similar to cheeses produced in conventional dairy (*Miotello, 2010*).

There are many ways to evaluate the quality of the cheese. Cheese quality may be defined as the degree of acceptability of the product to the consumer (*Peri, 2006)*. Quality criteria involve different characteristics, including: sensory (taste, aroma, texture, appearance), physical (sliceability, crumbliness, hardness, mouth-feel), cooking (extent of flow, stringiness), chemical (intact casein, free fatty acids, free amino acids), compositional/nutritional (protein, fat, calcium, lactose, sodium content), and safety (absence of pathogens, toxic residues, foreign material) (*Law and Tamime, 2010*).

The aim of the study was to determine the effect of organic standards and methods on the organic hard cheese production. In addition, this paper describe technology, analysed the chemical composition and some parameters of organic cheese ripening.

Material and methods

Eight samples of cheeses were taken from organic producer and analysed during the three months of production. Analysis of cheese samples was conducted at the Laboratory for quality control of feed and animal products, Department of Animal Science, Faculty of Agriculture, Novi Sad, Serbia.

Chemical and compositional analyses (titratable acidity, total solids, moisture, fat, protein, salt) of cheese samples were determined after 3 month of ripening. Total protein was determined by measuring total nitrogen in the cheeses using the Kjeldahl method. Dry matter was measured by drying the sample to a constant weight. Fat content was determined according to Gerber, and titratable acidity according to Soxlet-Henkel method. Salt analyses were run using ion selective electrode.

Basic steps in cheese making include milk acidification and coagulation, whey draining, heating and salting the cheese curd and ripening (Table 1). Even slight changes in these processes can lead to significant differences in the final cheese composition and properties. Control of these steps is crucial in the cheese making transformation and the changing raw material (milk) into different cheese types. The cheese making techniques are the factor that the most of the others can affect the sensory characteristics of dairy products.

Results are statistically processed using Microsoft Excel and showed as arithmetic mean, standard deviation and coefficient of variation.

Table 1. Production of organic hard cheese

Technological operations	Technological indicators
Organic milk	Fat: 3.80-3.84% Protein:2.93-3.19% Casein: 2.44-2.80 SFA: 2.16-2.61 USFA: 1.062-1.119 MUFA: 0.575-0.820% PUFA: 0.114-0.639%
Milk pasteurization	72ºC/15 sec
Milk cooling	32ºC
Adding the culture	thermophilic (TCC-20), mesophilic (CHN-22) cultures (80:20)
Adding $CaCl_2$	0.02%
Adding the rennet	after 20 min, 16g/1000 L of milk
Coagulation of milk	30-40 min
Curd cutting	10 min
Curd stirring	10 min
Hot water adding (42ºC)	30%
Stirring	35-40 min
Heating	42ºC, 15-20 min
Curds forming	10-15 min
Take out the cheese curd	Removing the cheese curd and placing into moulds.
Pressing	Lower in the beginning and then gradually increases (2-4-6 bar)
Salting: % of salt pH of brine temperature (ºC) time	 19-20 5.20 11-13 2 days
Draying of cheese	1 day/ 12ºC
Ripening and packaging	12-14ºC, 83-87% R, Ripening is from 3 month to 2 year. In the first 30 days it turned every day, and in the later period once a week.

Popović-Vranješ (2015)

Results and discussion

Quality assurance refers to the overall process of ensuring that the product complies with quality, manufacturing, ingredient and ethical standards required by the customer and by legislation. The assessment of quality depends on measurable criteria which provide information about the product in terms of its microstructure, composition, rheology, sensory properties and/or consumer acceptability (*Law and*

Tamime, 2010). The composition of cheese has an influence on all aspects of quality including sensory properties and texture.
The descriptive statistics variables of attributes of organic cheese samples are shown in Table 2 and Table 3, respectively. The results show that the analysed samples are very uniform in their composition and correspond to the *Serbian Regulations (2014)*. Coefficients of variations (Table 2. and Table 3.) for all observed parameters showed very small deviations indicating that the cheeses have constant quality.

Table 2. Composition of organic hard cheeses

Variables	Range (xmin-xmax)	Arithmetic mean	SD	CV (%)
Dry matter (%)	57.94-64.31	60.27	2.46	4.08
Fat (%)	28.43-32.50	30.51	1.54	5.03
Proteins (%)	21.53-25.70	23.59	1.25	5.31
Salt (%)	1.80-2.08	1.92	0.18	6.09
Moisture (%)	35.69-42.06	39.73	2.46	6.19

SD –standard deviation, CV- coefficient of variation, minimal (min) and maximal (max) values of variables

Cheese contains a high content of biologically valuable protein. The protein content of cheese depends on the variety. It varies inversely with the fat content of cheese and in our investigation the moisture content of cheeses increased with decreasing fat content, and the reduction in fat was compensated for by an increase in protein and salt. Protein content of cheese samples were from 21.5 to 25.7%. Most of the cheese varieties are rich in fat and also varies widely, mainly because of the type of milk. Fat affects cheese firmness, adhesiveness, mouth-feel, flavour. Regarding the fat in dry matter content (50.6%) (Table 3.), our chesses are classified as full-fat cheeses (*Serbian Regulations*, *2014*). Fat in dry matter contents ranged from 48.7 to 51.9, and these range is smaller in comparison with the recommended range for Cheddar cheese which is about 50% to 57% (*Lawrence et al*., 2004). Salt content in the cheese is very important because the relationship between low salt content and pasty, weak bodied cheese has been noted by a number of researchers (*Fox, 1987*).

Owing to the interaction of different compositional parameters (pH, total calcium and ratio of soluble-to-colloidal calcium, moisture, fat and protein), it is difficult to study the exact effects of altering any one compositional parameter, or targeted changes in a group of selected parameters on quality (*Law and Tamime,*

2010).The levels of moisture and salt, the pH and the cheese microflora regulate and control the biochemical changes that occur during ripening and hence determine the flavor, aroma and texture of the finished product. Thus, the nature and quality of the finished cheese are determined largely by the manufacturing steps. Texture of cheeses is related to a complex interaction between chemical composition and ripening parameters. The differences in water content and holes may have caused these differences, with particular regard to hardness (*Innocente et al*., 2002).

According to *Serbian Regulations (2014)* extra hard cheeses contain <51% water in non-fat substance and hard cheeses 49-56%. Values for analysed samples were from 52.49 to 60.10% (Table 3.). Based on these parameters we can conclude that our cheeses according to firmness were between semi-hard and hard cheeses. In accordance with *Lawrence et al. (1984)* optimal value of water on fat-free basis in hard cheese is 52-54%.

Table 3. Relationships between composition and the quality of cheese

Parameters	Range (xmin-xmax)	Arithmetic mean	SD	CV (%)
Fat in dry matter (%) (FDA)	48.68-51.90	50.61	1.27	2.52
Moisture in non-fat –substance (%) (MNFS)	52.49-60.10	57.13	2.55	4.46
Titriable acidity ºSH (TA)	29.28-33.91	31.51	1.55	4.93
S/M (%) (salt/moisture)	4.28-5.74	4.86	0.59	12.06

SD –standard deviation, CV- coefficient of variation, minimal (min) and maximal (max) values of variables

Mistry et al. (1993) estimated similar values of FDM and MNFS for Cheddar (51.8 and 53.7, respectively) and higher values for Swiss cheese (55.0 and 56.3%, respectively).

During the ripening or curing stage, varieties of cheeses acquire their own unique textures, aromas, appearances, and tastes through complex physical and chemical changes. These changes are significantly influenced by storage conditions and very important is controlled as much as possible temperature, humidity, and duration of ripening. Consequently, with the exception of Cheddar cheese, there have been very few published studies attempting to relate composition to quality of different cheese varieties. Five major studies have considered the effects of composition (including level of salt or S/M) and quality/grading scores of mature Cheddars cheese (*Law and Tamime, 2010*). The share salt/moisture moved in the range of 4.28 up to 5.74% which is important because the inhibition of the

utilization of lactose is carried out at ratio greater than 5.8%. Higher S/M, which is caused by higher salt content, inhibits starter culture activity, and results in lower lactic acid production, and a higher pH values (*Pastorino et al., 2003*). The recommended S/M range for Cheddar cheese is about 4.0% to 6.0% (*Lawrence et al., 2004).*

Physical properties of cheese are also influenced by acidity and the pH of the cheese which dictates the state of the calcium-phosphate-casein structure. *Kafili et* al., *(2009)* reported that titratable acidity of all samples did not change significantly in the first month of the ripening, but increased until the end of the ripening due to lactic acid production by microbial flora. Coefficient of variation for titratable showed low-value of variation (4.90%) (Table 3.). The higher titratable acidity results in smaller pH values and minimum pH of cheeses is usually reached within the first few days of maturation. It is regulated by the amount of lactose fermented to lactic acid and the buffering capacity of the curd during manufacturing of the cheese. Buffering capacity is determined by concentrations of undissolved calcium phosphate, caseins and lactate remaining in the cheese (*Lucey and Fox, 1993).*

Conclusion

The aim of this study was to examine the organic production on the composition and characteristics of organic hard cheeses. Cheese making technology has advanced considerably leading to cheese with more consistent composition and quality and production method varied to develop new types of cheese. Good quality of organic milk as a raw material is the first requirement of obtaining quality products. Milk from conventional production from animals that are sick, abused, suffering from mastitis or treated with antibiotics will not have a balanced composition. Many cheeses contain the same or similar components, but in different concentrations and ratios. In our study the moisture content of cheeses increased with decreasing fat content, and the reduction in fat was compensated for by an increase in protein and salt. Regarding the fat content in dry matter (50.6%) our chesses are classified as full-fat cheeses. Indicators of descriptive statistics varied in small limits and were quite balanced. According to water in fat-free matter (52.5 to 60.1%) our cheeses were in the range between hard and semi-hard indicating a lack of ripening control. The share salt/moisture moved in the range of 4.28 up to 5.74%. A smaller titratable acidity contributed to an increase in protein hydration and a higher in cheese moisture and this parameter was in optimal range (29.3-33.9%).

Proizvodnja, sastav i karakteristike organskog tvrdog sira

Anka Popović-Vranješ, Snežana Paskaš, Anka Kasalica, Marija Jevtić, Milka Popović, Branislava Belić

Rezime

Organski sirevi predstavljaju proizvode sa dodatom vrednošću koji obezbeđuju male proizvođače mleka sa održivim izvorom prihoda i poseduju potencijal za revitalizaciju farmi, obezbeđenje novih radnih mesta a takodje i nastanak novih varijeteta sireva sa jedinstvenom aromom i novim iskustvom potrošača. U ovom radu je opisana tehnologija proizvodnje tvrdog organskog sira. Proizvodnja organskih sireva mora biti u saglasnosti sa standardima i propisima organske proizvodnje. Punomasno organsko mleko koje ne sadrži rezidue pesticida, hormona i antibiotika predstavlja kvalitetan sirovi materijal za spremanje organskih sireva sa dodatom vrednošću. Istovremeno sa postojećim tehnologijama proizvođači razvijaju i kreiraju nove brendirane proizvode koji su više originalni i prepoznatljivi na tržištu. Cilj svake tehnologije je ponovljiv tehnološki protokol i dobijanje ujednačenog kvaliteta sira sa željenim osobinama.

Istraživanje je obuhvatilo sastav i varijable koje utiču na kvalitet organskih tvrdih sireva (kiselost, sadržaj soli, vode, masti u suvoj materiji i vode u bezmasnoj materiji sira). Ispitivani uzorci sira koji potiču iz različitog vremena proizvodnje su pokazali konstantan kvalitet i dobijeni parametri potvrđuju da analizirani sirevi po svojoj kvalifikaciji odgovaraju punomasnim tvrdim sirevima.

Acknowledgements

This research was funded by the Ministry of Education, Science and Technological development, Republic of Serbia, Project 31095 (2011-2016).

References

CODEX (1999): Guidelines for the production, processing, labelling and marketing of organically produced foods, 32, 1–35. Retrieved from: http://www.codexalimentarius.net/input/download/standards/360/cxg_032e.pdf

COPPA M., VERDIER-METZ I., FERLAY A., PRADEL P., DIDENNE R., FARRUGGIA A., MARTIN B. (2011): Effect of different grazing systems on upland pastures compared with hay diet on cheese sensory properties evaluated at different ripening times. International Dairy Journal, 21(10): 815–822.

FOX P.F. 1987. Significance of salt in cheese ripening. Dairy Industries International, 52 (9): 19-21.

INOCENNTE N., PITTIA P., STEFANUTO O., CORRADIN I. C. (2002): Correlation among instrumental texture, chemical composition and presence of characteristic holes in a semi-hard Italian cheese. Milchwissenschaft 57, 204–208.

KAFILI T., RAZAVI S. H., EMAM DJOMEH Z., NAGHAVI M. R.,

ALVAREZ-MARTIN P., MAYO B. (2009): Microbial Characterization of Iranian Traditional Lighvan Cheese Over Manufacturing and Ripening *via* Culturing and PCR-DGGE Analysis: Identificationand Typing of Dominant Lactobacilli. *Euro.* Food Reseserch Technology, 229(1): 83-92.

LAW B., TAMIME A. Y., (2010): Technology of cheese making. Second Edition.Wiley-Blackwell. A JohnWiley & Sons, Ltd. Publication. www.wiley.com/wiley-blackwell, pp. 260

LAWRENCE, R.C., CREAMER, L.K., GILLES, J. (1984): Texture development during cheese ripening, Journal of Dairy Science, 70(8): 1748-1760.

LAWRENCE R.C., GILLES J.,CREAMER L.K.,CROW V.L., HEAP H.A., HONORE C.G., JOHNSTON K. A., SAMAL P.K. (2004): Cheddar cheese and related dry-salted cheese varieties, In In Cheese: Chemistry, Physics and Microbiology, Vol. 2: Major Cheese Groups, 3rd Edition, (eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogam, and T. P. Guinee.), Elsevier Academic Press, Boston, MA, pp. 71-102.

LUCEY J., A., FOX P. F. (1993): Importance of Calcium and Phosphate in Cheese Manufacture: A Review. Journal of Dairy Science, 76(6): 1714-1724.

MARLER B. (2009): Comparing the food safety record of pasteurized and raw milk products–Part 3 (pp. 1–33). Retrieved from: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Comparing+the+Food+Safety+Record+of+Pasteurized+and+Raw+Milk+Products#0

MIOTELLO S.(2010): Organic animal production systems and quality of products from ruminants. PhD thesis. Universita degli Studi di Padova. Italy.

MISTRY V.V., ANDERSON D.L., (1993): Composition and microstructure of commercial full-fat and low fat cheeses. Food Structure, 12, 259-266.

NAPOLITANO F., BRAGHIERI A. (2010): Cheese liking and consumer willingness to pay as affected by information about organic production. Journal of Dairy Research, 77, 280–286.
NIELSEN J., LARSEN L., KNOCHEL S. (2001): Production of raw milk cheese from organic milk. Publishing Co. Orgprints.org, (November). Retrieved from http://orgprints.org/id/file/47342
PASTORINO A. J., HANSEN C.L., D. J. McMAHON D.J. (2003): Effect of pH on the chemical composition and structure-function relationships of Cheddar cheese. Journal of Dairy Science, 86: 2751 – 2760.
PERI C. (2006): The universe of food quality. Food Quality and Preference. 17, 3–8.
POPOVIĆ-VRANJEŠ A., (2015): Specijalno sirarstvo. Univerzitet u NovomSadu. Poljoprivredni fakultet, Departman za stočarstvo, Novi Sad. pp.403
SERBIAN REGULATIONS (2014): Pravilnik o kvalitetu i druigim zahtevima za mleko, mlečne proizvode, kompozitne mlečne proizvode i starter kulture, Sl. Glasnik RS, 34/2014.
TSIPLAKOU E., KOTROTSIOS V., HADJIGEORGIOU I., ZERVAS G. (2010): Differences in sheep and goats milk fatty acid profile between conventional and organic farming systems. The Journal of Dairy Research, 77(3): 343–349.

Received 6 December 2016; accepted for publication 20 December 2016

Biotechnology in Animal Husbandry 32 (4), p 403-412 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.04'639
DOI: 10.2298/ BAH1604403K

THE COMPOSITION OF GOAT MILK IN DIFFERENT TYPES OF FARMINGS

Denis Kučević*, Ivan Pihler, Miroslav Plavšić, Tamara Vuković

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg D. Obradovića 8, Novi Sad, 21000, Serbia
*Corresponding author: E-mail: denis.kucevic@stocarstvo.edu.rs
Original scientific paper

Abstract: Possible differences between compositions of raw goat's milk due to the types of farmings system (organic, conventional and traditional) were investigated. Farms were located in different parts of Republic of Serbia. All animals were loose housed. The capacity of the farm ranged from 50 to 750 French Alpine goats. A total of 72 bulk samples of raw milk were collected from each farm during the year. The principle of analysis of raw milk samples was in accordance to mid-infrared spectrometry and flow cytometry. The following parameters in raw Milk were analyzed: fat, protein, lactose, total solids, somatic cell count, milk urea, and contents of fatty acid: saturated, unsaturated, polyunsaturated and monounsaturated fatty acids. The fixed effect the types of farming and season (winter, spring, summer and fall) have shown a statistical significance difference ($P<0.05$) on all examined milk parameters except to the content of lactose, MU and the number of SCC ($P> 0.05$). The Composition of milk also affected by a number of other factors such as the nutrition of dairy goats, breed and farm management. It is recommended to consider all these factors during the comparison of different farming systems. The results that showed significant differences could be used to improve the breeding technology of dairy goats, and feeding strategies as well as emphasize the importance of using pastures.

Key words: milk composition, organic, conventional, traditional, dairy farming, Alpine goats

Introduction

Variability in goat milk production is caused by living conditions and, in particular, by nutrition. Because of its biological and energy value, goat milk has an advantage in relation to cow milk *(Božanić et al., 2002; Spruzs and Selegovska, 2004; Brito et al., 2011*). Consistent with previous study *(Park and Haenlein, 2010),* the average goat milk composition is the following: milk fat 3.8 %, proteins 3.5 % and lactose 4.1 %. The fat content can vary from 2.4 % to 7.8 %, which is

the component that varies the most *(Park and Haenlei, 2010; Antunac and Samaržija, 2000; Krajinović, 2006).* In goat milk, the content of total solids often varies and primarily depends on the goat breed *(Superchi et al., 2005).* Goat milk quality depends on many factors, such as: goat breed, age, health condition, body weight, lactation length, number of lactation, lactation stadium, length of the dry period, number of daily milking, workers who perform milking, hygiene, kidding, gestation, system of production, diet, accommodation, weather conditions etc. *(Park and Haenlein, 2010; Antunac and Samaržija, 2000; Mioč et al. 2008; Goetsch et al. 2011; Abbas et al. 2014; Krajinović and Pihler, 2014).* Contents of essential fatty acids and fatty acids (FA) are often considered as goat milk quality. In order to understand the impact of goat milk on human diet, it has to be determined what is made of *(Volkmann, 2012).* Different feeding strategies have different impact on the chemical composition of goat milk *(Toledo et al., 2002).* Different nutrients, such as pasture, hay, silage and concentrates, which are used in animal diet, along with various feeding strategies have great impact on the content of FA in milk. Organic raw milk compared with milk from conventional production has a better FA composition; i.e. contains more polyunsaturated fatty acids - PUFA with a higher proportion of omega-3 fatty acids and conjugated linoleic acid (CLA) *(Ellis et al. 2006; Prandini et al. 2009; Kučević et al. 2016).* The mentioned group of fatty acids (especially omega-3) has positive effects on human health. Their activity is associated with the improvement of neurological function *(Contreras and Raport, 2002),* decreases of diabetes, prevention of cardiovascular diseases, and improvement of the immune system *(Pariza, 2003).*

Materials and Methods

Animals and System of farming

The 12-mo research was carried out on three goat farms in the northern part of Serbia. All animals were housed in a free stall stable. The capacity of the farm ranged from 50 to 750 French Alpine goats. The first farm with the 60 goats was operated in accordance the conventional principles of farming (CF). The farm still does not produce their own feed; owners buy them instead. The diet corresponded to the daily milk yield of goats and is based on alfalfa and grass hay whereas the share of concentrate is 30-35 % (about 1 kg per day per goat, and it contains corn, barley, wheat and sunflower meal). The second farm with 50 goats was operated under the traditional farming system (TF). This is the only farm which had enabled fulltime grazing for goats. While grazing, goats are spent time on corn, soy and turnip stubbles. They were also consuming sudan grass, clover and forest species such as acacia, ash tree, vine, willow and wild strawberries. Beside this, goats were fed with alfalfa, clover and grass hay. The share of concentrate in the goat diet was

about 20 % and it was made out of soy, corn, barley and meal. A concentrate with higher percentage of selenium was added to the diet, too. The third farm is the farm which follows organic standards-certified organic farm (OF). On this farm hay and concentrate was produced for 750 French Alpine goats on 600 ha. Goats did not use a pasture. There was an outdoor space for goats, where they could express their natural behavior. Goats were fed with alfalfa, clover and grass hay. The share of concentrate was about 20% of overall diet. Concentrate was produced on farm land and it contained corn, barley, oats and triticale. For all three farms, the average water consumption was about 8 L/day/goat. Also, all three farms were using mineral and salt blocks as diet supplementation.

Sampling and Instrumental analysis

A total of 72 bulk samples of raw milk were collected during 12 months research (twice a month, 24 samples per farm). The milk analysis was performed in the Laboratory for Quality Control of Milk, the Faculty of Agriculture in Novi Sad, using a CombiFoss™FT+ analyzer for routine compositional raw milk analysis employing Fourier Transform Infrared "FTIR". This device is a combination instrument consisting of the MilkoScan™ FT+ and the Fossomatic™ FC, techniques comply with: ISO 9622 / IDF 141:2013 and the AOAC official method 972.16. Fossomatic was used for analysis of somatic cell counting in raw milk. The collection of samples is carried out in accordance with the regulations of the International Committee for Animal Recording (ICAR- AT_4). The following parameters were analyzed: fat, protein, lactose, total solids, somatic cell count (SCC), milk urea (MU), and contents of fatty acid (FA): Saturated (SFA), unsaturated (UFA), polyunsaturated (PUFA) and monounsaturated Fatty Acids (MUFA).

Statistical analysis

The data was evaluated by the *STATISTICA software (Ver. 13 StatSoft Company, 2016).* The average values and variability of examined parameters as well as the effect of factors (types of farmings and seasons as a fixed effect) on investigated milk traits were studied by means of the procedures PROC UNIVARIATE and PROC GLM-General linear model. Quantitatively dependent variables were (fat, protein, lactose, total solids, SCC, MU, SFA, UFA, PUFA, MUFA) by a fixed factors - types of farmings and seasons as independent variables. The model equation used for the evaluation was as follows:

$Yijk = \mu + Si + Rj + eijk$
where:

Yijk – dependent variable (fat, protein, lactose, total solids, SCC, MU, SFA, UFA, PUFA, MUFA);
μ - mean value of dependent variable;
Si - fixed effect of the System i (i = 1, 2, 3);
Rj - fixed effect of the Season j (j = 1, 2, 3, 4);
eijk - other random effects.

Logarithmic transformation was used in order to properly adjust the number of SCC to normal distribution, as follows:

SCC = Log2 (SCC /100000) +3

Results and Discussion

The fixed effect the types of framings (OF, CF and TF) and seasons (winter, spring, summer and fall) have shown a statistical significance difference ($P<0.05$) on fat, protein, total solids, SFA, UFA, PUFA and MUFA. Significant difference wasn't found in the number of SCC, content of lactose and MU ($P> 0.05$). The average results of raw milk composition are shown in Table 1.

Table 1. Results of raw milk composition in different types of farming

Milk components	Conventional dairy farming				Traditional dairy farming			Organic dairy farming		
	n	mean	SD	CV %	mean	SD	CV %	mean	SD	CV %
Fat (g/100 g)	72	3.95^{a}	0.73	18.4	4.16^{b}	0.93	22.3	3.08^{c}	0.42	13.6
Proteins (g/100 g)	72	2.79^{a}	0.26	9.3	3.05^{b}	0.18	5.9	3.76^{c}	0.40	10.6
Lactose (g/100 g)	72	4.09^{aaa}	0.05	1.2	4.07^{aaa}	0.17	4.1	4.16^{aaa}	0.14	3.3
T.solids (g/100 g)	72	11.76^{a}	0.85	7.2	12.26^{b}	0.95	7.7	11.97^{c}	0.88	7.3
Urea (mg/dL)	72	52.4^{aaa}	10.3	19.6	48.1^{aaa}	9.1	18.9	52.8^{aaa}	11.8	22.3
LogSCC	72	6.33^{aaa}	1.18	18.6	6.69^{aaa}	1.54	23.1	5.68^{aaa}	2.20	38.7

T. Solids - Total Solids; LogSCC - Logarithmically Somatic cell count; SD -Standard deviation; CV% - Coefficient of variation; ^{a,b,c}Values in the same row indicate significant differences at the level ($P<0.05$); ^{a,a,a}Values in the same row indicate insignificant differences ($P>0.05$);

The content of milk fat was the highest in the traditional farm (4.16 %) compared to the organic (3.08 %) and the conventional farm (3.95 %). The content of proteins was the highest in the milk from the organic farm (3.76 %). The lactose

content was the highest in the milk which comes from the organic farm (4.16 %), followed by the milk from the conventional (4.09 %) and traditional (4.07 %) farm. The highest content of total solids were found in the milk from the traditional farm (12.26%), followed by the milk from the organic (11.97 %) and conventional farm (11.76 %). The content of MU was the highest in OF (52.8 mg/dL) and the lowest in TF (48.1 mg/dL). The highest average number of SCC was found in the milk from the traditional farm (2.273, 735/mL; log 6.69). In the organic milk, average SCC was 2.026, 150/mL (log 5.68) and in the conventional one it was 1.384, 800/mL (log 6.33).

Comparing three farms in terms of milk fat content showed that there was a statistically significant difference between the organic and the conventional, and the organic and traditional farm ($P<0.05$). These results are in accordance with other research in which systems based on pastures result with a high content of fat *(Morand et al., 2007)*, because the diet rich in fibers has a major influence on milk fat content. It can be assumed that the greatest influence on this difference was made due to the share of concentrate and fresh grass in goat diet *(Goetsch et al., 2011; Abbas et al. 2014).* Fluctuation of milk fat content could be affected by a number of other factors such as breed, stage of lactation, season (agro-climatic conditions), loss of appetite etc. *(Park and Haenlein, 2010; Krajinović and Pihler, 2014; Maroteau et al., 2014; Rahmann, 2007).*Values of the coefficient of variation (CV) and standard deviation (SD) indicate that the variability of fat is under the influence of biological and breed characteristics of dairy animals too *(Kučević et al., 2011).*

Comparing these three farms in terms of protein content showed that there was statistically significant difference between the organic and conventional, and between the organic and traditional farm ($P<0.05$). The reason for higher protein content from the organic farm is the significantly higher share of high quality alfalfa and clover hay in diet. In addition, genetic variations have an impact on the content of protein too *(Brito et al., 2011).*

The analysis of variance showed no statistical significance between the three farms regarding somatic cell count, but it should be noted that the values from the traditional and organic farm were higher than the average. Differences in number of SCC in milk (especially high values) between farms depend on environmental factors, specifically slightly worse milking and breeding conditions *(Přidalová et al., 2009).* Right away after leaving the udder, the milk of healthy cows, kept in adequate breeding conditions, is almost sterile and contains the minimum number of microorganisms (8.933 CFU / ml) (*Kučević et al., 2013*). On the organic farm, the reason for high SCC in goat most likely depends on the frequent occurrence of mastitis. Inflammation of the mammary gland was accompanied by the changes in the number of SCC, mainly as an increase in SCC in diseased quarters of udder. To contamination came mainly during and after

milking (after leaving the udder) due to the activity of microorganisms from the environment.

The content of milk urea revealed the proteins/energy ratio in the diet i.e. it is balanced or not. It is used as an indicator of a rather higher amount of proteins in diet or lower amount of energy (carbon hydrates). The content of MU is linked to the protein intake through the diet. This fact can be perceived in research in the same way in relation to the highest content of MU (52.8 mg/dL) and proteins (3.76 %) on the organic farm. Higher values of MU indicate an the imbalance of protein and energy, but MU concentration was also influenced by a whole range of factors: feeding, breed, stage and number of lactations, body weight, daily production and chemical composition of milk, somatic cell count, season and milking *(Čobanović et al., 2015).*

The average content of SFA, UFA, PUFA, MUFA fatty acids in the raw milk (all types of farming) is presented in Table 2.

Table 2. Results of fatty acid in raw milk in different types of farming

Fatty acid (g/dL)	Conventional dairy farming				Traditional dairy farming			Organic dairy farming		
	n	mean	SD	CV %	mean	SD	CV %	mean	SD	CV %
SFA	72	2.01^{A}	0.35	17.4	2.00^{B}	0.30	14.9	2.24^{C}	0.38	16.9
UFA	72	0.78^{A}	0.10	12.8	1.01^{B}	0.21^{b}	20.7	0.59^{C}	0.10	16.9
MUFA	72	1.01^{A}	0.12	11.8	1.17^{B}	0.20	17.0	0.84^{C}	0.15	17.8
PUFA	72	0.38^{A}	0.02	5.2	0.33^{B}	0.02	6.0	0.31^{C}	0.03	9.6

SD - Standard deviation; CV% - Coefficient of variation; ^{A,B,C}Values in the same row indicate significant differences at the level (P<0.01);

According to the results in Table 2, it is evident that there was a highly statistically significant difference between the types of farming in all tested parameters (P<0.01). The milk produced in organic dairy farming had a significantly higher concentration of SFA (2.24 g/dL) and the lowest concentration of UFA (0.59 g/dL). The share of UFA (1.01 g/dL) and MUFA (1.17 g/dL) fatty acid was higher in the milk from the traditional farm and the content of PUFA was the highest in the milk from the conventional farm (0.38 g/dL).

The content of UFA in the organic (0.593 g/dL) compared to the conventional milk (0.780 g/dL) is contradictive to other results (24) where it been stated that milk from the certified organic farms contains higher concentrations of UFA than the milk from conventional farms with high inputs. The highest content of UFA and MUFA has been identified in the milk from the traditional farm which

is the only farm with fully organized grazing. This corresponds to the results gained from other research because a pasture has a great impact on the content of FA in milk by reducing content of SFA and increasing content of UFA *(Morand et al., 2007; Ferlay et al., 2007; Decandia et al., 2007; Tudisco et al., 2010; Kučević et al., 2016; Sampelayo et al., 2007).* On the other hand, this corresponds to the content of SFA, which is the lowest in the milk from the traditional (2.015 g/dL) and conventional (2.012 g/dL) farm, compared to the milk from the organic farm (2.248 g/dL). Fresh grass had a strong impact on FA content in milk by increasing the percentage of PUFA *(Morand et al., 2007; Aplocina and Spruzs, 2012)* which justifies the content of polyunsaturated PUFA in milk from the traditional (0.330 g/dL) and conventional (0.382 g/dL) farm, but not the content in the organic milk (0.311 g/dL).

Conclusion

The fixed effect the types of farming and season (winter, spring, summer and fall) have shown a statistical significance difference on all examined milk parameters except to the content of lactose, MU and the number of SCC. The Composition of milk also affected by a number of other factors such as the nutrition of dairy goats, breed and farm management. The contradictory results in the investigated farming systems are probably related to different feeding strategies and feed components (including pasture). Therefore, most of the authors, who had conducted similar studies, pointed out that the composition of raw milk is mostly influenced by nutrition. Regarding the diet of goat, special consideration should be given to the access to fresh grazing, silage type, cereal feeding etc., because nutritionals factor takes a greatly impact on the composition of goat's milk.

Sastav kozijeg mleka u različitim sistemima proizvodnje

Denis Kučević, Ivan Pihler, Miroslav Plavšić, Tamara Vuković

Rezime

Cilj rada je bio da se ispitaju razlike u sastavu kozijeg sirovog mleka dobijenog tokom godine u različitim sistemima proizvodnje (organska/ konvencionalna/tradicionalna). Farme za držanje mlečnih koza locirane su na severu Republike Srbije a životinje su uzgajane slobodnim sistemima držanja. Kapacitet farmi se kretao od 50 do 750 mlečnih koza rase francuska alpska. Ukupno je sakupljeno 72 zbirna uzorka sirovog mleka (2 puta mesečno po farmi). Uzorci su ispitani po metodi infracrvene spektrofotometrije i protočne citometrije a

od parametara u sirovom mleku su analizirani: mlečna mast, protein, laktoza, ukupna suva materija, ukupan broj somatskih ćelija, urea u mleku, sadržaj masnih kiselina (zasićene, nezasićene, polinezasićene i mononezasićene). Uticaj sistemskih faktora sistema proizvodnje i sezone je bio statistički značajan ($P<0,05$) na sve ispitivane parametre osim na sadržaj laktoze, uree i broja somatskih ćelija ($P> 0,05$). Na sastav kozijeg mleka utiče veliki broj drugih faktora kao što je način ishrane, rasa, farmski menadžment itd. Zato je za preporuku da se prilikom poređenja sastava mleka dobijenih iz različitih tipova proizvodnje, u razmatranjem obuhvate i pomenuti faktori. Rezultati istraživanja mogu poslužiti za unapređenje tehnologije odgajivanja mlečnih koza i unapređenja strategije ishrane, sa posebnim naglašavanjem korišćenja pašnjaka u ishrani koza.

References

ABBAS H.M., HASSAN F.A., EL-GAWAD M.A.A., ENABA K. (2014): Physicochemical Characteristics of Goat's Milk. Life Science Journal, 11: 1s.

ANTUNAC N., SAMARŽIJA D. (2000): Proizvodnja, sastav i osobine kozjeg mlijeka. Mljekarstvo, 50: (1) 53-66.

APLOCINA E., SPRUZS J. (2012): Influence of different feedstuffs on quality of goat milk. In Zinātniski praktiskās konference," Zinātne Latvijas Lauksaimniecības Nākotnei: Pārtika, Lopbarība, Šķiedra un Enerģija", Jelgava, Latvijas Lauksaimniecības Universitāte (LLU). Latvia, 23-24 February, p. 209-214.

BOŽANIĆ R., TRATNIK L., DRGALIĆ I. (2002): Kozje mlijeko: karakteristike i mogućnosti. Mljekarstvo, 52: (3) 207-237.

BRITO L.F., SILVA F.G., MELO A.L.P., CAETANO G.C., TORRES R.A., RODRIGUES M.T., MENEZES G.R.O. (2011): Genetic and environmental factors that influence production and quality of milk of Alpine and Saanen goats. Genetics and Molecular Research, 10: 3794-3802.

CONTRERAS M.A., RAPOPORT S.I. (2002): Recent studies on interactions between n-3 and n-6 polyunsaturated fatty acids in brain and other tissues. Current opinion in lipidology. 13: 267-272.

ČOBANOVIĆ KSENIJA, KUČEVIĆ D., TRIVUNOVIĆ S., PLAVŠIĆ, M. Variability of milk urea on Vojvodina's dairy farms. Sixth International Scientific Agricultural Symposium „Agrosym 2015" Jahorina, October 15 - 18. Book of proceedings 2015. p.1665-1671.

DECANDIA M., CABIDDU A., MOLLE G., BRANCA A., EPIFANI G., PINTUS S., ADDIS M. (2007): Effect of different feeding systems on fatty acid composition and volatile compound content in goat milk. Options Mediterraneennes, Series A, Advanced Nutrition and Feeding Strategies to Improve Sheep and Goat Production, 74: 129-134.

ELLIS K.A., INNOCENT G., GROVE-WHITE D., CRIPPS P., MCLEAN W.G., HOWARD C.V. (2006): Comparing the fatty acid composition of organic and conventional milk. Journal of Dairy Science, 89: 1938-50.
FERLAY A., BERNARD L., ROUEL J., DOREAU M. (2007): Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. European Journal Lipid Science Technology, 109: 828-855.
GOETSCH A.L., ZENG S.S., GIPSON T.A. (2011): Factors affecting goat milk production and quality. Small Ruminant Research, 101: (1) 55-63.
KRAJINOVIĆ, M. (2006): Ovčarstvo i kozarstvo. Univerzitet u Novom Sadu, Poljoprivredni fakulet Novi Sad.
KRAJINOVIĆ M., PIHLER I. (2014): Tehnologija kozarske proizvodnje. Univezitet u Novom Sadu, Poljoprivredni fakultet, 163-211.
KUČEVIĆ, D., TRIVUNOVIĆ S., RADINOVIĆ M., PLAVŠIĆ M., SKALICKI Z., PERIŠIĆ P. (2011): The effect of the farm size on milk traits of cows. Biotechnology in Animal Husbandry, 27: (3) 951-958.
KUČEVIĆ D., PLAVŠIĆ M., TRIVUNOVIĆ S., RADINOVIĆ M., KUČEVIĆ D. S. (2013): Effect of post - milking teat dipping on hygienic quality of cow's milk, Biotechnology in Animal Husbandry, Vol. 29, 4, 665-673.
KUČEVIĆ D., TRIVUNOVIĆ S., BOGDANOVIĆ V., ČOBANOVIĆ K., JANKOVIĆ D., STANOJEVIĆ D. (2016): Composition of Raw Milk from conventional and organic dairy farming. Biotechnology in Animal Husbandry 32: (2), 133-143.
MAROTEAU C., PALHIÈRE I., LARROQUE H., CLÉMENT V., FERRAND M., TOSSER-KLOPP G., RUPP R. (2014): Genetic parameter estimation for major milk fatty acids in Alpine and Saanen primiparous goats. Journal of Dairy Science, 97: (5) 3142-3155.
MIOČ B., PRPIĆ Z., VNUČEC I., BARAĆ Z., SAMARŽIJA D., PAVIĆ V. (2008): Factors affecting goat milk yield and composition. Mljekarstvo, 58: (4) 305.
MORAND-FEHR P., FEDELE V., DECANDIA M., LE FRILEUX Y. (2007): Influence of farming and feeding systems on composition and quality of goat and sheep milk. Small Ruminant Research, 68: (1) 20-34.
PARIZA M.W. (2003):The biological activities of conjugated linoleic acid, in Advances in Conjugated Linoleic Acid Research (2nd edn), ed. by Christie WW, S´ eb´ edio JL and Adlof RO. AOCS Press, Champaign, IL. 12-20.
PARK Y.W., HAENLEIN G.F.W. (2010): Milk production. Goat Science and Production, 12: (4) 275.
PRANDINI A., SIGOLO S., PIVA G. (2009): Conjugated linoleic acid (CLA) and fatty acid composition of milk, curd and Grana Padano cheese in conventional and organic farming systems. Journal of Dairy Research, 76: 278-82.

PŘIDALOVÁ H., JANŠTOVÁ B., CUPÁKOVÁ Š., DRAČKOVÁ M., NAVRÁTILOVÁ P., VORLOVÁ L. (2009): Somatic cell count in goat milk. Folia Veterinaria, 53: (2) 101-105.
RAHMANN G. (2007): Organic Sheep and Goat Farming. Tagungsreader der Pillnitzer Sommerakademie, 30-45.
SAMPELAYO M.S., CHILLIARD Y., SCHMIDELY P., BOZA J. (2007): Influence of type of diet on the fat constituents of goat and sheep milk. Small Ruminant Research, 68: (1) 42-63.
SPRUZS J., SELEGOVSKA E. (2004): Feeding of goats under conditions of organic farming. Veterinarija in zootechnika, 27: (49) 101-105.
SUPERCHI P., SUMMER A., SABBIONI A., MALACARNE M., FRANCESCHI P., MARIANI P. (2005): Feeding management and production factors affecting goat milk composition and quality. Titratable acidity and rennet-coagulation. Milchwissenschaft, 45: 361-362.
TOLEDO P., ANDERS A., BJÖRCK L. (2002): Composition of raw milk from sustainable production systems. International Dairy Journal; 12: 75-80.
TUDISCO R., CUTRIGNELLI M. I., CALABRÒ S., PICCOLO G., BOVERA F., GUGLIELMELLI A., INFASCELLI F. (2010): Influence of organic systems on milk fatty acid profile and CLA in goats. Small Ruminant Research, 88: (2) 151-155.
VOLKMANN A. (2012): Effects of two different forage to concentrate ratios on the milk performance of dairy goats and on the quality of goat milk and cheese. Master thesis. University of Natural Resources and Life Sciences, Vienna, Austria.

Received 26 September 2016; accepted for publication 20 November 2016

Biotechnology in Animal Husbandry 32 (4), p 413-423 , 2016 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 631.563'633.31
DOI: 10.2298/ BAH1604413D

THE EFFECT OF BACTERIAL INOCULANT ON CHEMICAL COMPOSITION AND FERMENTATION OF ALFALFA SILAGE

Snežana Đorđević[1], Violeta Mandić[2*], Dragana Stanojević[3]

[1]Faculty of Agriculture, University of Belgrade, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry Belgrade-Zemun, Belgrade, Republic of Serbia
[3]Biounik d.o.o., Research and Development Centre, Šimanovci, Republic of Serbia
[*]Corresponding author: violeta_randjelovic@yahoo.com
Original scientific paper

Abstract: Alfalfa silage is a useful source of protein for feeding ruminants. Therefore, managing alfalfa silage in livestock production systems is an important issue in order to maintain the silage quality and achieve maximum profitable production of milk and meat. The aim of this investigation was to estimate the effects of bacterial inoculant Silko, containing *Lactobacillus plantarum* (strains: LP1, LP2, LP3 and LP4) on chemical composition, energetic characteristics and fermentation alfalfa silage under field conditions in the commercial dairy farm, during the 2016. The first-cut alfalfa in the second year has been conserved in silage form. The silage mass was subdivided into two equal parts (control (silage without inoculant) and silages treated with bacterial inoculant Silko) and ensiled in trench silo. After 60 days of ensiling, the silages were analysed. Dry matter, ash, crude protein, lactic acid, acetic acid, total digestible nutrients value and relative feed value were significantly higher in silage treated with bacterial inoculant Silko compared to control. Contrary, alfalfa silage treated with a bacterial inoculant Silko had lower values of cellulose, acid detergent fibre, neutral detergent fibre, non-nitro extractive matter, pH, butyric acid, soluble nitrogen/total nitrogen and NH_3-N/total nitrogen than untreated silage. Results showed that bacterial inoculant Silko increases silage quality compared to control so that research should be directed toward the use of such prepared silage in ruminant diets and its impact on milk and meat production on farms.

Key words: alfalfa, chemical composition, energetic characteristics, fermentation parameters, inoculant, silage

Introduction

In Serbia, alfalfa is grown on an area of 109230 ha with a total annual production of 481003 tons and an average yield of 4.4 t ha^{-1} *(Statistical Yearbook*

of the Republic of Serbia, 2016). Alfalfa is important for the nutrition of all species of domestic animals, and it is used in various forms, such as hay, silage, dehydrated plants, less frequently as green food and for livestock grazing. In modern farming, alfalfa silage is a useful source of protein for feeding to cattle and sheep and a good supplement for maize silage. In Serbia, silage is an important feed for livestock in winter and early spring when reduces pasture production. However, the high protein content and low content of soluble carbohydrates in the fresh material (< 1.5%), low dry matter and high buffering capacity make it difficult to ensile. For these reasons, the application of chemical or bacterial additives is the important factor for ensiling alfalfa *(Repetto et al., 2011)*. The advantage of bacterial inoculants is that they leave no residues and does not adversely affect animal health and product quality and safety. For this reason, everywhere in the world largely suppressed chemical preservatives, regardless of their effectiveness. *McDonald et al. (1991)* stated that the bacterial inoculants safe, easy-to-use and noncorrosive to farm machinery, and do not pollute environment. During ensiling, LAB ferment water-soluble carbohydrates to organic acids, mainly lactic acid which reduce the pH and inhibit the growth of pathogenic and spoilage bacteria, yeast and moulds which influence on heating and spoilage silage and dry matter losses *(Zhang et al., 2009; Čabarkapa et al., 2010a)*. *Pahlow et al. (2003)* stated that LAB which found are silage is members of the genera *Lactobacillus*, *Pediococcus*, *Lactococcus*, *Enterococcus*, *Streptococcus* and *Leuconostoc*. *Lactobacillus* is a genus of Gram-positive organism which produces lactic acid and acidic environment (pH 5.5-6.5) *(Giraffa et al., 2010)*. *Kizilsimsek et al. (2007)*, *Zhang et al. (2009)* and *Zielińska et al. (2015)* reported that inoculation with LAB of the genus *Lactobacillus* can improve the fermentation of alfalfa silage, quality and aerobic stability. Also, many researches showed beneficial effects of silage inoculant on chemical composition and fermentation alfalfa silage *(Bolsen et al., 1996; Čabarkapa et al. 2010b; Silva et al., 2016; Tian et al., 2016)*. *Đorđević et al. (2011)* reported that addition of homofermentative bacterial inoculants to alfalfa silages reduced the content of NH_3-N and increased the lactic acid and pH compared to untreated silage.

The objective of this research was to determine the effects of bacterial inoculant Silko on chemical composition, energetic characteristics and fermentation alfalfa silage under field conditions in the commercial dairy farm.

Materials and Methods

The first-cut alfalfa cultivar Banat in the second year was harvested at initial flowering stage (May 2016), and after 24h wilting, the silage mass was chopped on about 20 mm chop length using chopper harvester. The silage mass was subdivided into two equal parts (control (silage without inoculant) and silages treated with bacterial inoculant Silko) and ensiled in trench silo. The liquid

inoculant was sprayed using a plant sprayer over the course of filling the silos. The inoculant was applied at recommended rate of 5 ml t^{-1} fresh material. The bacterial inoculant Silko contains homofermentative *Lactobacillus plantarum* (strains: LP1, LP2, LP3 and LP4). The number of colony forming units in inoculant is $1x10^{10}$ CFU/ml. After 60 days of ensiling, the silages were analyzed. Three composite samples were collected from each treatment. Composite sample included twelve samples which are collected with different locations in trench silo including from top to bottom and left to right, and were mixed in a clean plastic bucket to form a composite sample weighing about 1.5 kg. The samples were packed into plastic bags to avoid exposure to air and delivered to the laboratory.

The dry matter was determined as the difference in mass before and after the drying to constant mass in an oven at 105°C. The ash was determined heating the dry samples in an oven at 550°C for 2h. Crude fat (CF) content was determined according to Soxhlet method, crude protein (CP) according to Kjeldahl *(AOAC 1990)*, cellulose according to Weende method, neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to Van Soest method, soluble nitrogen/total nitrogen according to *Licitra et al. (1996)*, NH_3-N was determined by the distillation method using a Kjeltec 1026 analyser and the pH value was measured with a Hanna Instruments HI 83141 pH meter. Lactic acid (LA), acetic acid (AA) and butyric acid (BA) were analyzed with a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) according to *Faithfull (2002)*. Non-nitro extractive matter (NEM) was calculated by formula: 100% - % crude protein - % crude fat - % crude fibre - % ash - % moisture. Also, calculated total digestible nutrients value (TDN) and relative feed value (RFV) according to *Horrocks and Vallentine (1999)*, metabolic energy (ME) according to *Nauman and Bassler (1993)* and net energy for lactation (NEL) according to *Baležentienė and Mikulionien (2006)*:

TDN (%) = (-1,291 x ADF) + 101.35;

RFV (%) = Digestible Dry Matter (DDM) x Dry Matter Intake (DMI) x 0.775,

DDM (%) = 88.9 - (0.779 x % ADF) and DMI (%) = 120 / (% NDF);

ME (MJ kg^{-1}) = 14.07 + 0.0206 x crude fat (g kg^{-1}) – (0.0147 x crude fibre (g kg^{-1}) - 0.0114 x crude protein (g kg^{-1}) ± 4.5 %;

NEL (MJ kg^{-1}) = 9.10+0.0098 x crude fat (g kg^{-1}) – 0.0109 x crude fibre (g kg^{-1}) - 0.0073 x crude protein (g kg^{-1}).

Data were subjected to an ANOVA using Statistica version 10, a Randomized Complete Block Design and Duncan's Multiple Range Test was used to compare differences among treatment means ($P < 0.05$).

Results

Chemical composition

Data of ANOVA in Table 1 shows that bacterial inoculant Silko had highly significant effect on content of dry matter, ash, crude protein, cellulose, acid detergent fibre (ADF), neutral detergent fibre (NDF), and non-nitro extractive matter. Values of dry matter (434.4 g kg^{-1}), ash (101.50 g kg^{-1}) and crude protein (202.61 g kg^{-1}) were significantly higher in silage treated with bacterial inoculant Silko than in silage without inoculant (419.9 g kg^{-1}, 86.48 g kg^{-1} and 169.54 g kg^{-1}, respectively). Contrary, values of cellulose (295.70 g kg^{-1}), ADF (351.78 g kg^{-1}), NDF (408.61 g kg^{-1}) and non-nitro extractive matter (404.33 g kg^{-1}) were significantly higher in silage without inoculant than in silage treated with bacterial inoculant Silko (271.03 g kg^{-1}, 314.38 g kg^{-1}, 393.10 g kg^{-1} and 379.90 g kg^{-1}, respectively). The addition of inoculant did not alter crude fat content. Generally, addition of inoculant has improved the chemical composition of alfalfa silage.

Table 1 Chemical composition of untreated silage and silage treated with bacterial inoculant Silko

Item	Control	Silko	M	F test
Dry matter (g kg^{-1})	419.9^{b}	434.4^{a}	427.2	**
Ash (g kg^{-1} DM)	86.48^{b}	101.50^{a}	93.99	**
Crude fat (g kg^{-1} DM)	38.27	38.16	38.22	ns
Crude protein (g kg^{-1} DM)	169.54^{b}	202.61^{a}	186.07	**
Cellulose (g kg^{-1} DM)	295.70^{a}	271.03^{b}	283.36	**
Acid detergent fibre (ADF) (g kg^{-1} DM)	351.78^{a}	314.38^{b}	333.08	**
Neutral detergent fibre (NDF) (g kg^{-1} DM)	408.61^{a}	393.10^{b}	400.85	**
Non-nitro extractive matter (g kg^{-1} DM)	404.33^{a}	379.90^{b}	392.12	**

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability and ns - not significant

Energy characteristics

Total digestible nutrients value (TDN) (60.76%) and relative feed value (RFV) (152.38%) have significant higher in silage treated with bacterial inoculant than control (55.94% and 139.97%, respectively) (Table 2). Metabolic energy (ME) and net energy for lactation (NEL) were not affected by inoculation treatment.

Table 2. Energy characteristics of untreated silage and silage treated with bacterial inoculant Silko

Item	Control	Silko	M	F test
Total digestible nutrients value (TDN) (%)	55.94[b]	60.76[a]	58.34	**
Relative feed value (RFV) (%)	139.97[b]	152.38[a]	146.18	**
Metabolic energy (ME) (MJ kg^{-1})	8.58	8.56	8.57	ns
Net energy for lactation (NEL) (MJ kg^{-1})	5.01	5.04	5.02	ns

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability and ns - not significant

Fermentation parameters

Data of ANOVA in Table 3 show that silage inoculant had significant effect on all fermentation parameters. The lactic acid (86.00 g kg^{-1} DM) and acetic acid (8.45 g kg^{-1} DM) were higher in silage treated with inoculant Silko compared to control (79.92 g kg^{-1} and 5.69 g kg^{-1}, respectively). The pH (4.69), butyric acid (0.021 g kg^{-1} DM), soluble N/TN (343.43 g kg^{-1} TN) and NH_3-N/TN (21.83 g kg^{-1} TN) were lower in silage treated with inoculant Silko compared to control (4.80, 0.026 g kg^{-1}, 350.68 g kg^{-1} and 27.90 g kg^{-1}, respectively).

Table 3. Fermentation parameters of untreated silage and silage treated with bacterial inoculant Silko

Item	Control	Silko	M	F test
pH	4.80[a]	4.69[b]	4.74	*
Lactic acid (g kg^{-1} DM)	79.92[b]	86.00[a]	82.96	**
Acetic acid (g kg^{-1} DM)	5.69[b]	8.45[a]	7.07	**
Butyric acid (g kg^{-1} DM)	0.026	0.021	0.024	*
Soluble N/TN (g kg^{-1} TN)	350.68[a]	343.43[b]	347.06	*
NH_3-N/TN (g kg^{-1} TN)	27.90[a]	21.83[b]	24.87	**

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$), ** - significant at 1% level of probability and * - significant at 5% level of probability

Discussion

The low content of soluble carbohydrates (<1.5%) in fresh alfalfa material makes it difficult to ensiling. Various types of chemical or bacterial additives have been developed in order to improve the ensiling process. There are a large number of bacterial inoculants for ensiling on the market. This research showed beneficial effects of bacterial inoculant (Silko) on silage quality. It is believed that the most efficient type of homofermentative lactic acid bacteria *Lactobacillus plantarum*

(strains: LP1, LP2, LP3 and LP4) which most effectively transforms water soluble carbohydrates into lactic acid. Inoculant Silko had a positive effect on chemical composition alfalfa silages in terms of higher dry matter content, crude protein and mineral elements, and lower cellulose, ADF, NDF, and non-nitro extractive matter. Generally, inoculant Silko has improved the chemical composition of alfalfa silage. *Jatkauskas et al. (2015)* reported that bacterial inoculants improve chemical composition alfalfa silage by increasing content of dry matter, crude protein and soluble carbohydrates. Dry matter content was higher in silage treated with inoculant Silko. This can be explained by the fact that lactic acid fermentation is slow in control, due to small number of lactic acid bacteria on living plants, even by providing optimal initial conditions. *Doležal et al. (2012)* concluded that optimal dry matter content 350-400 g kg^{-1} for alfalfa silage. In our study, the dry matter content of alfalfa silages was higher than optimal content. High quality alfalfa silage has crude protein minimum 200 g kg^{-1} of dry matter. Crude protein in treated silage (202.61 g kg^{-1}) was higher than untreated silage (169.54 g kg^{-1}) and belongs to a group of high quality silage. In control, the higher crude protein content can be explained by harvesting alfalfa in early phase when share of leaves was equal to or greater than the share of stems. *Bijelić et al. (2015)* reported that crude protein content (179 g kg^{-1}) in alfalfa silage in early harvest phase was higher than phase of late harvest (146.2 g kg^{-1}). The reduction of fractions ADF and NDF in treated silage evidenced favorable anaerobic conditions for a fermentation process. The NDF and ADF are important quality parameters of silage. High contents of NDF and ADF in silage adversely affect the quality and decreased digestibility. *Temel et al. (2015)* reported that the NDF and ADF are undesired structures in fodder crops. Degradation cell-wall content (NDF and ADF) during the fermentation improves silages digestibility and animal performance *(Bolsen et al., 1996)*. *McDonald et al. (1991)* pointed that homofermentative bacteria degrade the cellular walls of forage during the ensiling process.

The chemical composition of treated silage was improved due to a reduction ADF and NDF, as well as increases in energy content. Silage treated with Silko inoculant had higher TDN and RFV than untreated silage. TDN is directly related to digestible energy and is often calculated based on ADF. Higher TDN and RFV values indicate higher forage quality. They are indication of good chemical composition of treated silage. *Horrocks and Vallentine (1999)* reported that the RFV value is greater than 151 is considered prime. In our study the RFV (152.38%) in treated silage was higher than 151. The energy content (ME and NEL) were not affected by the use of the silage inoculant. Contrary, *Sánchez et al. (2014)* reported that ME and NEL contents increased in inoculated alfalfa silage. According to *Juraček et al. (2016)* the average value of NEL in alfalfa silages in Slovakia farms is 4.83 MJ kg^{-1} of DM, while in our study the value of NEL was higher (in average for both silage is 5.04 MJ kg^{-1} of DM).

The lower values of pH indicate that fermentation was initiated effectively by added *Lactobacillus plantarum* strains. The lower pH in inoculated silage is important for conserving of nutrients and promoting homofermentative lactic acid bacteria. Generaly, the main effect of silage inoculant was the increased production of lactic acid with significant reduction of pH *(Jatkauskas and Vrotniakiene, 2011; Hashemzadeh-Cigari et al. 2011; Sánchez et al., 2014)*. The content of lactic acid and acetic acid were significantly higher while soluble N/TN and NH_3-N/TN significantly lower in inoculated silage than control. These indicate efficient fermentation and minimal dry matter loss. Inoculation resulted in lower protein degradation. Many researches showed that silages treated with inoculants containing of *Lactobacillus plantarum* had lower pH and NH_3-N/TN, and higher content of lactic acid than untreated silages *(Saarisalo et al., 2006; Jatkauskas et al., 2013; Jatkauskas et al., 2015)*. In control, the high level of NH_3-N/TN indicating protein degradation from proteolytic enzymatic activity contained within the crop. In treated silage, NH_3-N/TN content decreased due to the lower pH and more lactic acid produced.

The primary goal of rapid fermentation and stabilization of a plant material is to produce higher levels of lactic acid rather than acetic acid. The content of acetic acid was significantly higher in inoculated silage than control. Also, *Zhang et al. (2009)* and *Sánchez et al. (2014)* concluded that the inoculated alfalfa silage had more lactic acid and acetic acid content than the control. Many studies have indicated that acetic acid has anti-fungal properties, reduces aerobic spoilage of silage and growth of moulds and yeasts *(McDonald et al., 1991; Schmidt et al., 2009; Čabarkapa et al. 2010a, b*). Otherwise acetic acid is produced naturally during fermentation, with or without inoculants. *Seglar (2003)* reported that the presence of butyric acid is the result of Clostridial activity. Clostridia spores degrade lactic acid to butyric acid. *Pahlow et al. (2003)* concluded that to prevent Clostridial activity should be reached lower pH value, which was achieved in the treated silage with Silko. Therefore the higher content of butyric acid was detected in control than treated silage, but in both silages concentration of butyric acid is <0.05% of dry matter. According to the content of butyric acid, the investigated silages are good quality. Generally, fermentation characteristics in treated silage indicate good silage quality.

Conclusions

Results showed that values of dry matter, ash, crude protein, lactic acid, acetic acid, total digestible nutrients value and relative feed value significantly increased in treated silage with inoculant Silko. On the other hand, values of cellulose, acid detergent fibre, neutral detergent fibre, non-nitro extractive matter, pH, butyric acid, soluble nitrogen/total nitrogen and NH_3-N/total nitrogen

significantly decreased in treated silage. Generally, results showed that bacterial inoculant Silko improves chemical, nutritional quality and fermentation quality of alfalfa silage. Adding bacterial inoculant Silko may be a promising management practice to improve fermentation, conserve more nutrients and increase their availability to the ruminants.

Uticaj bakterijskog inokulanta na hemijski sastav i fermentaciju silaže lucerke

Snežana Đorđević, Violeta Mandić, Dragana Stanojević

Rezime

Silaža lucerke je koristan izvor proteina za ishranu preživara. Stoga, proizvodnja silaže lucerke u stočarstvu predstavlja važno pitanje kako bi se održao kvalitet silaže i postigla maksimalna profitabilna proizvodnja mleka i mesa. Cilj ovog istraživanja je bio da se proceni efekat bakterijskog inokulanta Silka koji sadrži *Lactobacillus plantarum* (sojevi: LP1 LP2, LP3 i LP4) na hemijski sastav i fermentaciju silaže lucerke u terenskim uslovima na komercijalnoj farmi goveda u 2016. godini. Prvi otkos lucerke u drugoj godini je konzerviran u obliku silaže. Silažna masa je podeljena na dva jednaka dela (kontrola (silaža bez inokulanta) i silaža tretiranih bakterijskim inokulantom Silko) i silirana u rovu silosu. Silaža je analizirana 60 dana nakon siliranja. Sadržaj suve materije, pepela, sirovih proteina, mlečne i sirćetne kiseline, ukupna svarljiva hranljiva materija i relativna hranljiva vrednost značajno su veći u silaži tretiranoj bakterijskim inokulantom Silko nego u kontroli. Suprotno, silaža lucerke tretirana sa bakterijskom inokulantom Silko imala je niže vrednosti za celulozu, ADF, NDF, bezazotne ekstraktivne materije, pH, buternu kiselinu i udeo rastvorljivog i amonijačnog azota u ukupnom azotu nego kontrola. Rezultati su pokazali da bakterijski inokulant Silko povećava kvalitet silaže u odnosu na kontrolu, tako da bi dalja istraživanja trebalo da budu usmerena ka korišćenju ovako pripremljene silaže u ishrani preživara i njen uticaj na proizvodnju mleka i mesa na farmama.

Acknowledgment

The research was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, projects ‘Silage Inoculants’ and TR 31053.

References

AOAC (1990): Association of Official Analytical Chemists, Washington DC, USA, 1, 14, 684.
BIJELIĆ Z., TOMIĆ Z., RUŽIĆ-MUSLIĆ D., KRNJAJA V., MANDIĆ V., PETRIČEVIĆ M., CARO-PETROVIĆ V. (2015): Silage fermentation characteristics of grass-legume mixtures harvested at two different maturity stages. Biotechnology in Animal Husbandry, 31, 2, 303-311.
BOLSEN K. K., ASHBELL G., WEINBERG Z. G. (1996): Silage fermentation and silage additives. Asian Australasian Journal of Animal Sciences, 9, 5, 483-493.
BALEŽENTIENĖ L., MIKULIONIENĖ S. (2006): Chemical composition of galega mixtures silages. Agronomy Research, 4, 2, 483-492.
ČABARKAPA I., PALIĆ D., PLAVŠIĆ D., JEREMIĆ D. (2010a): The influence of a bacterial inoculant on reduction of aerobic microflora during ensiling of alfalfa. The Book of abstracts of the 9th International symposium of animal biology and nutrition, Bucharest, Romania, 23-24 September 2010, 38-39.
ČABARKAPA I., PALIĆ D., PLAVŠIĆ D, VUKMIROVIĆ Đ., ČOLOVIĆ R. (2010b): The influence of a bacterial inoculant on reduction of aerobic microflora during ensiling of alfalfa. Food and Feed Research, 1, 23-26.
DOLEŽAL P. (2012): Feed Conservation. Olomouc: Petr Baštan (in Czech).
ĐORĐEVIĆ N., GRUBIĆ G., STOJANOVIĆ B., BOŽIČKOVIĆ A. (2011): The influence of compression level and inoculation on biochemical changes in lucerne silages. Journal of Agricultural Sciences, 56, 1, 15-23.
FAITHFULL N. (2002): Methods in Agricultural Chemical Analysis: A Practical Handbook, CABI Publishing, Wallingford.
GIRAFFA G., CHANISHVILI N., WIDYASTUTI Y. (2010): Importance of lactobacilli in food and feed biotechnology. Research in Microbiology, 161, 6, 480-487.
HASHEMZADEH-CIGARI F., KHORVASH M., GHORBANI G. R., TAGHIZADEH A. (2011): The effects of wilting, molasses and inoculants on the fermentation quality and nutritive value of lucerne silage South African Journal of Animal Science, 41, 4, 377-388.
HORROCKS R. D., VALLENTINE J. F. (1999): Harvested Forages. Academic Press, London, UK.
JATKAUSKAS J., VROTNIAKIENE V. (2011): The effects of silage inoculants on the fermentation and aerobic stability of legume-grass silage. Zemdirbyste-Agriculture, 98, 4, 367-374.
JATKAUSKAS J., VROTNIAKIENE V., OHLSSON C., LUND B. (2013): The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agricultural and Food Science, 22, 137-144.

JATKAUSKAS J., VROTNIAKIENE V., LANCKRIET A. (2015): The effect of different types of inoculants on the characteristics of alfalfa, ryegrass and red clover/ryegrass/timothy silage. Zemdirbyste-Agriculture, 102, 1, 95-102.
JURÁČEK M., BÍRO D., ŠIMKO M., GÁLIK B., ROLINEC M. (2016): The quality of farm-scale alfalfa silages. Acta fytotechn zootechn, 19, 2, 54-58.
KILIÇ A. (1986). Silo Yemi (Öğretim, Öğrenim ve Uygulama Önerileri). Bilgehan Basımevi, Izmir, Turkey, 340.
KIZILSIMSEK M., SCHMIDT R.J., KUNG L. Jr. (2007): Effects of a Mixture of Lactic Acid Bacteria Applied as a Freeze-Dried or Fresh Culture on the Fermentation of Alfalfa Silage. Journal of Dairy Science, 90, 12, 5698–5705.
LICITRA G., HERNANDEZ T. M., VAN SOEST P. J. (1996): Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 51, 347-358.
MCDONALD P., HENDERSON A. R., HERON S. J. E. (1991): The biochemistry of silage. 2nd edn. Chalcombe Publ, Bucks, UK.
NAUMAN C., BASSLER R. (1993): Die chemische Unter-schung von Futtermilteln. Methodenbuch. Band III. VDLUFA. Damstadt., 256.
PAHLOW G., MUCK R. E., DRIEHUIS F. (2003): Microbiology of ensiling. In: BUXTON, D.R.; MUCK, R.E.; HARRISON, J.H. (Eds.) Silage science and technology. Madison: American Society of Agronomy, Crop Science Society of America. Soil Science Society of America, 31-93.
REPETTO J. L., ECHARRI V., AGUERRE M., CAJARVILLE C. (2011): Use of fresh cheese whey as an additive for Lucerne silages: Effects on chemical composition, conservation quality and ruminal degradation of cell walls. Animal Feed Science and Technology, 170, 160-164.
SAARISALO E., JAAKOLA S., SKYTTÄ E., JALAVA T. (2006): Screening and selection of lactic acid bacteria strains suitable for ensiling grass. Journal of Applied Microbiology, 102, 327-336.
SÁNCHEZ D. J. I., SERRATO C. J. S., RETA S. D. G.,, OCHOA M.E. REYES G.A. (2014): Assessment of ensilability and chemical composition of canola and alfalfa forages with or without microbial inoculation. Indian Journal of Agricultural Research, 48, 6, 421-428.
SCHMIDT R., HU W., MILLS J., KUNG L. (2009): The development of lactic acid bacteria and *Lactobacillus buchnery* and their effects on the fermentation of alfalfa silage. Journal of Dairy Science, 92, 5005–5010.
SEGLAR B. (2003): Fermentation analysis and silage quality testing. Proceedings of the Minnesota Dairy Health Conference, College of Veterinary Medicine, University of Minnesota, 29 May, Minnesota, 119-136.
SILVA V. P., PEREIRA O. G., LEANDRO E. S., DA SILVA T. C., RIBEIRO K. G., MANTOVANI H. C., SANTOS S. A. (2016): Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical

composition of alfalfa silage in tropical conditions. Journal of Dairy Science, 99, 3, 1895-1902.
STATISTICAL YEARBOOK OF THE REPUBLIC OF SERBIA, 2016.
TEMEL S., KESKIN B., YILDIZ V., KIR A. E. (2015).Investigation of dry hay yield and quality characteristics of commonvetch (*Viciasativa* L.) cultivars for in Igdir plain download conditions. Iğdır University Journal of the Institute of Science and Technology, 5, 3, 67-76.
TIAN J., LI Z., YU Z., ZHANG Q., LI X. (2016): Interactive effect of inoculant and dried jujube powder on the fermentation quality and nitrogen fraction of alfalfa silage. Animal Science Journal, doi: 10.1111/asj.12689.
ZHANG T., LI L., WANG X., ZENG Z., HU Y., CUI Z. (2009): Effects of *Lactobacillus buchneri* and *Lactobacillus plantarum* on fermentation, aerobic stability, bacteria diversity and ruminal degradability of alfalfa silage. World Journal of Microbiology and Biotechnology, 25, 965-971.
ZIELIŃSKA K., FABISZEWSKA A., STEFAŃSKA I. (2015): Different aspects of *Lactobacillus* inoculants on the improvement of quality and safety of alfalfa silage. Chilean journal of agricultural research, 75, 3, 298-306.

Received 6 December 2016; accepted for publication 19 December 2016

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)

Acknowledgment – for example:

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 6885.

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

11th International Symposium
"Modern Trends in Livestock Production"
11th – 13th October 2017, 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 **11th International Symposium on Modern Trends in Livestock production**, which will be held **from 11th to 13th October 2017 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. Breeding, Selection, Genetics, and Reproduction of Farm Animals
2. Nutrition of Farm Animals
3. Animal Welfare and Health Care
4. Organic Livestock Production
5. Technology and Quality of Animal Products
6. Protection of the Environment and Bidoversity in Animal Production
7. Livestock Production and Food Security in a Context of Climate Change
8. Livestock Feed and Ecology

OFFICIAL LANGUAGE

The official language of the Symposium is English.

REGISTRATION AND PAYMENTS

Registration and submission of abstracts and 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 2017 (Early registration)**	**After 30th June 2017 (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** €

The first author of the Invited paper does not pay Registration Fee

IMPORTANT DATES

Deadline for abstract submission **January, 31st 2017**
Deadline for full paper submission **May, 31st 2017**

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:	**DEUTDEFF** **DEUTSCHE BANK AG** **TAUNUSANLAGE 12** **60262 FRANKFURT AM MAIN** **GERMANY**
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 2017.**

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

Accommodation bookings forwarded directly to the hotel by filling HOTEL RESERVATION FORM which is attached to this notice.

On behalf of
Organizing Committee

Dr. Milan M. Petrović
Principal Research Fellow
Serbia

On behalf of
International Scientific Committee

Prof. Dr. Martin Waehner
Germany