UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

VOL 29, 2
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2013

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2013

Biotechnology in Animal Husbandry 29 (2), p 183-404, 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

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, - M24 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 29 (2), p 183-191 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 619:616.24'632
DOI: 10.2298/BAH1302183V

ECONOMIC IMPACT OF PARATUBERCULOSIS ON MILK PRODUCTION

B. Vidić, S. Savić, V. Vidić, M. Jovičin, N. Prica

Scientific veterinary institute "Novi Sad", Rumenački put 20, Novi Sad
Corresponding author: branka@niv.ns.ac.rs
Review paper

Abstract: Paratuberculosis (Johne's disease) is a chronic inflammatory bowel disease, primarily affecting ruminants. The aetiologic agent is *Mycobacterium avium* subsp. ***paratuberculosis*** (MAP). The disease is characterised by persistent diarrhea, weight loss and protein-losing enteropathy. Paratuberculosis can cause significant economic loss in affected herds, as a result of reduced milk yield, increased incidence of mastitis, altered milk constituents, increased somatic cell counts, poor feed conversion, increased susceptibility to disease in general, reduced reproductive efficiency, premature culling and reduced cull cow values. The economic impact of paratuberculosis includes production losses due to sub-clinical and clinical cases, losses due to increased replacement of animals and costs of control measures. Due to the fact that most cases of paratuberculosis are subclinical and precise prevalence data are often lacking, it is difficult to assess the economic consequences of paratuberculosis. For instance, estimates of milk production losses are inconsistent. Some studies found equivalent or even higher milk productions in test-positive animals. Other studies showed losses in test-positive animals of up to 19.5% of the 0 to 305 days-in-milk production, depending on parity.

Key words: paratuberculosis, cattle**,** economic impact, milk production

Introduction

Paratuberculosis (PTB) is infective granulomatous enteritis of ruminants. The disease is also called Johne's disease and it is an incurable illness. The causative agent is *Mycobacterium avium subspecies paratuberculosis* (MAP). The clinical symptoms that can be found are persistent diarrhea, weight loss and protein-losing enteropathy. Due to these clinical signs, paratuberculosis can cause significant economic loss in affected herds, like reduced milk yield, increased incidence of mastitis, altered milk constituents, increased somatic cell counts, poor feed conversion, decreased immune response in general, reduced reproductive

efficiency, premature culling and reduced cull cow values. In cattle clinical signs usually appear in the period between 2-6 years of age. Most often it is noticed on one animal and the disease spreads slowly. Serology examinations showed that there are up to 30% of infected animals. Paratuberculosis is present in many countries of Europe, USA, Australia, Canada, Japan, Southern America and in some countries in Africa (*Vidić et al., 2001; Cvetnic et al., 2002; Dufour et al, 2004; Hendrick et al., 2005).*

The problem of detecting subclinical infections is present. This fact, as well as complex procedure in establishing laboratory diagnosis, enabled permanent spreading of the infection in the herds of ruminants, so implemented control measures were not sufficiently effective. Animals are infected through food and water contaminated with the feces of the infected animals (*Vidić et al., 2002).*

Diagnostic procedure can be done in clinical form of the disease and when detecting subclinical infection. Diagnostics of paratuberculosis has to overcast monitoring of the herd and reliable diagnosing of individual animals. Not one diagnostic procedure has given reliable diagnosing of individual animals, and determining prevalence, so far. Diagnostics is based on determining causative agent in feces using direct microscopy, cultures, applying DNA test and PCR. Serology methods are the cheapest and fastest methods for agent detection, but there is a problem with the sensitivity and specificity of method (*Vidić et al., 2010)*. The control program is based on reducing agent transmission on susceptible animals, elimination of infected animals, hygienic measures and vaccination.

Healthy cattle which are infected are common source of *M. paratuberculosis* in dairy herds. The incubation period is very long, so infected cattle may show no signs of the infection for many years, and also the serological tests can be negative and/or faecal culture tests, too (*Sweeney, 1996*). These facts should be taken into consideration when purchasing new animals. Sometimes, paratubercusosis can lead to great economic losses with the collapse of the farm (*Benedictus et al., 1987).* However, some data say that paratubercusosis caused less economic losses than other severe diseases *(Stott et al., 2005*).

In many countries with developed agricultural practices, economic outcomes of paratubercusosis were investigated in dairy herds *(Nordlund et al., 1996; Johnson-Ifearulundu and Kaneene, 1997; Vidić et al.,2011a).* An accurate assessment of economic impact of MAP is still impossible.

More importantly than direct losses, a milk price reduction for infected herds can result from consumer concerns about the zoonotic potential of paratuberculosis. The issue of a potential role of MAP in the pathogenesis of Crohn's disease in humans has not yet been resolved. If MAP is implicated, then milk is a possible vehicle of transmission of the organism to humans, because MAP has been detected in raw milk and might not be effectively inactivated by pasteurisation

Classification of economic losses

When disease occurs, the production results and the prices are both affected - increased veterinary input aimed at disease control may result in increased national output of livestock products, which consecutively may result in decreased prices for output (*Bennett, 2003; Losinger, 2005).*

Cost of the diasease can be defined as direct disease cost. Direct disease cost can be shown as $C = (L + R) + T + P$, where L is the value of the loss in expected output due to the presence of a disease, R is the increase in expenditures on non-veterinary resources due to a disease (farm labor etc.), T is the cost of inputs used to treat disease and P is the cost of disease prevention measures. Indirect impact of the disease was not included in this model, only some of them were described: effects on human health, animal welfare, and international trade (*Bennett, 2003*).

Losses attributable to paratuberculosis in animals can be classified in two groups. First group are the ones caused by the presence of clinically ill animals which are calculated as a sum of physical loss of a cow and its calf, loss of a female calf that is important for future reproduction, male calves are fattened before they reach slaughter body weight. The amount of these losses is determined by the number of culled and subsequently replaced animals. There are also other financial losses, like costs of veterinary services and laboratory testing. The second group of losses are the ones caused by subclinically infected animals, which are difficult to estimate in comparison with losses caused by clinical cases (decrease of milk production) *(Dufour et al, 2004).*

The most commonly used classification of categorizing economic losses to direct losses and indirect losses is used by several authors *(Bennett, 2003; Groenendaal, 2005).*

Direct economic losses include: mortality of clinically ill animals and decreased slaughter value or complete distraint of slaughtered animals, reduced milk production in quantity and quality (changes in milk parameters, increased somatic cells counts, increased incidence of mastitis), decreased pregnancy rate and increased post-partum complications (decreased fertility rate within the herd), poor feed conversion in clinically and in subclinically infected animals, decreased productive age length, increased predisposition to other chronic diseases in herds affected with paratuberculosis (chronic arthritis, rumenitis, dermatitis, mastitis, etc.).

Indirect economic losses include: doubtful future income caused by prematurely culled animals, increased expenses for idle production, increased expenses for herd replacement, expenses for paratuberculosis-testing, ineffective veterinary care (treatment of chronic diarrhea that is usually fatal in high-producing animals or slaughter of such animals) and veterinary services for animals culled due to infection, expenses for the control programs, lost genetic value of highly valuable animals, which are culled from a herd due to suspected infection, trade restrictions, lost reputation of a farm with animals infected with paratuberculosis.

Economic impact of respective diagnostic methods and production loss

The diagnostic test for discriminating infected from presumed non-infected animals represents an important factor influencing the assessment of production loss associated with paratuberculosis (*Hendrick et al., 2005; Vidić et al.,2011b).* In a study of two herds, MAP-positive cows, as determined by faecal culture results, produced 18.8% less milk than did MAP-negative herdmates *(Spangler et al., 1992).* However, results obtained in the same herds using enzyme-linked immunosorbent assay (ELISA) revealed no significant differences in milk production between MAP-positive and MAP-negative cows.

Production outcomes associated with the PTB-status may vary, depending on the diagnostic test used because of differences in their accuracy and stadium of infection. Thus, comparison of ELISA and fecal culture results for the same MAP-infected animals by the use of the kappa statistics indicated that two tests have detected different subgroups of animals (*Collins et al., 1991).* The difference in mature equivalent (ME) of milk production between ELISA-positive and ELISA-negative cows was as high as 376 kg per lactation *(Nordlund et al., 1996*). The results of this study demonstrated association of subclinical MAP infection diagnosed by USDA-licensed MAP-ELISA with an average 3.95% (ranging between 1.44 and 6.46%) reduction in milk production.

Hendrick et al. (2005) investigated and compared results of three diagnostic tests (faecal culture, milk ELISA and serum ELISA) and their effects on milk production. They found that cows with positive results of bacteriologic culture of faeces or milk ELISA produced 457 or 548 kg less milk in a 305-day lactation compared with negative herd mates. Similar associations were found between results of bacteriologic culture of faeces and milk ELISA test status and 305-day fat and protein production. The only association found for cows with positive results of the serum ELISA was a significant reduction in 305-day protein production, compared with sero-negative cows.

Most tests for paratuberculosis have high specificity but low sensitivity, which results in a very small proportion of non-infected animals being falsely classified as positives and a high proportion of infected animals being falsely classified as negatives. An outcome of these misclassifications is that production differences between test positive and negative animals and herds will underestimate the actual losses. As infected animals are more likely to test positive late in the course of the disease, the measured losses more accurately reflect those associated with advanced infection.

On the other hand, *Johnson et al. (2001)* assumed that the key to the inconsistent results presented in the literature regarding subclinical MAP-infection and milk production might not be in the method of diagnosis but in the parity of the cows in the study.

Influence of paratuberculosis on milk production

Successful control of the disease in herds can be done with the contribution of test-and-cull strategies in a reasonable period of time, but some alternatives must be considered since culling of all positive animals is not necessarily always required. The assessment of the economic benefit of paratuberculosis "test-and-cull" programs must be based of comprehensive analysis of subclinical production losses. Such programs could be considered cost-effective if subclinical paratuberculosis infection has highly reduced milk production, even if the prevalence within the herd is low. In case of minimal milk production loss, the effectiveness of "test-and-cull" practice is apparent only in herds with high prevalence of the infection. The authors consider the losses of milk production under 6% as minute losses suggesting that the factors such as herd size, contact of adult animals with calves and the level of herd milk production have a little effect on profitability and "test-and-cull" program *(Collins and Morgan, 1991).*

In order to prevent the risk of further contamination and spread of infection from subclinically infected animals to healthy ones, the culling of test-positive animals should be considered even when decrease in milk production has not been established. The short-term economic losses must be compared to the risk of increasing the herd prevalence to paratuberculosis, which may cause severe long-term economic losses *(Johnson et al., 2001).*Prevention and control programs require a complex approach. Milk production losses should be evaluated along with the range of other losses in order to decide on applying the "test-and-cull" strategy and other tools for improving the herd management.

If there is an impact of paratuberculosis on milk constituents, it has not yet been elucidated. The differences establishedso far with respect to lactation average percentages of fat and protein were not found significant. The lactation percentage of fat content ranged between 2.51 to 5.31 in PTB positive and 2.06 to 6.80 in negative cows. The lactation percentage of protein content ranged between 2.58 to 3.73 in PTB positive cows and 2.43 to 4.42 in negative cows *(Nordlund et al., 1996).* Other authors also did not confirm statistically significant differences in fat and protein content in milk of PTB positive cows and healthy cows (*Johnson et al, 2001; Lombard et al, 2005; Sweeney et al.1996)* reported significantly decreased daily milk fat and milk protein production in the infected cows.

Mastitis that occurs during a PTB disease is one of the most common reasons for removing animals from the herd. In one of the investigated herds, mastitis was the reason for removal of 3.6% of the non-infected and 22.6% of the infected cows with unapparent paratuberculosis *(McNab et al. ,1991a).* There is a positive correlation between MAP-positive status and lower prevalence of mastitis. Paratuberculosis was associated with economic benefit due to lower rates of mastitis in positive cows, but a final was financial loss, because of reduced milk production and increased culling rates *(Wilson et al., 1995). Staphylococcus aureus,* as mastitis pathogen was significantly more (x2, $P < 0.001$) detected in

negative cows to paratuberculosis, than in cows positive to paratuberculosis. Significantly higher percentage of positive cows to paratuberculosis, were affected with mastitis caused by coagulase-negative *Staphylococcus sp.* Compared to negative animals to paratuberculosis from the same herd (x2, $P < 0.001$). *Serratia sp.* mastitis was diagnosed only in negative cows to paratuberculosis *(Wilson et al, 1993).*Some authors have studied the association between subclinical PTB infection and increased somatic cell count (*McNab et al, 1991a*). Others established lower somatic cell count in cows infected with paratuberculosis, up to the third lactation when in contrast, this value was higher *(Wilson et al, 1993).*

Conclusions

Economic losses caused by paratuberculosis in dairy cattle herds represent a severe problem for farmers and also for dairy industry. Analysis of economic consequences of paratuberculosis occurence in a farm is of great importance, in order to establish the programs of disease control, enabling certification of PTB-free herds and improving the position of farmers and owners of infected herds.

A decreased milk production in connection with PTB infection and was found by numerous researchers. The rate of milk production decrease was associated with the respective method for disease classification.

Impact of PTB infection on milk production should be considered as one of many factors that influence milk production.Effects of PTB infection on the occurrence of mastitis and somatic cell count still needs to be explored. Some authors found an association between paratuberculosis and increased mastitis, whereas in other studies a low incidence of mastitis (or no association at all) was established. More investigation is necessary to confirm or exclude involvement of paratuberculosis. The control of the transmission of PTB infection is time-consuming and requires considerable financial assets. Also, non-significant statistical differences in the distribution of the causative agent of PTB infection within the organism of various dairy, beef and dual-purpose cattle breeds were found. PTB infections may be a cause of significant economic losses in beef and dairy cattle herds. Furthermore, reduction of the milk price may occur. The losses may be so high that a national PTB eradication program will be economically attractive.

It is concluded that the likelihood of paratuberculosis occurring in a herd and its economic impact are important issues in risk management of paratuberculosis in dairy herds. Measures that can be taken to reduce this likelihood and impact include closed herd management, preventive management measures, test-and-cull of infected animals and participation in quality assurance programmes. Keys to success include realistic expectations of the results of paratuberculosis control, development of a quality assurance programme that is

appreciated by farmers and incentives for farmers to participate in such a programme.

Acknowledgment

This study was supported by grant TR31084 from Serbian Ministry of Education, Science and technological development.

Ekonomski uticaj paratuberkuloze na proizvodnju mleka

B.Vidić, S.Savić, V. Vidić, M. Jovičin, N. Prica

Rezime

Paratuberkuloza (Džonova bolest) je hronično zapaljenje creva koje se prvenstveno javlja kod preživara. Etiološki agens je *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Oboljenje karakteriše uporna dijareja, gubitak težine i enteropatija sa gubitkom protein. Paratuberkuloza može dovesti do značajnih ekonomskih gubitaka u obolelom zapatu, kao rezultat smanjenog lučenja mleka, povećanom broju mastitisa, promena sastava mleka, povećanje broja somatskih ćelija, slaba konverzija hrane, povećana prijemčivost za bolesti uopšte, smanjenje reproduktivne sposobnosti, prevremeno isključivanje životinja i smanjene vrednosti teladi.

Ekonomski uticaj paratuberkuloze obuhvata gubitke u proizvodnji kod sub kliničkih i kliničkih slučajeva oboljenja, gubitke zbog intenzivnije zamene životinja i troškova mera kontrole. Većina slučajeva paratuberkuloze čine subklinička oboljenja, a preciznih podataka o prevalence oboljenja nema. Iz ovih razloga, teško je proračunati ekonomske posledice pojave paratuberkuloze u zapatu. Procene gubitaka u proizvodnji mleka su promenjivi. U nekim istraživanjima je nađena ista ili čak veća proizvodnja mleka kod životinja pozitivnih na paratuberkulozu. Druga istraživanja prikazuju gubitke kod životinja pozitivnih na paratuberkulozu i do 19.5%, od 0 do 305. dana proizvodnje mleka, u zavisnosti od pariteta.

References

BENEDICTUS G., DIJKHUIZEN A.A., STELWAGEN J. (1987): Economic losses due to paratuberculosis in dairy cattle. Veterinary Record, 121, 142–146.

BENNETT R. (2003): The ‘Direct costs’ of livestock disease: The development of a system of models for the analysis of 30 endemic livestock diseases in Great Britain. Journal of Agricultural Economics, 54, 55–71.
COLLINS M.T., MORGAN I.R. (1991): Economic decision analysis model of a paratuberculosis test and cull program. Journal of the American Veterinary Medical Association, 199, 1724–1729.
COLLINS M.T., SOCKETT D.C., RIDGE S., GOODGER W.J., CONRAD T.A., THOMAS C.B., CARR D.J. (1991): Estimation of a commercial enzyme-linked immunosorbent assay for Johne’s disease. Journal of Clinical Microbiology, 29, 272–276.
CVETNIC Z., BRLEK K., TRSTENJAK J., OCEPEK M., SPICIC S., MITAK M., KRT B. (2002): Economic importance of paratuberculosis in dairy cattle breedings. In: Proceedings of the Seventh International Colloquium on Paratuberculosis. Bilbao, Spain, 11th to 14th June, 2002, ISBN 0-9633043-5-6 (pbk.: alk. paper), 486–487.
DUFOUR B., POUILLOT R., DURAND B. (2004): A cost/benefit study of paratuberculosis certification in French cattle herds. Veterinary Research, 35, 69–81.
GROENENDAAL H. (2005): Control programs for Johne’s disease. Advances in Dairy Technology, 17, 81–94.
HENDRICK S.H., KELTON D.F., LESLIE K.E., LISSEMORE K.D., ARCHAMBAULT M., DUFFIELD T.F. (2005): Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. Journal of the American Veterinary Medical Association, 227, 1302–1308.
JOHNSON Y.J., KANEENE J.B., GARDINER J.C., LLOYD J.W., SPRECHER D.J., COE P.H. (2001): TheeffectofsubclinicalM. paratuberculosis infection on milk production in Michigan dairy cows. Journal of Dairy Science, 84, 2188–2194.
JOHNSON-IFEARULUNDU Y., KANEENE J.B. (1997): Epidemiology and economic impact of subclinical Johne’s disease: a review. Veterinary Bulletin, 67, 437–447.
LOMBARD J.E., GARRY F.B., MCCLUSKEY B.J., WAGNER B.A. (2005): Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows. Journal of the American Veterinary Medical Association, 227, 1975–1981.
LOSINGER W. (2005): Economic impact of reduced milk production associated with Johne’s disease on dairy operations in the USA. Journal of Dairy Research, 72, 425–432.
McNab W.B., Meek A.H., Duncan J.R., Brooks B.W., Van Dreumel A.A., Martin S.W., NIELSEN K.H., SUGDEN E.A., TURCOTTE C. (1991A): An estimation of selected screening tests for bovine paratuberculosis. Canadian Journal of Veterinary Research, 55, 252–259.

NORDLUND K.V., GOODGER W.J., PELLETIER J., COLLINS M.T. (1996): Associations between subclinical paratuber-culosis and milk production, milk components, and somatic cell counts in dairy herds. Journal of the American Veterinary Medical Association, 208, 1872–1876.
SPANGLER E., BECH-NIELSEN S., HEIDER L.E. (1992): Diagnostic performance of two serologic tests and fecal culture for subclinical paratuberculosis, and associations with production. Preventive Veterinary Medicine, 13, 185–195.
STOTT A.W., JONES G.M., HUMPHRY R.W., GUNN G.J. (2005): Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom. Veterinary Record, 156, 825–831.
SWEENEY R.W. (1996): Transmission of paratuberculosis. Veterinary Clinics of North America: Food Animal Practice, 12, 305–312.
VIDIĆ B., GRGIĆ Ž., BJELAJAC B., TRKULJA R. (2001): Ispitivanje rasprostranjenosti paratuberkuloze kod goveda i ovaca = Investigations of spread of paratuberculosi in cattle and sheep.Veterinarski glasnik, ISSN 0350-2457, 55, 1/2, 9-16.
VIDIĆ B., BOBOŠ S., GRGIĆ Ž.(2002): Paratuberkoloza u goveda = Bovine paratuberculosis.Savremena poljoprivreda, ISSN 0350-1205, 51, 3/4, 273-277.
VIDIĆ B., GRGIĆ Ž., SAVIĆ S., MILANOV D., PRICA N.(2010): Aktuelni pristup diagnostici paratuberkuloze u goveda = Current approaches to diagnosis of paratuberculosis in cattle.Arhiv veterinarske medicine, ISSN 1820-9955, 3, 1, 73-83.
VIDIĆ B., GRGIĆ Ž., SAVIĆ S., PRICA N., ŠEGULJEV Z. (2011a): Current Approaches in Laboratory Diagnostic of Paratuberculosis.Congress Proceedings, 19th International Congress of Mediterannean Federation of Health and Production of Ruminants, May 25-28, 2011, Belgrade, Chief Editors Biljana Radojičić, Stanko Boboš, , str.156-157, ISBN 978-86-7522-034-3.
VIDIĆ B., SAVIĆ S., PRICA N.(2011b): Paratuberculosis in cattle - the outspread, diagnostics and control = Paratuberkuloza kod goveda - raširenost, dijagnostika i kontrola.New Perspectives and Callenges of Sustainable Livestock Production.Book 1, 3rd International Congress, Belgrade, 5-7 Oktober 2011, Chairman Martin Wahner, Beograd, Institute for Animal Husbandryl, 335-344.
WILSON D.J., ROSSITER CH., HAN H.R., SEARS P.M. (1993): Association of Mycobacterium paratuberculosis infection with reduced mastitis, but with decreased milk production and increased cull rate in clinically normal dairy cows. American Journal of Veterinary Research, 54, 1851–1857.
WILSON D.J., ROSSITER CH., HAN H.R., SEARS P.M. (1995): Financial effects of Mycobacterium paratuberculosis on mastitis, milk production, and cull rate in clinically normal cows. Agri-Practice, 16, 12–18.

Received 28 May 2013; accepted for publication 26 June 2013

Biotechnology in Animal Husbandry 29 (2), p 193-210 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.084.52'635
DOI: 10.2298/BAH1302193S

IMPROVEMENT PRODUCTION EFFICIENCY AND CARCASS QUALITY THROUGH FEED RESTRICTION PROGRAMS IN BROILER CHICKENS

M. Sahraei

Member of Scientific board in Agriculture and Natural Resources Center of Ardabil Province.Ardabil, Iran.
Corresponding author: m.sahraei2009@gmail.com
Review paper

Abstract: The main objective of broiler rearing industry is production of high quality broiler carcasses that will be acceptable for consumers; Acceptability depends on the quality and quantity of edible parts of carcasses, and the amount of muscle mass in carcass. Feeding strategy in growing broiler chickens should be to produce animals with maximum lean body mass, highest feed conversion ratio and maximum body weight. Continuous genetic selection and improvement in nutrition have led to a very fast growth rate in modern strains. That early-life fast growth rate is accompanied by a number of problems, such as body fat deposition, high incidence of metabolic disorders, high mortality, and high incidence of skeletal diseases. The feed restriction programs is on of the main techniques in growth curve manipulation for increasing production efficiency in broiler chicken in alleviate the incidence of some metabolic diseases and can be used to produce a leaner bird and reduce the unfavorable effects of fat on human health, and to reduce fat deposition in broiler carcasses could be profitable in broiler chickens production. This article implicated on new findings in about different feed restriction programs effects on production efficiency and carcass quality in broiler chickens.

Key words: broiler chicken, feed restriction, carcass quality, performance

Introduction

During the last 50 years, the amount of time required to reach market weight, and the quantity of feed needed to produce a pound of meat, have been reduced by 50% (*Anthony, 1998*). While concomitant significant improvements have been accomplished in husbandry practices, disease prevention and nutrition, it has been estimated that 90% of the phenotypic changes in poultry have come from

genetic progress (*Havenstein et al., 1994 a*).Unfortunately this growth rate is accompanied by increased body fat deposition, high mortality and high incidence of metabolic diseases and skeletal disorders (*Zubair and Leeson, 1996).* These situations more commonly observed in fast growing broilers that are *ad libitum* fed that led to fat deposition in carcass (*Pasternak and Shalev, 1983; Nir et al., 1996*). This fact is of economical concern because fat represent an undesirable and uneconomical product. To produce leaner bird and reduce the unfavorable effect of fat deposition in carcass on human health, there is interest in reduce fat deposition in broiler carcasses. Thus feed restriction programs have been proposed to overcome these problems. Early feed restriction programs used to reduce abdominal and carcass fat in broiler chickens rely on the phenomenon called compensatory growth or catch up growth to produce market body weight similar to control groups, Compensatory growth or catch-up growth is defined as abnormally rapid growth relative to age. An enhanced rate of growth, exceeding the normal rate of gain, occurs when growth has been retarded by nutritional deprivation and followed by *ad libitum* feeding. This phenomenon has long been used as an effective method to reduce growth rates and changing body composition of most animals (*McMurtry et al., 1988*). Also *Osbourn and Wilson (1960)* said that compensatory growth as being the stage of rapid growth, rather than age, exhibited by mammals and birds after a period of nutritional deprivation. How over About 60-70 % of the expenditures involved in poultry production is feeding costs. As such, the most reasonable phase in reducing the cost of broiler chicken production would be find possible methods, which are cheap, adequate and readily available for feeding livestock. One such method is restricting the amount of daily feed offer for sometime (*Novele et al., 2009*). The main reason for controlling feed intake in broilers is to prevent high fat deposition in broiler chickens when feeding in extra of the broiler chickens requirements for maintenance and production is converted to the fat (*Fontana et al., 1992*) does affect the carcass quality (*Zubair and Leeson, 1996*). Excessive fat is one of the main problems faced by the broiler industry these days, since it not only reduces carcass yield and feed efficiency but also causes rejection of the meat by consumers (*Kessler et al., 2000*) and causes difficulties in processing (*McMurtry et al., 1988*). For overcome in these problems almost in many studies of feed restriction in broiler chickens have been impacted on feed efficiency and body fat deposition. Until very recently, feed restriction was thought to increase feed efficiency and reduce body fat deposition (*Lee and Lesson 2001; Jones and Farrell, 1992; Sahraei and Mohammadi, 2012*). The use of this concept to address problems of high carcass fat requires more studies on the nutrition of the broiler chicken during the period of growth compensation.

Compensatory growth

In general, compensatory growth is defined as the abnormally rapid growth relative to age within a breed of an animal after early growth retardation. The terms "catch-up growth" and "compensatory growth" are used in same concept. When is occurs catch up growth follows refeeding after a periods of under nutrition or recovery from illness. The mechanisms of these phenomena have been studied by a number of investigators (*Osbourn and Wilson, 1960; Mosier, 1986). Benschop (2000)* indicated that the key mechanisms in compensatory growth are decreased maintenance costs, increased feed intake, increased efficiency of growth and in some instances increased digesta load. The reduction in maintenance costs would then allow for comparatively more energy for growth upon realimentation, thus contributing to the compensatory growth responses (*Ryan, 1990*). Increased feed intake has been demonstrated by many researchers as the main mechanism that drives compensatory growth. *Zubair and Leeson (1994a)* reported that restricted-refed broiler chickens have shown higher feed intake relative to body weight when compared to the *ad libitum* control. Hence, higher feed intake as related to body weight, and its associated digestive adaptations seem to be important contributing factors to any growth compensation. Birds with retarded growth due to undernutrition can achieve a growth rate higher than normal for chronological age after removal of the feed restriction (*Plavnik and Hurwitz, 1985*). Owing to the increased efficiency of protein deposition because of the concomitant water deposition that results in more gain per gram protein deposited than lipid deposited, higher rates of protein deposition during realimentation would have a significant impact on the overall growth rates (*Benschop, 2000). Zubair and Leeson (1994b)* showed that other adaptation observed by the restricted-refed broiler chickens is the relative enlargement of digestive organs, especially the gizzard, crop, pancreas and liver which enhance feed intake and help support compensatory growth. But , this finding is not supported by the findings of *Subsilla et al., (1994),* who applied food restriction of 75% and 50% of *ad libitum* intake to broiler chickens from day 5 to 11 days of age and could not find any differences in proportional liver weight during the experiment.

Feed Restriction Definition

Feed restriction is method of feeding that is time, duration and amount of feed were limited, have an impact on whether a bird is capable of achieving the same body weight as unrestricted birds (*Ballay et al., 1992; Yu and Robinson, 1992*). In general, feed restriction included of quantitative and qualitative restriction that is in quantitative to limiting the amount of feed daily given to the

animals whereas a qualitative restriction is related to nutrient dilution in the diet(Lesson and Zubair,1997**)**

Feed Restriction Programs

Quantitative and qualitative feed restriction are procedures that con be applied to manipulate the feeding strategies of poultry in order to decrease growth, and metabolic rate to some extent and so alleviate the incidence of some metabolic diseases as well as improving feed conversion in broiler chickens. These methods include: physical feed restriction, limiting the level of consumption of feed in time (skip-a-day feeding) or reducing the time of illumination of feeding (*Religious et al., 2001*), diet dilution, chemical methods of feed restriction and use of low protein or low energy diets (*Zubair and Lesson, 1996*).

Physical feed restriction

This method is one of the common procedure was used in controlling feed intake in poultry. Physical feed restriction supply a calculated amount of feed per bird, which is often just enough to meet maintenance requirements (*Plavnik and Hurwitz, 1989*). But practical application of physical feed restriction is not simple due to the problems of regularly weighing birds, and calculating feed consumption on a daily basis. Moreover, it is necessary to provide sufficient feeder space in order to prevent competition among restricted birds and to prevent unequal growth of birds within a flock. Also in this method should be attention to educate consuming of micronutrient, coccideoastat and etc. Physical feed restriction programs for broilers have been extensively studied (*Santos et al., 1993b; Scheideler and Baughman, 1993; Sahraei and Mohammadi, 2012)*. Severity of feed restriction, length of restriction, and age at marketing are the main factors to take into account in a feed restriction program for broilers. Quantitative feed restriction has been observed to reduce mortality and culling (*Fontana at al., 1992; Robinson et al., 1992*), improve feed conversion ratio (*Deaton, 1995; Lee and Lesson, 2001)* and allow a complete recovery of body weight if the degree of restriction was not too severe and slaughter ages were extended beyond 6 weeks (*Deaton, 1995; Plavnik and Hurtwiz, 1988b). Dozier et al. (2002),* referred to feed restriction programs of yielding inconsistent results in the literature and that variation maybe partially attributed to differences in bird management, lighting, strain and ventilation. *Plavnik and Hurwitz (1988a)* showed that full compensatory gain with males but not females after early feed restriction. From their findings, it can be concluded that with females feed restriction should be started from 5 to 7 days of age and the duration should not exceed 5 days to achieve complete recovery of final body weight and optimum feed efficiency. Although the level of early feed restriction is an important factor influencing the broiler chicken response, early feed restriction at 30% of ad libitum intake was not able to

influence broiler chicken performance at market age of 49 days (*Giachetto et al., 2003*).

Skip-a-day feeding

Skip-a-day deprivation of feed is a technique for restricting early growth and has not been extensively studied in broiler chickens (*Dozier et al., 2002*). But these programs providing limited allotments are commonly used in broiler breeder's growth restriction. Removing feed for 8-24 hour periods during the starter period reduces early rapid growth and meat yield in broiler chickens. Skip-a-day feed removal has been reported in other studies to decrease early growth and reduce the incident of ascites without affecting final body weight *(Arce et al., 1992; Ballay et al., 1992).Oyedeji and Atteh (2005)* and *Oyedeji et al. (2003),* reported reduction in feed intake after exposing the birds to fasting on every other day. *Oyedeji and Atteh (2005)* showed that skip-a day feeding for 3 weeks starting at day-old would improve carcass quality and reduce sudden death syndrome which is often associated with birds that are on *ad libitum* feed intake.

Lighting programs

Birds are very sensitive to light. Light allows the birds to establish rhythmcity and synchronize many essential functions, including body temperature and various metabolic steps that facilitate feeding and digestion (*Olanrenwaju et al., 2006*). Light intensity, color, and the photoperiodic regime can affect the physical activity of broiler chickens (*Lewis and Morris, 1998)*. In the common production methods, broiler chickens are raising under 23 h Light per day, because it is thought that under this light regimen feed intake is greater and therefore growth rate is suitable. Although lighting programs are not categorized in the literature as a feed restriction method it has been applied. It is known that by changing Lighting periods by either reducing the hours of light or developing intermittent schedules feed utilization is improved (*Buys et al., 1998; Apeldoorn et al., 1999*). The incidence of leg abnormalities is also lowered by reducing the hours of Light per day (*Classen and Riddell, 1989*) as is mortality and specifically sudden death syndrome (*Blair et al., 1993*). The so called step-don and step-up lighting programs (*Classen and Riddell, 1989***)** have attained popularity because of reduced incidence of leg abnormalities, sudden death syndrome and mortality while maintaining the same market weight for age. Broilers under different reduced lighting programs therefore, will reduce their feed intake, and so this program can be included within the definition of feed restriction. However, broilers do learn to eat during darkness when hours of lighting are low (*Morris, 1986***).** *Buyse et al. (1994)* studied the effect of intermittent (step-up and step-down programs) and continuous lighting on the performance of female broilers. Lower cumulative feed intake and significantly improved feed conversion was observed in chickens under an intermittent program **(**1L: 3D from 8 to 49 days) compared with those under a

continuous lighting schedule. These results are in agreement with *Buyse et al. (1996),* who showed improved feed conversion and compensatory growth in male broiler chickens at 41 days with a light schedule from day 7 of 1L: 3D repeated six times daily. The use of lighting programs has the advantage of reducing electricity costs, the incidence of leg abnormalities and sudden death syndrome, and of improving feed efficiency with no reduction of weight at market age. Genotype, sex, feeder space, diet composition and stocking density are the main aspects that can interact with the lighting program (*Buyse et al., 1994),* and affect the broiler's final performance.

Diet dilution

The most problems form of physical feed restriction is usually considered to be maintenance allowance, described by *Plavnik and Hurwitz (1989)* at 1.5 kcal ME/gBW$^{0.67}$/d. But for very young birds, this means a very small quantity of feed is distributed daily, and so this leads to the alternate concept of diet dilution. Therefore many investigators have used diet dilution as an alternative method of nutrient restriction because of the advantage of attaining a more consistent growth pattern within a flock. In this method diets are mixed with non-digestible ingredients such as fiber, and so are of reduce nutrient density. The use of diluted diets relies upon the fact that broiler chickens eat close to their physical intake capacity (*Newcombe and Summers, 1984). Jones and Farrell (1992)* used 50 to 65% diet dilution with rice hulls in order to retard early growth. This technique appeared to be successful, and even though these birds ate more feed, adjustment was insufficient to normalize nutrient intake, and so growth rate was reduced. In many of these physical feed restriction or diet dilution studies, there are reports of reduced body fat deposition, although this effect seems variable. The most consistent feature of all these studies, regardless of method of implementation, is improved feed efficiency. *Griffiths et al. (1977)* lowered the energy of a broiler chicken diet to 2233 kcal ME/kg DM from 3087 kcal ME/kg DM of feed by substituting ground yellow corn with oat meal as the main ingredient. Chickens fed the low energy diet consumed significantly more feed than those fed the high energy diet. When fed the low energy diet from 0 to 3 weeks of age, the chicks were not significantly different in body weight or in abdominal fat pad development from the *ad libitum* birds at 4 weeks of age.

Sahraei and Shariatmadari (2007) used different levels of finisher diet diluted with sand and wheat bran (wt:wt) (in levels 7, 14, 21 or 28%) of Arian strain have showed that feed intake in different levels was more than control birds. But live weight (at 45 ages), body weight gain only in 28% levels were less than control birds. *Cabel and Waldroup (1990)* observed that diluting the starter diet with sand from 5 to 11 days of age moderately restricted growth, which was completely recovered by 49 days of age.

Use of low protein or low energy diets

For retardations of growth rate in broiler chickens can be used of diets with low energy and protein concentrations**.** This method has an advantage in that it does not need any additional labor of weighing the feed, and is accomplished by lowering the level of either protein or energy. In normal conditions broilers are given 22%, 20%, and 18% of crude protein in the starter, grower, and finisher periods respectively, and 3200 kcal ME kg diet (*NRC, 1994*). When broilers are fed with low nutrient dense diets they will increase their feed intake in an attempt to maintain nutrient intake (*Leeson and Summers, 1997*). Feeding broilers with combinations of high density diets (21 -2-25.9% protein,13.9-14.3 MJ ME/kg) produces greater live bird performance and carcass meat components until 50 days of age, but the rate of gain was lower when compared with combinations of low-high density diets (1 9.1-22.5% protein, 12.7-1 3.1 MJ MEkg),(*Walker et al., 1995*). The study of *Plavnik and Hurwitz (1990)* showed that broilers fed *ad libitum* with a 9.4% crude protein diet from 8 to 14 days markedly reduced their feed intake and weight gain by about 57% and 41% respectively. This reduction in feed intake may have been due to of a protein and amino acid deficiency, since other nutrients were at normal levels. But *Rosebrough and McMurtry (1993)* showed the effect of 6 days of diet energy restriction in broiler chickens, the restriction period was from 6 to 12 days and was designed to only support the maintenance requirements for body weight. Body weight at 54 days was achieved for birds given feed *ad libitum* from day 13 to 54, and for those fed *ad libitum* from 21 days onward. Feed efficiency was not significantly different between restricted and unrestricted birds. *Leeson and Summers (1997)* utilized finisher diets varying in energy level from 2700 to 3300 kcal ME kg and showed no significant difference in body weight at 49 days. There was increased feed intake by birds fed the lower energy level diets. Fisher (1984) reported those broilers chickens tend to increase their feed intake to make up for deficiencies when fed diets that are marginally deficient in crude protein. *Leeson et al. (1996)* reported that diluting commercial broiler chicken diets from 35 to 49 days of age with oat hulls and sand, which led to the diets deficient in energy content, caused a significant reduction in body weight at 42 days of age, although the growth was compensated thereafter. Birds seemed to maintain energy intake, therefore there was increased feed intake with energy deficient diet. *Coon et al. (1981)* comparing the performance of male and female broiler chickens fed low or high energy rations for 56 days, found a significant improvement in the feed conversion ratio using a diet with high energy level.

Feed textures

Feed forms such as pellet, crumble, mash and particle size also influences broiler growth and development (*Reece et al., 1985, Jones et al., 1995*). Broilers fed crumble-pellet diets show improved weight gain, feed intake, and feed conversion ratio compared to birds fed mash (*Calet, 1965).* Also, the consumption

of mash feed at different phases of the broiler's growth may be employed as a method of limiting feed intake. Birds offered mash spend more time consuming their feed compare to birds fed pellets (*Savory, 1974*), and therefore, expend more energy in this process. *Andrew (1991)* suggested that the improvement in growth rate due to eating pellets is related to some extent to the increase in bulk density of pullets which in some situations increases nutrient intake, which increases nutrient intake in some situations. *Nir et al. (1995)* fed male and female broilers to 49 days with mash or crumble diets during the starter and grower periods, and mash or pellets for the finisher period. Males showed a significant increase in body weight and improved feed conversion when fed pelleted compared to mash diets. On the other hand, the improvement in performance was not evident for females, which showed no significant difference either in body weight or feed conversion ratio at 49 days of age. Mortality was higher in birds fed pelleted diets. These results are in agreement with those of *Jones et al. (1995)* and *Hamilton and Proudfoot (1995)* where an improved weight gain and feed conversion at 6 weeks of age were obtained in birds fed pelleted compared to mash diets. The improvement in broiler performance with pelleted diets may be attributable to a greater digestibility of carbohydrates together with increased daily nutrient intake (*Hamilton and Proudfoot, 1995*), better nutrient availability *(Nir et al., 1995*), and or less feed wastage *(Calet, 1965, Savory, 1974).* Because chicks fed pelleted diets spend less time and energy feeding, they were less active than mash-fed birds (*Nir et al., 1994*), and so spend less energy for maintenance.

Chemical Methods

The other method that has been used to reduce feed intake in broilers is the use of chernicals or phamacological agents. It has an advantage of equally distributing the feed among flock and so decreasing the variations in growth than can take place with physical feed restriction. Restriction of feed intake of broiler chickens by chemical methods was suggested by *Fancher and Jensen (1988).* Also *Pînchasov and Jensen (1989)* used 1.5 or *3%* glycolic acid as an anorectic agent from 7 to 14 days in order to suppress the feed intake of chicks. Feed intake was severely reduced, resulting in 22% and 50% weight reduction with 1.5% or 3.0% glycolic acid inclusion respectively. *Oyawoye and Krueger (1990)* showed that 400 and 300 mg of phenylpropanolamine hydrochloride or monensin sodium per kg of diet, respectively, significantly decreased body weight of the broiler chickens at 4 weeks of age. *Pinchasov and Elmaliah (1994)* used of 1 or 3% of acetic and propionic acids in the diet and found that weight gains of chemically restricted birds were close to those obtaining under a recommended program of quantitative feed restriction for female broiler breeders between 2 to 6 weeks of age. *Savory et al.(1996)* used of 50g/kg of calcium propionate as an appetite suppressor and showed that weight gains of chemically restricted birds were close to those

obtaining under a recommended program of quantitative feed restriction for female broiler breeders between 2 to 6 weeks of age.

Effect of feed restriction on carcass quality and production efficiency

The use of total feed restriction at an early age to elicit compensatory growth, improved feed efficiency and reduced abdominal fat pad has received considerable attention. Researchers (*Naji et al., 2003*) suggested that physical feed restriction at early age of birds for a short period stimulated compensatory growth so that at the market age feed restricted birds performed similarly to those of the full fed groups. *Novel et al. (2009)* also reported that early period 75% *ad libitum* restriction feeding gave an economic advantage over *ad libitum* feeding mainly by enhancing feed utilization and able to attain. But feed restriction can exert negative effects on the body weight at marketing age (*Pinchasov and Jensen, 1989*) and on the relative weight of breast muscle (*Reece et al., 1986). Plavnik and Hurwitz (1989)* used a severe feed restriction program at 6 to 7 days of age for a one-week period in birds and indicated the birds were much reduced in weight by two weeks of age, as compared to the control birds, but they body weights in market age were equal, feed efficiency was improved. *Osbourn and Wilson(1960)* showed compensatory growth in poultry, following a period of growth retardation by early feed restriction. Weight compensation by 42 days of age, *Plavnik and Hurwitz 1985,1990), McMurtry (1989),* and *Pinchasov and Jensen (1992)* reported that birds subjected to feed restriction compensated for BW upon resumption of *ad libitum* feeding. On the other hand, *Yu et al. (1990)* and *Fattori et al. (1991)* indicated the ineffectiveness of feed restriction in chickens. *Yu et al. (1990),* in an experiment conducted on chicks in which restriction started after 1wk of age and through d 14, reported that after refeeding *ad libitum*, no compensatory growth was observed. Other researchers (*Osbourn and Wilson., 1960; Summers et al.,1990*) observed that even though feed-restricted birds had lower fat content in their carcass, they showed similar feed efficiency as those birds fed *ad libitum*. Many contradictory results concerning body fat deposition are also seen in the literature. Feed restricted birds have been shown to have lower carcass fat content at market age than birds fed *ad libitum* (*Cherry et al,1978; Washburn and Bondari, 1978*) However, in recent reports *Fontana et al.,1992 and Scheideler and Baughman (1993)* observed no effect of feed restriction regimens on carcass fat content. *Sizemore and Siegel (1993)* tested the effects of early energy restriction, while keeping protein and other nutrients constant, on different female broiler crosses. According to study of *Zubair and Leeson (1994),* most weight loss during early feed restriction in birds can be normally compensated by 20 to 25 d of the refeeding period. *Zorrila et al. (1993),* observed a linear increase in body weight gain when diet energy levels were increased. On the other hand, a linear decrease in carcass weight and breast meat yield was observed with birds fed both protein

and energy deficient diets. These results suggested that birds can grow quite well on low energy diet but a period of 7 days is necessary to adjust their feed intake (*Leeson et al.,1996). Babu et al. (1986)* reported comparable feed intake, weight gain and feed: gain ratio for broiler chickens subjected to low crude protein diets compared with those on higher crude protein diets. In contrast, *Plavnick and Hurwitz (1990)* reported that broiler chickens fed low crude protein diets gained the least body weight and did not recover the body weight as measured at 56 days of age. *Onbasilar et al. (2009)* observed that 4 h daily feed removal had no significant effects on body weight, feed intake, feed efficiency, and carcass characteristics. The study of *Fanooci and Torki (2010)* showed that no significant difference in the overall FCR (9-49 d) between chicks fed the restricted and non-restricted control diet, except for chicks fed on 20% restricted diet that had the highest FCR during the experiment. It was concluded that dietary inclusion of wood caracole up to 10% to restrict broiler diets would not have deleterious effect on performance of broiler chicks with no adverse effect on abdominal fat and visceral and carcass measurements. Improved meat quality attracts more and more attention from consumers, and excessive fat deposition is one of the important factors of poor meat quality of broilers. Some studies have shown that feed restriction could decrease fat content and increase protein deposition in carcasses, thus resulting in the improved carcass composition (*Jones and Farrell, 1992; Nielsen et al., 2003*). However, a lot of research has failed to reduce fat with feed restriction (*Zubair and Leeson, 1996; Lippens et al., 2000*). Variability in response to a period of undernutrition likely relates to the vast range of techniques used to impose growth regulation. *Osbourn and Wilson (1960)* conclude that compensatory growth following undernutrition was influenced by duration, timing, and severity of undernutrition, together with realimenation nutrition.

Conclusion

In general, the potential of feed restriction programs as a management's tool, related to decreasing the carcass fat deposition, reduce maintenance requirements and improvement of feed efficiency in broiler chickens production. Also can be lead to economical saving in cost of feeding in broiler chicken production, thus may be usefulness for commercial broiler chicks production farms.

Poboljšanje efikasnosti proizvodnje i kvaliteta trupa kroz programe ograničenja ishrane brojlerskih pilića

M. Sahraei

Rezime

Osnovni cilj industrije proizvodnje brojlera je proizvodnja visoko kvalitetnih trupova pilića koji će biti prihvatljivi za potrošače. Prihvatljivost zavisi od kvaliteta i kvantiteta jestivih delova trupova, i količine mišićne mase u polutkama. Strategija ishrane u uzgoju pilića treba da bude proizvodnja životinja sa maksimalnim prinosom mesa, najvišom konverzijom hrane i maksimalnom telesnom težinom. Kontinuirana genetska selekcija i poboljšanje ishrane doveli su do veoma brzog rasta savremenih sojeva. Taj brz porast u ranom period života je praćen brojnim problemima, kao što su taloženje masti u telu, visoka učestalost metaboličkih poremećaja, visok mortalitet, i visoka učestalost oboljenja kostiju. Program ograničenje ishrane je jedan od glavnih tehnika manipulacije krive rasta za povećanje efikasnosti proizvodnje brojlerskih pilića i ublažavanja pojave nekih metaboličkih bolesti i može da se koristi za proizvodnju mesnatijih pilića čime će se smanjiti negativni uticaj masti na zdravlje, a manje taloženje masti u trupovima brojlerskih pilića može biti profitabilno u proizvodnji brojlera. Ovaj rad predstavlja nova saznanja u vezi sa efektima različitih programa ograničenja ishrane na efikasnosti proizvodnje i kvalitet trupa brojlera.

References

ACAR N., MORAN E.T., J.R., BILGILI, S.F. (1991) Live performance and carcass yield of male broilers from two commercial strain crosses receiving rations containing lysine below and above the established requirement between six and eight weeks of age. Poult. Sci.70: 2315-2321.

ACAR N., SIZEMORE F.G., LEACH, G.R., WIDEMAN, R.F., OWEN R.L., BARBATO G.F. (1995) Growth of broiler chickens in response to feed restriction regimens to reduce ascites. Poult. Sci. 74 : 833-843.

ANTHONY, N.B.(1998) A review of genetic parameters in poultry: Efforts to improve meat quality. J. Muscle Foods. 9:25-33.

APELDOORN EJ, SCHARAMA JW, MACHALY MM, PARMENTIER HK(1999) Effect of melatonin and lighting schedule on energy metabolism in broiler chickens. Poult.Sci. 78: 223-229.

ARCE J, M BERGER CL, COELLO (1992) Control of ascites syndrome by feed restriction techniques. J. Appl. Poult. Res. 1:1–5.
ANDREWS J. (1991) Pelleting: review of why, how, value, and standards. Poult. Dig. 50: 64-71.
BALLAY, M., DUNNINGTON, E.A., GROSS, B.W., SIEGEL P.B. (1992) Restricted feeding and broiler performance: age at initiation and length of restriction. Poult. Sci. 71: 440-447.
BENSCHOP, D. (2000) Compensatory growth in ruminants - an overview. Proceedings of the 2000 Course in Ruminant Digestion and Metabolism ANSC 6260. University of Gulph, Ontario,p. 1-16.
BLAIR R., R.C. NEWBERRY, GARDINER E. E. (1993) Effects of lighting pattern and dietary tryptophan supplementation on growth and mortality of broilers. Poult. Sci. 72:495–502.
BOWES V.A., JULIAN R.J., JULIAN L.S., STIRTZINGER .L, STIRTZINGER T. (1988) Effect of feed restriction on feed efficiency and incidence of sudden death syndrome in broiler chickens. Poult. Sci., 67(7): 1102-1104.
BUYSE J, DECUYPERE E. (1988) The influence of intermittent light on broiler performance and on patterns of food intake. Pages 133-134 in: Leanness in restrice Birds. Genetics, Metabolic and Hormonal Aspects.
BUYSE J., E. DECUYPERE, MICHELS H. (1994) Intermittent lighting and broiler production. 1. Effect on female broiler performance. Archive fur Geflugelkunde, 58: 69-74.
BUYSE J., R KUHN DECUYPERE E. (1996) The use of intermittent lighting in broiler raising. 1. Effect on broiler performance and efficiency of nitrogen retention. Poult. Sci.75:589-594.
BUYS N., J. BUYSE, M. HASSANZADEH-LADMAKHI, DECUYPERE E. (1998) Intermittent lighting reduces the incidence of ascites in broilers: an interaction with protein content of feed on performance and the endocrine system. Poult. Sci. 77:54-61.
CABEL M.C., WALDROUP P.W. (1990) Effect of different nutrient-restriction programs early in life on broiler performance and abdominal fat content. Poultry Science 69:652-660.
CALET C. (1965) The relative value of pellets versus mash and grain in poultry nutrition. World Poult. Sci.J., 21: 23-52.
CHERRY J.A., P.B. SIEGEL BEANE W.L. (1978) Genetic-nutritional relationships in growth and carcass characteristics of broiler chickens. Poult. Sci. 571482-1487.
CLASSEN H.L., RIDDELL C. (1990) Early growth rate and lighting effects on broiler skeletal disease. Poult. Sci.69(Suppl. 1):35. (Abstr.)
COON C.N., BECKER W.A., SPENCER J.V. (1981) The effect of feeding high energy diets containing supplemental fat on broiler weight gain, feed efficiency, and carcass composition. Poultry Science 60, 1264-1271.

CORZO, A., KIDD, M.T. (2004) Starter dietary lysine level and strain cross effects on performance and carcass traits of broiler females. Revista Brasileira Ciencia Avicola. Vol.6,no.2, p.93-97. ISSN 1516-635X.
DEATON, J.W. (1995) The effect of early feed restriction on broiler performance. Poult. Sci. 74:1280-1286.
DICKERSON, G.E. (1978) Animal size and efficiency: basic concepts. Anim. Prod. 27367-379.
DOZIER W.A., R.J. LIEN, J.B. HESS, S.F. BILGILI, R.W. GORDON, C.P. LASTER S.L. VIEIRA (2002) Effects of Early Skip-a-Day Feed Removal on Broiler Live Performance and Carcass Yield. J. Appl. Poult. Res. 11:297–303.
FANCHER B. I., JENSEN L.S. (1988) Induction of voluntary feed intake restriction in broiler chicks by dietary glycolic acid supplementation. Poult. Sci. 67:1469–1482.
FANOOCI and TORKI (2010) Effects of Qualitative Dietary Restriction on Performance, Carcass Characteristics,White Blood Cell Count and Humoral Immune Response of Broiler Chicks Global Veterinaria 4 (3): 277-282.
FATTORI, T. R., H. R. WILSON, R. H. HARMS, R. D. MILES (1991) Response of broiler breeder females to feed restriction below recommended levels. 1. Growth and reproductive performance. Poult. Sci. 70:26–36.
FONTANA, E.A., WEAVER, W.D., WATKINS, JR., B.A., DENBOW, D.M (1992) Effect of early feed restriction on growth, feed conversion, and mortality in broiler chickens. Poult. Sci. 71:1296 – 1305.
FISHER, C. (1984) Fat deposition in broilers pp. 437-470 in: Fats in animal nutrition. J. Wiseman, ed. Proc. Easter School in Agricultural Science, University of Nottingham (37th),Butterworth, London, U.K.
FONTANA, E.A., WEAVER, W.D., WATKINS, JR.B.A., DENBOW, D.M. (1992) Effect of early feed restriction on growth, feed conversion, and mortality in broiler chickens. Poult. Sci.71:1296 – 1305.
GEORGE, Q. (2007) Reduction of Early Mortality in Broiler Chickens through Nutrition and Management:Champion Feed service limited: www.championfeeds.com, www.championfeeds.com., pp. 1-2.
GIACHETTO, P.F., GUERREIRO, E.N., FERRO, J.A., FERRO, M.I.T., FURLAN, R.L., MACARI, M. (2003)Desempenho e perfil hormonal de frangos alimentados com diferentes niveis energeticos apos restrição alimentar. Pesquisa Agropecuaria Brasileira 38(6): 697-702.
GONZALES E., BUYSE, J., LODDI, M.M., TAKITA, T.S., BUESE N., DECUYPERE, E. (1998) Performance,incidence of metabolic disturbances and endocrine variables of food-restricted male broiler chickens. Br.Poult. Sci. 39:671-678.
GRIFFITHS, L., LEESON, S., SUMMERS, J.D. (1977) Fat deposition in broilers: Effect of dietary energy to protein balance and early life caloric restriction on productive performance and abdominal fat pad size. Poult. Sci. 56: 638-646.

JANG, I. S., S. Y. KANG, Y. H. KO, Y. S. MOON, S. H. SOHN (2009) Effect of Qualitative and Quantitative Feed Restriction on Growth Performance and Immune Function in Broiler Chickens Asian-Aust. J. Anim. Sci. Vol. 22, No. 3 : 388 - 395

JONES, G.P.D., FARRELL, D.J. (1992)Early life food restriction of the chicken. I. Methods of application, amino acid supplementation and the age at which restriction should commence. Br.Poult. Sci. 33:579 - 587.

JONES, F.T., ANDERSON, K.E., FERKET, P.R. (1995) Effect of extrusion on feed characteristics and broiler chicken performance. J. Appl. Poult. Res. 4:300-309.

JULIAN, R.J. (2000) Physiological management and environmental triggers of the ascites syndrome. Avian Pathol. 29:519–527.

HAMILTON, R. M. G., PROUDFOOT, F. G. (1995) Ingredient particle size and feed texture: effects on the performance of broiler chickens. Anim. Feed Sci. Technol. 51: 203–210.

HAVENSTEIN, G. B., P. R. FERKET, S. E. SCHEIDELER, B. T. LARSON (1994) Growth,livability, and feed conversion of 1957 vs 199 1 broilers when fed Typical"1957 and 1991 broiler diets. Poultry Sci. 73: 1785-1794.

KESSLER, A.M., SNIZEK, JR.P.N., BRUGALLI, I. (2000) Manipulação da quantidade de gordura na carcaça de frangos. In: Anais da Conferência APINCO de Ciência e Tecnologia Avícolas. APINCO, Campinas, SP, Brazil, pp. 107-133.

KYRIAZAKIS, I., STAMATARIS, C., EMMANS, G.C., WHITTEMORE, C.T. (1991) The effects of food protein content on the performance of pigs previously given foods with low or moderate protein contents. Anim. Prod. 52: 165-173

LEESON, S., SUMMERS, J.D., CASTON, L.J. (1991) Diet dilution and compensatory growth in broilers. Poult. Sci. 70:867-873.

LEESON, S., CASTON, L., SUMMERS J.D. (1996) Broiler response to energy or energy and protein dilution in the finisher diet. Poult. Sci. 75: 522-528.

LEESON, S., ZUBAIR, K. (1997) Nutrition of the broiler chicken around the period of compensatory growth. Poult. Sci. 76: 992-999.

LEESON, S., SUMMERS, D.J., CASTON, L.J. (1992) Response of broilers to feed restriction or diet dilution in the finisher period. Poult. Sci. 70:867-873.

LEESON, S. (1996) Nutrition and Broiler Carcass Quality. Hubbard Farms Technical Report.

Lee, K. H., and S. Lesson (2001) Performance of broilers fed limited quantities of feed or nutrients during seven to fourteen days of age. Poult. Sci. 80:446–454.

LEWIS, P.D., MORRIS, T.R. (1998) Responses of domestic poultry to various light sources. Word's Poult. Sci. J. 54:72-75.

LIPPENS, M., G. ROOM, G. DE GROOTE, E. DECUYPERE (2000) Early and temporary quantitative food restriction of broiler chickens. 1. Effects on performance characteristics, mortality and meat quality. Br. Poult. Sci. 41:343–354.

MALLARD, J., M. DOAPIRE (1988) Strate ‘es of selection for leanness in meat production. Pages 8~in :Leanness in Domestic Birds. Genetics, Metabolic and Hormonal Aspects. Leclercq and C.C. Whitehead, ed.Buttemrths, London, UK.
MARKS, H. L. (1979) Growth rate and feed intake of selected and nonselected broilers. Growth 43:80-90.
MCMURTRY, J. P., ROSEBROUGH, R.W., PLAVNIK, I. CARTWRIGHT, A.I. (1988) Influence of early plane of nutrition on enzyme systems and subsequent tissue deposition. pp. 329-341In: Bio-mechanisms Regulating Growth and Development (G. L. Steffens and T. S. Rumsey,ed). Betsville Symposia on Agricultural Research, Klumer Academic Publishers, Dordrecht, the Netherlands.
MOLLISON, B., W. GUENFTR, B.R BOYCOTT (1984) Abdominal fat defintion and sudden death -syndrome in broilers: the effect of restricted intake, early caloric fat restriction, and calorie:protein ratio. Poult.Sci. 63:1190-1200.
MORRIS, T.R. (1986) Light requirement of the fowl. In: environmental control in poultry production. Carter, T.C. (Ed), Edinburgh, Oliver and Boyod, pp. 15-39.
NRC (1994) Nutrient Requirements of Poultry. 9th Revised edition, National Academy Press, Washington, DC.
NEWCOMBE, M., J.D. SUMMERS (1984) Effect of pervious diet on feed intake and body weight gain of broiler and Leghorn chicks. Poult. Sci. J., 63:1237-1242.
NIR, I., R. HILLEL, I. PTICHI, G. SHEFET (1995) Effect of particle size on performance. 3. Grinding pelleting interactions. Poult. Sci. 74:771–783.
NIR, I., NITSAN, Z., DUNNINGTON, E.A., SIEGEL, P.B. (1996) Aspects of food intake in young domestic fowl: Metabolic and genetic considerations. World Poult. Sci.J. 52: 251-266.
NIELSEN, B. L., M. LITHERLAND, F. NØDDEGAARD (2003) Effect of qualitative and quantitative feed restriction on the activity of broiler chickens. Appl. Anim. Behav. Sci. 83:309–323.
NOVELE DJ, NG’AMBI JW, NORRIS D, MBAJIORGU CA (2009) Effect of different feed restriction regimes during the starter stage on productivity and carcass characteristics of male and female Ross 308 broiler chickens. Int. J. Poult. Sci., 8(1): 35-39.
ONBASILAR, E. E., YALCIN, S., TORLAK, E. OZDEMIR, P. (2009) Effects of early feed restriction on live performance, carcass characteristics, meat and liver composition, some blood parameters, heterophile lymphocyte ratio, antibody production and tonic immobility duration. Trop. Anim. Health and Prod. 41: 1513-1519.
OLANREWAJU, H.A., THAXTON, J.P., DIZIER W.P., PURSUEL, J., ROUSH, W.B., BRANTON, S.L. (2006) A review of lighting programs for broiler production. International Journal of poultry Science 5(4): 301-308.
OSBOURN, D.F., P.N. WILSON (1960) Compensatory growth after undernutrition in mammals and birds. Bio. Rev. 35:325 - 3633.

OYEDEJI, J.O. ATTEH, J.O (2005) Response of broilers to feeding manipulations. Inter. J. of Poult. Sci. 4(2): 91-95.
OYAWOYE, E.O., KRUEGER, W.F. (1990) Potential of chemical regulation of food intake and body weight of broiler breeder chick. Br. Poult. Sci. 31:735-742.
PASTERNAK, H., B.A. SHALEV (1983) Genetic economic evaluations of traits in a broiler enterprise: reduction of food intake due to increased growth rate. Br. Poult. Sci. 24531-536.
PINCHASOV, Y., JENSEN, L.S. (1989) Comparison of physiological and chemical means of feed restriction in broiler chicks. Poultry Science 68:61-69.
PINCHASOV, Y., ELMALIAH, S. (1994) Broiler chick responses to anorectic agents: 1.dietary acetic and propionic acids and the digestive system. Phar. Bioch. Behavior. 48: 371-376.
PLAVNIK, I., HURWITZ, S. (1985) The performance of broiler chicks during and following a severe feed restriction at an early age. Poultry Science 64:348 – 355.
PLAVNIK, I., MCMURTRY, J.P., ROSEBROUGH, R.W. (1986) Effect of early feed restriction in broilers. I. Growth performance and carcass composition. Growth 50:68 – 76.
PLAVNIK, I., HURWITZ, S. (1988a) Early feed restriction in chicks: Effects of age, duration and Sex. Poultry Sc. 67: 1407-1413.
PLAVNIK, I., HURWITZ, S. (1988b) Early feed restriction in male turkeys: Growth pattern, feed efficiency and body composition. Poultry Sci.67: 1407-1413.
PLAVNIK, I., HURWITZ, S. (1989) Effect of dietary protein, energy and feed pelleting on the response of chicks to early feed restriction. Poult. Sci. 68:1118 – 1125.
PLAVNIK, I., HURWITZ, S. (1990) Performance of broiler chickens and turkeys poults subjected to feed restriction or to feeding of low sodium diets at an early age. Pout. Sci. 69(4)945-952.
PLAVNIK, I., HURWITZ, S. (1990) Performance of broiler chickens and turkeys poults subjected to feed restriction or to feeding of low-protein or low-sodium diets at an early age. Poult. Sci. 69:945-952.
PLAVNIK, I., HURWITZ, S. 1991) Response of broiler chickens and turkey poults to foodrestriction of varied severity during early life. Br. Poult. Sci. 32:343 – 352.
REECE, F.N., B.D. LOLT, J.W. DEATON, S.L BRANBN (1986) Meal feeding and broiler performance. Poult. Sci. 65:1497-1501.
REDDY, C.V. (2000) Maintaining growth and production. Poult. Inter. 39(2): 36-40.
RELIGIOUS, K.B., TESSERAUD. S., PICCADY, O. A. (2001) Food neonatale and early development of table fowl. 2001, INRA. Production. Animal., 14, 219-230.
ROBINSON, F. H L CLESSEN, J.A HPNSON, AND D.K. ONDERKP (1992) Growth performance, feed efficiency and the incidence of skeletal and metabolic

disease in fa-fed and feed restricted broiler and roaster chickens. J. Appl. Poultry Res. 1:33-41.
ROSEBROUGH, R.W., MCMURTRY, J.P. (1993) Energy repletion and lipid metabolism during compensatory gain in broiler chickens. Growth Dev. and Aging. 57:73-83.
RYAN, W. J., WILLIAMS, I. H., MOIR, R.J. (1993) Compensatory growth in sheep and cattle.1. Growth pattern and feed intake. Aust. J. Agric Res. 44: 1623-1633.
SAHRAEI, M F SHARIATMADARI (2007) Effect of different levels of diet dilution during finisher period on broiler chickens performance and carcass characteristics. Int. J. Poult. Sci., 6(4): 280-282.
SAHRAEI, M. MOHAMMADI, M.H. (2012)Effect of Physical Feed Restriction in Finisher Period on Carcass Traits and Broiler Chickens Performance Global Veterinaria 9 (2): 201-204, 2012
SALEH, E.A., WATKINS, S.E., WALDROUP, A.L., WALDROUP, P.W. (2005) Effects of early quantitative feed restriction on live performance and carcass composition of male broiler grown for further processing. Journal of Appl. Poult. Res. 14:87-93.
SANTOSO, U., TANAKA, K. OHTANI, S. (1995) Early skip-a-day feeding of female broiler chicks fed high-protein realimentation diets. Performance and body composition. Poult. Sci. 74:494-501.
SAVORY C.J. (1974) Growth and behavior of chickens fed on pellets or mash.Br. Poult. Sci. 15:281-286.
SCOTT, M.L., NESHEIM, M.C., YOUNG R.J. (1982) Nutrition of the chicken. Third edition, M.L. Scott and Associates, publishers Ithaca, New York. pp1-406
SCHEIDELER, S.E. BAUGHMAM, G.R. (1993) Cumputarized early feed restriction programs for various strains of broilers. Poult. Sci 72: 236-242.
SIZEMORE, F. G., H.S. SIEGEL (1993) Growth, feed conversion, and carcass composition in females of four broiler crosses fed starter diets with different energy levels and energy to protein ratios. Poult. Sci. 72:2216–2228.
SUMMERS, J.D., S. LEESON (1985) Poultry Nutrition Handbook. Department of Animal and PoultryScience, Ontario Agricultural Science, Guelph, Ontario.
SUMMERS, J.D., SPRATT, D. ATKINSON, J.L. (1990) Restricted feeding and compensatory growth for broilers. Poult. Sci. 69:1855-1861.
SUMMERS, J.,D., D. SPRATT, J.L ATKINSON (1990) Restricted feeding and compensatory growth for broilers. Poultry Sci. 69:855-1861.
SUSBILLA, P.J., FRANKEL, T.L., PARKINSON, G., GOW, C.B. (1994) Weight of internal organs and carcass yield of early food restricted broilers. Br. Poult. Sci. 35:677-685.
WALKER, A. W., J. WISEMAN, N. J. LYNN, D. R. CHADES, (1995) Recent findings on the effects of nutrition on the growth of specific broiler carcass

components. In: Recents advances in animal nutrition. Nottingham University Press. Nottingham, England,
WASHBURN, KW., K. BONDARI (1978) Effects of timing and duration of restricted feedin on compensatory growth in broilers. Poultry Sci. 5710?3-1021.
WILLIAMS, B., S. SOLOMON, D. WADDINGTON, B. THORP, C.FARQUHARSON (2000) Skeletal development in the meat-type chicken. Br. Poult. Sci. 41:141–149.
WILSON, L.J., WEAVER, JR, W.D., BEANE, W.L., CHERRY, A. (1984) Effects of light and feeding space on leg abnormalities in broilers. Poult. Sci. 63:565-567.
WILSON K.J., BEYER R.S. (2000) Poultry Nutrition Information for the Small Flock. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. http://www.oznet.ksu.edu
WILSON, M. (2005) Production focus (In; Balancing genetics, welfare and economics in broiler production). Vol 1 (no. 1). pp 1. Publication of Cobb-Vantress, Inc.
WINICK, M., NOBEL, A. (1966) Cellular response in rats during malnutrition at various ages. J. of Nutr. 89:300 – 306.
YU, M.E., ROBINSON, F.E., CLANDINI, M.T. BODNAR, L. (1990) Growth and body composition of broiler chickens in response to different regimes of feed restriction. Poult.Sci. 69(12): 2074-2081.
YU, M.E. AND ROBINSON, F.E. (1992) The application of short-term feed restriction to broiler chicken production: A review. J. of Appl. Poult. Res.1:147 – 153.
ZUBAIR, A.K. AND LEESON, S. (1996) Compensatory growth in the broiler chicken: a review. World's Poult. Sci. 52:189-201.
ZORRILA, F.F., CUCA, M.G. AND ÁVILA, E.G. (1993) Efecto de niveis de energia, lisina y proteina endietas para pollos de engorda en iniciacion. Veterinaria Mexico, Mexico. 24: 311-316.

Received 10 May 2013; accepted for publication 15 June 2013

Biotechnology in Animal Husbandry 29 (2), p 211-222 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 619:616.24'632
DOI: 10.2298/BAH1302211J

IMMUNOSUPPRESSION – POSTPARTUM DISEASES OF DAIRY COWS

M. Joksimović Todorović, V. Davidović

University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: miratodo@agrif.bg.ac.rs
Review paper

Abstract: Periparturient period in dairy cows is accompanied by numerous physiological, metabolic and nutritive changes. The way in which they occur and develop have a great influence on lactation performances, subclinical and clinical postparturient diseases and reproductive disorders, thus affecting the profitability as well. During periparturient period dairy cows may subject to the immune system function disorders. This immunosuppression leads to increased number of severe cases of infections (mastitis, metritis, retained placenta and other health disorders). The cause of periparturient immunosuppression is unknown, although it is a subject of numerous studies which suggest that immune disfunction may be provoked by both metabolic and endocrine factors. Numerous authors suggest that negative energy balance, non-esterified fatty acids (NEFA), ketone bodies and hypocalcaemia play a significant role in the development of immunosuppression. It is well known that glucocorticoids exert an immunosuppressive action. The changes in the levels of estradiol and progesterone may have either a direct or indirect influence on immune defence. However, their effect on the development of this immunofunction is not long, since the changes in their concentrations are short-lasting. Some nutrients are involved in the function of immune cells thus minimizing the inflammatory damages, due to more efficient immune response. Numerous tissue damages during the infection are possible, as a consequence of oxidative stress – reaction of unstable oxidised molecules with tissue lipids, proteins and DNA. A great number of antioxidants are important for animal health, however, optimal concentrations and forms of these supplements for maximizing the immune function and tissue protection are still unknown.

Key words: dairy cows, immunosuppression, oxidative stress, antioxidants-application, post-parturient diseases.

Introduction

During periparturient period in dairy cows, three weeks prior to and three weeks after calving the reduction in immune functions is perceived. The occurred immune disfunction results in the increased number of infections and their severity in this period (*Waldron, 2010*). The cause of this immune disfunction is unknown, however numerous factors may be involved. Metabolic disorders and change in the level of some hormones (glucocorticoids, estradiol and progesterone) most likely affect the decrease in immune function (*Waldron and Revelo, 2008*). Glucocorticoids, are known as immunosuppressors, and since their level increases in the calving period they are partially responsible for periparturient immunosuppression. The changes in the level of estradiol and progesterone at calving may have both direct and indirect effect on immune response (*Weber et al., 2001*). The changes in the level in any of these hormones do not match with an entire period of immunosuppression, suggesting that some other factors may also be responsible for its incidence.

The infections of mammary gland (mastitis) and uterus (metritis) are common in dairy cows during periparturient period. Other health disorders that occur in this period are milk fever and ketosis. Although the etiologies of infections and metabolic disorders are different, there is a significant relationship between their activities. In cows infected by milk fever, clinical mastitis is observed five times more in comparison with the cows that have no such health problem (*Curtis et al., 1985*).

During inflammatory diseases, immune cells produce reactive oxygen species (ROS) (*Sordillo and Aitken, 2009*). Leukocytes use these toxic components as a part of arsenal when destroying the pathogens. However, those same molecules can also cause the damage of mammary tissue. Although very efficient against pathogenes an unfavourable feature of these oxidants is non-selectivity when there is a case of the „damaging“ of cells, thus being frequent and significant damage of some tissues. This happens when there is a disbalance between the increased production of ROS and available antioxidative defence, necessary to reduce ROS accumulated during periparturient period. In this period the cows are exposed to strong oxidative stress. Previous studies indicated the importance of oxidative stress in etilogy of diseases in dairy cows. The studies made in the last ten years support the concept that oxidative stress is a significant factor in the development of disfunction of animal immunity and inflammatory response and that it may increase the sensitivity of individuals to different health problems, particularly during the transitional period (*Sordillo, 2005; Wilde, 2006*).

Selenium and E vitamin are the most studied antioxidants in relation to the health status in young cows, since their addition into the rations during

periparturient period can reduce the severity and duration of disease occuring in this period.

Metabolic and nutritional challenges of periparturient dairy cows

The research in the field of both human and veterinary medicine has indicated a close relation between nutrition, inflammation and sensitivity to ailments (*Calder, 2008; Wood et al., 2009*). Certain physiological changes occuring in cows during transitional period may affect the nutritive status and contribute to the increase in sensitivity to some diseases. In this period the needs for energy are elevated, provoked by the beginning of lactation and reduced intake of dry matter, what leads the individual into the state of negative energy balance. Approximately 85% glucose goes directly into the mammary gland for the synthesis and secretion of milk. Moreover, the need for aminoacids increases, because the liver utilizes them as gluconeogenetic substrate, what results in mobilizartion of protein from skeleton muscles.The consequence of negative energy balance is the mobilisation of fats from body depos and departure of unsaturated fatty acids into the blood. Continued lipolysis results in transformation of NEFA into triglycerides, and their accumulation in liver may result in the disease called "fatty" liver. In recent years the importance of NEFA for inflammatory response has been pointed out, although the major mechanisms of their effects are still the subject of research (*Sordillo et al., 2009*). It is thought that there are several mechanisms by which NEFA can regulate inflammatory response. Fatty acids may be built into membrane lipids and affect some important cell functions (*Martins de lima et al., 2007*).

Effects of metabolic challenges on immunocompetence

Negative energy balance in dairy cows may affect the creation of immunosuppression (*Waldron, 2010*). However, in cattle, experimentaly induced energy balance in individuals has a small effect on the creation of adhesive molecules on the surface of leukocytes (*Perkins et al., 2001*). Some studies suggest that nutritive balance and metabolic disorders have no strong influence on the creation of immunosuppression but that some other changes are likely to be responsible for this immune disfunction. In contrast to these opinions, some researchers suggest that certain components are connected with negative energy balance concluding that only hypoglycaemia has no influence on the incidence of immunosuppression (*Nonnecke et al., 1992*), while hyperketonemia has a multifold negative effect on immune functions (*Suriyasathaporn et al., 2000*). Ketosis may increase the danger of the incident of mastitis in cows with immunosuppression, because immune cells suffer negative effects and in this way the defence

mechanisms of the mamary gland are weakened. In cows having ketosis blastogenesis of lymphocytes is in supremacy, the activity of respiratory burst is reduced along with neutrophils chemotaxic ability, and also, the titre of γ-interferon and TNF-α in aorta endotheliumel cells in cattle are reduced as well. Certain studies suggest that calcium metabolism may affect the immunecompetence. Significant quantities of calcium are necessary for the synthesis of milk and start of lactation, therefore the hypocalcemia is frequent in dairy cows. Calcium is important also for intracellular metabolism and a signal ion for numerous cells including leukocytes so that the low level of calcium in calving period may contribute to the incidence of immunosuppression. *Kimura et al. (2006)* said that the low level of calcium content in mononucleus leukocytes may lead to immunosuppression. The authors state that the level of intracellular calcium is a more sensitive indicator of metabolic stress than the level of calcium in blood.

Oxidative stress

Oxidative stress is also an important factor which provokes disfunction of inflammatory response in metabolic stress in cows during periparturient period (*Sordillo and Aitken, 2009*). It happens when reactive oxygen species (ROS) are present in higher concentration, while enzyme and non-enzyme antioxidants are deficient. The increased generating of ROS during periparturient period results in spending important antioxidative defence mechanisms (*Gitto et al., 2002*). Reactive oxygen species are primarily formed as the end products of mitochondrial respiratory chain or by the activation of NADPH oxidase (*Sordillo and Aitken, 2009*). The most important ROS forms are: superoxide anion ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2) and the most reactive hydroxil radical ($OH^{\cdot}$). Great quantities of superoxide anions and hydrogen peroxide are produced during oxidative burst, when the phagocyte cells are stimulated by NADPH oxidase for destroying pathogenic agents (*Valko et al., 2007*). Hydroxil radical breaks DNA chains, modifies purine and pirimidin bases and molecules of desoxiribosis. DNA oxidative damages lead to transcription changes and permanent damages in humans, however, such an influence in dairy cows is unknown. Oxidative stress in dairy cows during transitional period is connected with a significant increase of lipid peroxidation (*Castillo et al., 2006*).

Physiological stress is associated with very rapid differentiating of secretory parenchyma, intensive growth of mammary gland, onset of abundant synthesis and secretion of milk, high energy requirements and the need for oxygen (*Gitto et al., 2002*). Increased needs for oxygen lead to the conditions which are the basis for ROS generating. For that reason the oxygen which is essential for aerobic organisms can be also called “oxygen paradox”. Production of free radicals is

controlled by antioxidative defense system. Antioxidants can delay, prevent or remove oxidatively damaged target molecules (*Halliwell and Gutteridege, 2007*). Antioxidative defence system can be divided into enzyme (superoxide dismutasis (SOD), gluthatione peroxidase (GSH-Px) and catalase and non-enzyme mechanisms (vitamin E and selenium) (*Sharma et al., 2011*). Abundant production of free radicals and ROS weakens the defence, leads to damage of macro molecules and disorder of metabolic processes (*Trevisan et al., 2001; Joksimović Todorović and Davidović, 2007*). When ROS is produced faster than it can be neutralised by antioxidants, the result thereof is oxidative stress.

Antioxidants - application

It is thought that Se and vitamin E are the key ingredients of food performing oxidative protection. Vitamin E is an integral component of all lipid membranes, it acts inside them and neutralises free radicals, as well as those produced by lipid peroxidation. It represents the first line of cellular defence against free radicals.

Selenium performs its biological role in organism through enzyme gluthatione peroxidase (GSH-Px). The activity of this enzyme depends on the level of selenium in food, what can be used as a reliable indicator of selenium biological available (*Mihailović et al., 1991*). However, at certain concentrations of selenium, the activity of GSH-Px reaches plateau, so that further increase of the selenium level results in no increase of the activity of this enzyme. High selenium levels added into food (> 5 mg/kg) lead to no linear increase of the activity of this selenoenzyme. In the first days the selenium activity increases in animal blood plasma, but only 10 days later a significant fall occurs therein (*Joksimović Todorović and Jokić, 2005; Joksimović Todorović et al., 2005*).

In the nutrition of domestic animals the organic selenium in the form of selenomethionine (present in plants in different concentrations) and non-organic selenium in the form of sodium selenite or selenate are used. The difference between these two forms of selenium is in metabolic path and efficiency of action. High levels of selenite are more toxic than the same levels of organic selenium (*Mihailović et al., 1996a,b; Mihailović et al., 1997; Todorović et al., 1999*). It can generally be said that adequate selenium levels in food are crucial factors in ensuring animal good health and their high productivity (*Jokić et al., 2005; Joksimović Todorović et al., 2006*).

Antioxidative status may be one of the factors which influences the reproductive functions in dairy cows. Selenium and vitamin E reduce the incidence of postparturient ailments in dairy cows – retained placenta, metritis and ovarian cysts and increase the level of conceptions. Neutrophile granulocytes in dairy cows blood contain very low catalase. Reduced function of neutrophile

granulocytes is connected with high levels of superoxide (O^-). Inadequate protection from ROS leads to reduction in the function of neutrophile granulocytes, what causes frequent diseases. Primary function of Se is to secure the immunological defence and to augment the migration of neutrophile granulocytes towards inflammatory region, where it will phagocyte and destroy present bacteria. Selenium deficiency leads to reduction in both number and disorder of functions of all cells of immune system. There are some disorders observed in maturing of lymphocytes, and in the function of mature forms as well. The activity of T lymphocytes and NK cells (killer cells) is decreased, as well as the synthesis of some antibodies, particularly IgM class. Selenium intensifies the response to antigenic stimulation, and the activity of NK cells.

Mastitis

Dairy cows are exposed to numerous genetic, physiological and external factors, which can endanger the immunity and increase the frequency of mastitis (*Sordillo, 2011*). Selection in the direction of maximum milk production enhances metabolic stress provoked by increased synthesis and secretion of milk and reduces the resistance to mastitis.

Mammary gland is protected by defence mechanisms – natural and acquired immunity. Natural immunity is dominant during various infections. It is being activated rapidly on the site of infection by numerous stimulants, but it is not enlarged by re-exposure to that same cause. If natural mechanism of defence functions adequately, many pathogenes can be easily removed in a short period. If pathogenes are not completely eliminated, then a specific immunological system is activated (*Sordillo and Streicher, 2002*).

The use of antioxidants in dairy cows reduces the duration and intensity of diseases. Accurate mechanisms of improving the health of mammary gland are not fully known, and they are brought into connection with their antioxidative functions, including cellular signals and reduction of oxidative damages of mammary tissue. Antioxidants in dairy cows enhance the resistance to mastitis, ensure phagocyte capacity of neutrophiles, increasing the hemotaxis on the place of infection (*Spears and Weiss, 2008*).

Retained placenta

Retained placenta is the most frequent disease in dairy cows perceived in 4-18% calved individuals. Rejection of placenta occurs in the period from 2-8 hours after calving. Retention of placenta longer than 12 hours represents the state of illness. Fetal placenta is regarded as an “alien tissue” and is being rejected after parturition. Numerous factors including mechanical, nutritional, management and

infectious causative agents may affect the incidence of this disease. Mechanical causal factors comprise difficult delivery, caesarean section, uterus torsion, abortion, still born calf, emphysema fetus and twinning. Nutritional causes comprise deficit of energy, protein, selenium, iodine, vitamins A and E and calcium, during pregnancy. Management causes involve stress, external factors and overweight. Incidence of retained placenta is frequent (about 50% animals) in relation to zoonoses such as brucellosis, salmonellosis, leptospirosis and listeriosis (*Gunay et al., 2011*). Certain studies show that the retained placenta is one of the major causes of endometritis in cows (*Han and Kim, 2005*). Retained placenta is the cause of reduced fertility, and therefore reduced fertility rate, more difficult conception and can prolong calving interval (*Bella and Roberts, 2007*). Selenium and vitamin E promote the function of neutrophiles, affect their migration and hemotaxic activity. These nutrients elevate the number of neutrophiles in placenta and affect the weakening of links between fetomaternal joint (cotyledon and caruncle). Elevated concentration of cortisol is a response to stress and inflammation of pregnant uterus. Cortisol reduces the function of thermophiles or hinders absolutely their activity and therefore provokes the incidence of this disease.

Abomasum dislocation

Abomasum dislocation in high productive diary cows affect the physiology of abomasum. In 95% cases abomasum moves either to left or right thoracoabdominal position. Enlarged abomasum, can also change the position in cranial direction. In that case, dislocated abomasum is placed between the reticulum and diaphragm (*Zadnik, 2003*). The studies pointed to the fact that three days before the incidence of clinical symptoms the production of milk significantly reduces. In case of left dislocation of abomasum milk production is reduced by 16.6%, while in case of right 43.5%. Animals are less active, and their body mass reduces by 15.9, ie. 22.9%. During the manifestation of clinical symptoms the milk production is reduced by 60.5% (*Antanaitis et al., 2009*).

This ailment is provoked by stress, nutritional diseases and metabolic defects. High productive dairy cows fed large quantities of cereals may have an abomasum atony. Factors which may lead to atony include metabolic diseases (hypocalcemia and ketosis), postparturient diseases (mastitis, metritis, retained placenta and milk fever). In individuals with abomasum dislocation, possible complications in ketosis and metritis are increased. In animals with left abomasum dislocation the movements of rumen are reduced to up to two to a half movement in a minute, while in some cases the rumen is atoned. Gas production is increased due to prolonged fermentation in abomasum (mostly methane and carbon dioxide are being accumulated). Defecation is considerably reduced while feces is usually small. The incidence of leukocytosis and neutrophilia in abomasum left dislocation

is regarded as an immunological response to endotoxemia provoked by this disease (*El-Attar et al., 2007*). It is thought that abomasum dislocation is connected with hemoconcentration, electrolytes imbalance, liver disorders and hypocalcemia. Therefore it is necessary, in the first 4 weeks after parturition, to maintain energy balance, electrolytes balance and normocalcemia by properly planned diet (*Mokhber Dezfouli et al., 2011*). The first lactation is highly risky what can be a consequence of poor social and nutritive adaptation.

Conclusion

Periparturition diseases in dairy cows are a source of constant concern for producers, nutritionists and vets. The researchers generally think that the immunosupression is the cause of incidence of these diseases, although there are some different viewpoints on what can cause immune disfunction. However, they all agree that rations supplemented by certain nutrients prevents the development of postparturient diseases. A proper nutritive profile ensures animal metabolic soundness and it seems to be the best strategy in preserving and improving the immune functions. Moreover, hygiene and minimizing the stress are the most important in prevention of diseases in dairy cows.

Acknowledgment

This work was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, project TR 31086: Optimisation of technological procedure and zootechnical resources on farms with the aim of improving the sustainability of milk production.

Imunosupresija – postpartalna oboljenja mlečnih krava

M. Joksimović Todorović, V. Davidović

Rezime

Peripartalni period kod mlečnih krava prate brojne fiziološke, metaboličke i nutritivne promene. Način na koji se one dešavaju i kako se odvijaju imaju veliki uticaj na laktacione performanse, subklinička i klinička postpartalna oboljenja, reproduktivne poremećaje, a sami tim utiču i na rentabilnost.

U toku peripartalnog perioda mlečne krave podležu poremećaju funkcije imunskog sistema. Ova imunosupresija dovodi do povećanja broja i ozbiljnosti infekcija (mastitis, metritis, zadržavanje posteljice i drugi zdravstveni poremećaji).

Uzrok peripartalne imunosupresije nije poznat, ali je predmet brojnih istraživanja koja sugerišu da je imunska disfunkcija uzrokovana metaboličkim i endokrinim faktorima. Brojni autori navode da negativni energetski bilans, nezasićene masne kiseline (NEFA), ketonska tela i hipokalcemija, imaju značajnu ulogu u razvoju imunosupresije. Poznato je da glukokorikosteroidi imaju imunosupresivno dejstvo. Promene nivoa estradiola i progesterona takođe mogu imati direktan i indirektan uticaj na imunsku odbranu. Međutim, oni nemaju dugotrajan uticaj na razvoj ove imunodisfunkcije, jer promene njihovih koncentracija kratko traju.

Pojedini nutricienti su uključeni u funkciju imunskih ćelija i tako minimiziraju inflamatorna oštećenja, zahvaljujući efikasnijem imunskom odgovoru. Moguća su brojna oštećenja tkiva za vreme infekcije, kao posledica oksidativnog stresa – reakcije nestabilnih oksidisanih molekula sa tkivnim lipidima, proteinima i DNA. Brojni antioksidansi su važni za zdravlje životinja, međutim optimalne koncentracije i forme ovih suplemenata za maksimiziranje imunske funkcije i zaštite tkiva, su još uvek nepoznate.

References

ANTANAITIS R., ŽILAITIS V, KUČINSKAS A. (2009): Šliuzo dislokacijos ankstyvosios diagnozes bei pooperacinio pasvekimo galimbyių ivertinimas kompiuterine bandos valdymo programa. Veterinaria IR Zootechnika (Vet Med Zot), T 45(67), 3-7.

BELLA M.J., ROBERTS D.J. (2007): The impact of uterine infection on a dairy cows performance. Theriogenology, 68(7), 1074-1079.

CALDER P.C. (2008): The relationship between the fatty acid composition of immune cells and their function. Prostaglandins, Leukotrienes and Essential Fatty Acids, 79, 101-108.

CASTILLO C., HERNANDEZ J., VALVERDE I., PEREIRA V., SOTILLO J., ALONSO M.L., BENEDITO J.L. (2006): Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in Veterinary Science, 80, 133-139.

CURTIS C.R., ERB H.N., SNIFFEN C.J., SMITH R.D., KRONFELD D.S. (1985): Path analysis of dry period nutrition, postpartum metabolic and reproductive disoders, and mastitis in Holstein cows. J. Dairy Sci., 68, 2347-2360.

EL-ATTAR H.M., YASSEIN M., ABD EL-RAOF, GHANEM M.M. (2007): Alterations in the clinical, hematological and biochemical pictures in abomasal displacement in cows in Egypt. Vet.Med. J., 16, 102–109.

GITTO E., REITER R.J., KARBOWNIK M., TAN D.X., GITTO P., BARBERI S., BARBERI I. (2002): Causes of oxidative stress in the pre- and perinatal period. Biol. NeonatE, 81, 46-57.

GUNAY A., GUNAY U., ORMAN A. (2011): Effects of retained placenta on the fertility in treated dairy cows. Bulgarian Journal of Agricultural Science, 17(1), 126-131.
HALLIWELL B., GUTTERIDEGE J.M.C. (2007): Free radicals in biology and medicine. 4th Ed.Oxford University Press. Grune Strottan, New York.
HAN Y.K., KIM I.H. (2005): Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. J. Vet. Sci., 6, 53-59.
JOKIĆ Ž., JOKSIMOVIĆ TODOROVIĆ M., DAVIDOVIĆ V. (2005): Organski selen u ishrani pilića u tovu. Biotehnologija u stočarstvu, 21(1-2), 79-89.
JOKSIMOVIĆ TODOROVIĆ M., JOKIĆ Ž. (2005): Uticaj visokih nivoa neorganskog selena na aktivnost glutation peroksidaze (GSH-Px) u krvnoj plazmi brojlera. Biotehnologija u stočarstvu, 21 (3-4), 125-131.
JOKSIMOVIĆ TODOROVIĆ M., JOKIĆ Ž., SINOVEC Z. Uticaj visokih nivoa organskog selena na aktivnost glutation peroksidaze (GSH-Px) u krvnoj plazmi brojlera. Veterinarski glasnik 2005; 59(3-4): 383-390.
JOKSIMOVIĆ TODOROVIĆ M., JOKIĆ Ž., HRISTOV S. (2006): The effect of different levels of organic selenium on body mass, bodyweight gain, feed conversion and selenium concentration in some gilts tissues. Acta Veterinaria, 56(5-6): 489-495.
JOKSIMOVIĆ TODOROVIĆ M., DAVIDOVIĆ V. (2007): Selenium, oxidative stress. Proceeding of Third Symposium of Livestock Production with International Participation, Ohrid, Macedonia, 12-14. September 2007: 527-530.
KIMURA K., REINHARDT T.A., GOFF J.P. (2006): Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J. Dairy Sci., 89: 2588-2595.
MARTINS DE LIMA T., GORJO R., HATANAKA E., CURY-BOAVENTURA M.F., PORTIOLISILVA E.P., PROCOPIO J., CURI R. (2007): Mechanisms by which fatty acids regulate leucocyte function. Clinical Science, 113, 65-77.
MIHAILOVIĆ M., TODOROVIĆ M., ILIĆ V. (1991): Effects of dietary selenium of glutatione peroxidase activity and body weight of growing turkeys. Acta Veterinaria, 23, 75-80.
MIHAILOVIĆ M., TODOROVIĆ M., JOVANOVIĆ M., PALIĆ T., JOVANOVIĆ J., PEŠUT O., KOSANOVIĆ M. (1996a): Toxicity of organic and inorganic selenium to chicken. Ninth Intrnat. Symp. of Trace Elements in Man and Animals (Tema 9) - Banff Alberta - Canada 1996. Trace Elements in Man and Animals, 9, 554-555.
MIHAILOVIĆ M., TODOROVIĆ M., JOVANOVIĆ M., PALIĆ T., JOVANOVIĆ J., PEŠUT O., KOSANOVIĆ M. (1996b): Toxicity of sodium selenite and selenized yeast to chicken. The Sixt Intrnat. Symp. on selenium in Biol. and Med. - Peking NR Kina 1996. Program and Abstract, 125.

MIHAILOVIĆ M., TODOROVIĆ M., JOVANOVIĆ M., PALIĆ T., JOVANOVIĆ J., PEŠUT O. (1997): Toxicity of selenium to broilers. XIth International Congres of the World Poultry Association 18-22 August, Budapest, Hungary.
MOKHBER DEZFOULI M., EFTEKHARI Z., SADEGHIAN S., BAHOUNAR A., JELOUDARI M. (2011): Evaluation of hematological and biochemical profiles in dairy cows with left displacement of the abomasum. Comp. Clin. Pathol., DOI 10.1007/s00580-011-1382-5.
NONNECKE B.J., FRANKLIN S.T., YOUNG J.V. (1992): Effects of ketones, acetate, and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. J. Dairy Sci., 75: 982-990.
PERKINS K.H., VANDEHAAR M.J., TEMPELMAN R.J., BURTON J.L. (2001): Negative energy balance does not decrease expression of leukocyte adhesion or antigen-presenting molecules in cattle. J. Dairy Sci., 84: 421-428.
SHARMA N., SINGH N.K., SINGH O.P., PANDEY V., VERMA P.K. (2011): Oxidative Stress and Antioxidant Status during Transition Period in Dairy Cows. Asian-Aust. J. Anim. Sci., 24(4): 479-484.
SORDILLO L.M., STREICHER K.L. (2002): Mammary gland immunity and mastitis susceptibility. J Mammary Gland Biol. Neoplasia, 7: 135-146.
SORDILLO L.M. (2005): Factors affecting mammary gland immunity mastitis susceptibility. Livestok Prod. Sci., 98: 89-99.
SORDILLO L.M., AITKEN S.L. (2009): Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol., 128: 104-109.
SORDILLO L.M., CONTRERAS G.A., AITKEN S.L. (2009): Metabolic factors affecting the imflammatory response of periparturient dairy cows. Animal Health Research Reviews, 10(1): 53-63.
SORDILLO L.M. (2011): New Concepts in the Causes and Control of Mastitis. Mammary Gland Biol. Neoplasia, 16: 271-273.
SPEARS J.W., WEISS W.P. (2008): Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J., 176: 70-76.
SURIYASATHAPORN W., HEUER C., NOORDHUIZE-STASSEN E.N., SCHUKKEN Y.H. (2000): Hyperketonemija and the impairment of udder defense: a review. Vet. Res., 31: 397-412.
TODOROVIĆ M., MIHAILOVIĆ M., HRISTOV S. (1999): Effects of excessive levels of sodium selenite on daily weight gain, mortality and plasma selenium concentration in chickens. Acta Veterinaria, 49(5-6): 313-320.
TREVISAN M., BROWNE R., RAM M., MUTI P., FREUDENHEIM J., CAROSELLA A.N., ARMSTRONG D. (2001): Correlates of markers of oxidative status in the general population. Am. J. Epidemiol., 154: 348-356.
VALKO M., LEIBFRITZ D., MONCOL J., CRONIN M.T., 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.
ZADNIK T. (2003): Review of anterior displacement of the abomasum in cattle in Slovenia. Veterinary Record, 153(1): 24-25.
WALDRON M.R., REVELO X.S. (2008): Causes and Effects of Periparturient Immunosuppression. WCDS Advances in Dairy Tehnology, 20: 97-109.
WALDRON M.R.(2010): Impact of Metabolic and Oxidative Stressors on Periparturient Immune Fuction and Health. Penn State Dairy Cattle Nutrition Workshop, 33-9.
WEBER P.S., MADSEN S.A., SMITH G.W., IRELAND J.J., BURTON J.L. (2001): Pre-translational egulation of neutrophil L-selection in glucocorticoid-challenged cattle. Vet. Immunol. Immunopathol., 83: 213-240.
WILDE D. (2006): Influence of macro and micro minerals in the periparturient period on fertility in dairy cattle. Anim. Peprod. Sci., 96: 240-249.
WOOD L.G., SCOTT H.A., GARG M.L., GIBSON P.G. (2009): Innate immune mechanisms linking non-esterified fatty acids and respiratory disease. Progress in Lipid Research., 48: 27-43.

Received 9 April 2013; accepted for publication 10 June 2013

Biotechnology in Animal Husbandry 29 (2), p 223-232 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.07'635
DOI: 10.2298/BAH1302223P

IMPORTANT STEPS FOR QUALITATIVE RISK ASSESSMENT OF CAMPYLOBACTER PRESENCE IN POULTRY MEAT IN SERBIA

J. Petrović [1], I. Stojanov [1], D. Pešić Mikulec [2], D. Milanov [1], M.Velhner[1], D. Stojanović [1]

[1]Scientific vetrinary institute Novi Sad, Rumenački put 20, Novi Sad
[2]Institute of veterinary Medicine of Serbia, Auto put za Zagreb 3, Novi Beograd
Corresponding author: jelenap@niv.ns.ac.rs
Review paper

Abstract: The aim of this paper was to answer some questions important for a qualitative risk assessment in poultry production related to *Campylobacter*. These steps are described: hazard identification, hazard characterisation, exposure assessment and risk characterization. Hazard identification: *Campylobacter* is important cause of food borne diseases. Hazard characterization: human campylobacteriosis is assumed to be dose-independent based on some studies on healthy human volunteers. Exposure assessment: *Campylobacter* colonisation and spread of *Campylobacter* on poultry carcasses were described. Our results indicate high prvalence of *Campylobacter spp*. in intestines of poultry, the clinical symptoms of campylobacteriosis usually depends of the age and additional immunosupresive factors, such as *Salmonella*. According to our experimental results artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after 5 days, with slight decrease in next 20 days and return to starting level in next 20 days. According to our experimental results, slaughtering of *Campylobacter* positive flock appears where 100% birds (with 3.02 log cfu/g feces) is contaminated lids to 50% contaminated carcasses. High prvalence of *Campylobacter spp*. was also found on poultry carcasses in our slaughterhuoses. Risk characterization: on the basis of the performed examinations it can be concluded that even there is high exposure of human population to *Campylobacter* from poultry meat; the incidence of human campilobacteriosis is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis.

Key words: risk assessment, *Campylobacter*, poultry

Introduction

Although various foods can serve as a source of food borne illness, meat and meat products are important sources of *Campylobacter*. The *Campylobacter* is most frequently reported cause of zoonotic diseases in EU in 2007, incidence was 45.2 cases per 100 000 population *EFSA* (2009, 2010). This bacteria is widespread within the poultry production in Europe. *C. jejuni* is frequent commensal in poultry and cattle, and *C. coli* in swine and poultry. The most common way people become infected with zoonotic enteric pathogens is through the ingestion of food contaminated with animal feces (contamination usually occurs during processing).

An understanding of thermophilic *Campylobacter* and specifically *C. jejuni* in broiler chickens is important from public health. The food safety risk analysis usually is used as a tool for the control of biological, chemical and physical hazards associated with foods. Risk analysis comprises of three functions: risk assessment, risk management and risk communication. Risk assessment can be quantitative and qualitative.

The aim of this paper was to answer some questions important for a qualitative risk assessment in poultry production related to *Campylobacter*. The qualitative risk assessment attempts to understand how the incidence of human campylobacteriosis is influenced by various factors during poultry breeding and broiler meat production "from farm to table". In the estimation of risks, the following steps are usually involved: hazard identification, hazard characterization, exposure assessment and risk characterization. During the research *Campylobacter* colonisation and spread of *Campylobacter* on poultry carcasses were examined.

Materials and methods

Experimental design: The control group (group A) consisted of 30 chickens which were not artificially infected with *Campylobacter*. The group B consisted of 32 chickens which were artificially infected with 6.77 log cfu *C. jejuni* ATCC 29428 per chicken on day 21 of life. Total count of *Campylobacter* was examined in chicken feces five times in the following twenty days. The chickens were slaughtered in the poultry abattoir on day 42 of life (10 chickens from the control group and 10 from the infected group). The prevalence of *Campylobacter* on carcasses and livers were determined immediately after processing.

The identification and total number of *Campylobacter* was determined according to procedures to the standard ISO 10272 and ISO 4833 procedures.

Results and discussion

Hazard identification

The genus *Campylobacter* now comprises 17 member species most of which are microaerophils, i.e. grow preferentially in low oxygen concentrations. The majority of cases of human campylobacteriosis are caused by two species: *C. jejuni* and the closely-related *C. coli*. These two species are often referred to as the "thermophilic" *Campylobacter* as they grow preferentially at 42°C *EFSA* (2009, 2010). Thermophilic *Campylobacter spp* are a leading cause of zoonotic enteric disease in most developed countries. They are usually indirectly transmitted to humans through the consumption of contaminated food. The principal reservoir of these organisms is the digestive tract of food producing animals. In the vast majority of cases, the organisms are constantly shed in feces by asymptomatic animals *Stojanov et al*. (2011). Handling raw poultry and eating poultry products are important risk factors for sporadic campylobacteriosis *EFSA* (2010). In our study hazard was identified as risk of human campylobacteriosis associated with thermophilic *Campylobacter* in poultry meat.

Hazard characterization

Hazard characterization provides a description of the public health outcomes following infection, including sequelae, pathogen characteristics influencing the ability of organism to elicit infection and illness, host characteristics that influence the acquisition of infection, and food-related factors that may affect the survival of *Campylobacter* in the human gastrointestinal tract.

Food poisoning data usually are obtained after an outbreak but in the case of campylobacteriosis there is no necessery data from outbreak, because this disease occurs in sporadic cases.

Until the mid of XX century there was a strong belief that human infections caused by campylobacteria are rare, and the organism was considered an opportune human pathogen. The researches dating from last 30 years established the importance of *Campylobacter* species in the pathogenesis of human diseases *Stojanov et al.*(2008a). Human campylobacteriosis occurs sporadically, as small-scale family infections or epidemics in particular communities. Campylobacteriosis is characterized by poor general health status of the patient, recurrent high body temperature, tremor, headache, abdominal pain, vomiting and diarrhea. Occurrence and severity of these symptoms is determined by the virulence of the organism and by the immune status of the patient. Results of some recent research revealed that campylobacter infections mostly affect the newborns and children, as well as patients with an immune deficiency syndrome or malignant diseases of the immune system *Zonios et al*. *(2005)* and *EFSA (2005).*

Human campylobacteriosis is assumed to be dose-independent based on some studies on healthy human volunteers *WHO* (*2002).*

Exposure assessment

Risk models which estimate the exposure to *Campylobacter* from poultry meat include two important steps at beginning: flock prevalence and concentration of campylobacter on poultry carcasses before cooling.

Exposure assessment at farm level

The occurrence of zoonotic pathogens et farm varies depending on the range of factors including the organism, geographical factors, production practice. Some animal production conditions facilitate the spread of bacteria, like poor infection control *Stojanov et al.* (2008a). *Campylobacter* colonisation in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalence from 18 to 90%. Higher prevalence was obtained in southern countries *Barrios et al. (2006).*

Initial introduction of *Campylobacter* into poultry flock still remains poorly understood and the phenomenon may be multi-factoral. *Campylobacter* usually colonise chickens in the third week of life. The level of colonisation and the spread of *Campylobacter* between animals depends on different factors: breeding conditions, flock size, hygiene measures, carry over from previous flock, air and water contamination, immune response of animals, other infected livestock on the farm, mechanical transmission via insects and birds *Barrios et al*. (*2006); Stojanov et al.* 2011).

According to our experimental results *Stojanov et al. (2011)* infection of the one day old chickens induces diarrhea, while infection of the three days old chickens with 10^9 cfu does not induce diarrhea. If diarrhea occurs, it is usually 6 hours after infection and lasts for 10 days. The distal part of intestines usually are affected (jejunum and cecum), pathological changes are not characteristic and are described as enlargement of the intestine, as a consequence of accumulation of the water and jelly content inside the intestine. Hemorrhages are not always present.

According to our experimental results *Stojanov et al. (2011)* artificial infection of chickens with 4 log cfu *C. jejuni* and 4 log cfu *Salmonella entritidis* per chicken on day 14 of life leads to watery diarrhea and traces ov blood in first two weeks after the infection. Campylobacteriosis can be expected if an additional factor is present, which affects the immune system and its ability to cope with the infection.

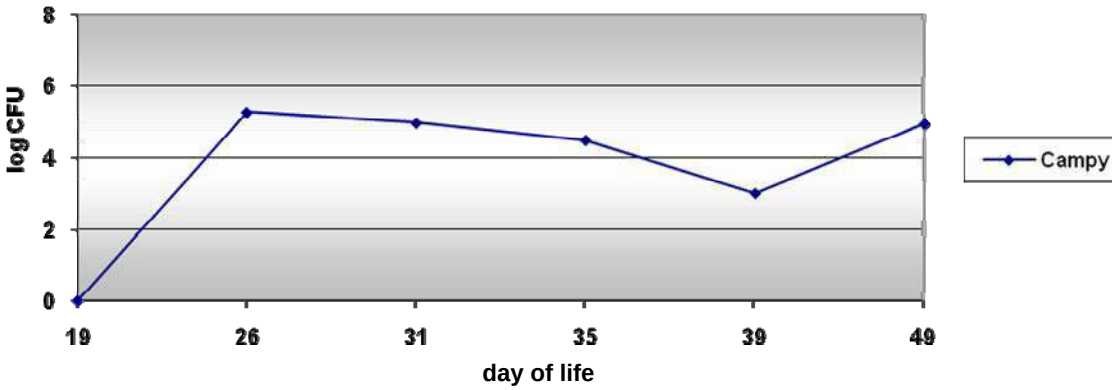
The clinical symptoms usually depend of the strain, number of bacteria, stress and immune suppression *Kazwala et al. (1992).* In spite of widespread nature of infections caused by *Campylobacter*, the clinical symptoms and pathology changes caused by these bacteria are rather rare. Table 1 presents findings of *Campylobacter spp*. in intestines from poultry, pigs and calves in Serbia.

According to our quantitative examinations of *Campylobacter* contamination and spread in poultry flock, artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after

only 5 days, with a tendency to decrease 4.97 log cfu/g (31st day of life), 4.49 log cfu/g (35th day of life) and 3.02 log cfu/g (39th day of life). After this day the increase in *C. jejuni* count was noticed 4.95 log cfu/g (49 th day of life). Tendency of decrease in *Campylobacter* count was found also in the work of *Van Boven et al. (2003)*. The results are shown in Graph 1.

Table 1. The findings of *Campylobacter spp*. in the samples originating from poultry, pigs and cattle *Stojanov et al*. (2008b)

Material	No of samples	*C. jejuni/coli* positive	%
Poultry	60	44	73.33
Pigs	12	7	58.33
Calves	6	4	66.66



Graph 1. Average number of *C. jejuni* log cfu/g feces by days of life

In Slovenia a nation-wide genotyping study was performed by PFGE to determine the genetic profiles of 500 isolates of *C. jejuni* obtained from humans (n=156), animals (n=133) and food (n=214). The isolates exhibited marked genetic diversity, however, identical PFGE profiles were identified for animal, human and food isolates, indicating the animals as a direct infection source for humans. The isolates from animals of the same owner, bred in different time periods, exhibited different PFGE profiles suggesting the *C. jejuni* strains do not persist on the farm *Ocepek et al. (2011)*.

Exposure assessment at slaughterhouse

According to our experimental results the prevalence of *Campylobacter* - contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g feces) to 50% of chicken carcasses - Table 2.

The *Campylobacter* can be transmitted to the production facility and can contaminate the processing environment and the final product. According to our results *Petrović et al*. (2007a, b, c) the occurrence of *Campylobacter* is very frequent in poultry carcasses in slaughterhouses in Vojvodina region (Table 3). The influence of production management is great, since in poultry abattoirs the prevalence varies from 11.43 to 90.00% carcasses *Petrović et al. (2008a, b, c).* The prevalence of *Campilobacter* positive carcasses increased during evisceration and decreased uppon the method of chilling.

Table 2. Prevalence of *Campylobacter* on broiler carcasses

Sample	No samples	Group A control *C. jejuni* posit (%)	Group B infected *C. jejuni* posit (%)
Liver	10	0 (0.00%)	5 (50.00%)
Carcase	10	0 (0.00%)	5 (50.00%)
Total	20	0 (0.00%)	10 (50.00%)

Table 3. Occurrence of *Campylobacter* in poultry samples *Petrović et al.* (2007a,b,c)

Sample		Abattoir mark/ Prevalence of *Campylobacter* (%)						
		A	B	C	D	E	F	G
	liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71
	carcasse	90.00	14.28	51.43	20.01	68.57	11.43	31.43

Risk characterization

This step links the probability and magnitude of exposure to *Campylobacter* associated with consumption of meat to adverse outcomes that might occur. *Campylobacter* is frequently found in feces of live animals in poultry farms. Overall *Campylobacter* contamination decreased through processing with temporary increases occurring during poultry transport and evisceration. According to the Report of the Institute of public health of Serbia »dr Milan Jovanović Batut«, in Serbia in 2010 there were 357 cases of *Enteritis Campylobacterialis* diagnosed

with the incidence of 4.88. Incidence in Vojvodina was between 5,22 in 2007 and 11,25 in 2008. *Hrnjak Cvjetković et al. (2011).* While in the EU in 2005 the incidence rate of 38.2-51.6 cases per 100 000 population was noticed. But despite high exposure of population to *Campylobacter* in Serbia, the incidence of human campylobacteriosis from raw meat is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis. *Enteritis Campylobacterialis* is a sporadic disease; symptoms usually do not require hospitalisation and many sick people do not go to the doctor and only small number of ill people has laboratory confirmation of campylobacteriosis. Also significant factor of exposure to the decrease are cooking habits in Serbia: meat is usually well cooked.

Conclusions

Hazard identification: termophilic *Campylobacter spp* are important cause of zoonotic enteric illness. Hazard characterization: it is assumed to be dose-independent based on some studies on healthy human volunteers. Exposure assessment: Initial introduction of *Campylobacter* into poultry flock still remains poorly understood and the phenomenon may be multi-factoral. The clinical symptoms usually depend of the age of chickens, stress and immune suppression. Campylobacteriosis can be expected if an additional factor is present, such as *Salmonella* which affects the immune system and its ability to cope with the infection. The high prevalence of *Campylobacter spp*. in intestines from poultry, pigs and calves was found. The artificial infection of chickens with 6.77 log cfu *C. jejuni* per chicken on day 21 of life leads to 5.26 log cfu/g feces after 5 days, with slight decrease in next 20 days and return to starting level in next 20 days. Also the prevalence of *Campylobacter* -contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g feces) to 50% of chicken carcasses. Identical PFGE profiles were identified for animal, human and food isolates, indicating the animals as a direct infection source of *Campylobacter* for humans. Risk characterization: even there is high exposure of human population to *Campylobacter* in Serbia; the incidence of human campylobacteriosis from raw meat is low, mainly because there is a lack of evidence and confirmation of human campylobacteriosis.

Acknowledgement

Research was finansed by the Ministry of Education and Science, Republic of Serbia project TR31071.

Neophodni koraci za kvalitativnu procenu rizika od prisustva kampilobakterija u mesu živine u Srbiji

J. Petrović, I. Stojanov, D. Pešić Mikulec, D. Milanov, M.Velhner, D.Stojanović

Rezime

Cilj ovog rada je da odgovori na neka od pitanja koja su neophodna da bi se izvršila kvalitativna procena rizika vezana za kampilobakterije u proizvodnji brojlera u Srbiji. Opisani su sledeći koraci: identifikacija hazarda, karakterizacija hazarda, procena izloženosti i karakterizacija rizika. Identifikacija hazarda: kampilobakterije su važan uzročnik alimentarnih oboljenja. Karakterizacija hazarda: na osnovu studija na zdravim dobrovoljcima pretpostavlja se da je humana kampilobakterioza nezavisna od koncentracije kampilobakterija u hrani. Procena izloženosti: opisana je kroz ispitivanja kolonizacije kampilobakterijama kod živine širenje na klanično obrađene trupove. Naši rezultati ukazuju na visoku prevalencu kampilobakterija u crevima živine, klinički simptomi kampilobakterioze obično zavise od starosti i dodatnih imunosupresivnih faktora, kao što je prisustvo *Salmonella*. Prema našim eksperimentalnim rezultatima veštačka infekcija pilića sa 6.77 log cfu *C. jejuni* 21. dana života nakon pet dana dovodi do kolonizacije od 5.26 log cfu/g fecesa, sa laganom tendencijom opadanja u narednih 20 dana i povratkom na početni nivo u sledećih 20 dana. Prema našim eksperimentalnim rezultatima, klanje jata u kom je prevalenca kampilobakterija 100% (sa 3.02 log cfu/g feces) dovodi do pojave 50% kontaminiranih trupova. Ustanovljena je visoka prevalenca termofilnih *Campylobacter spp*. na klanično obrađenim pilićima u našim klanicama. Karakterizacija rizika: na osnovu izvršenih ispitivanja u našoj zemlji može se zaključiti da iako je visoka izloženost humane populacije kampilobakterijama iz živinskog mesa, incidenca humane kampilobakterioze je mala, ovakvi podaci prvenstveno ukazuju na nedovoljnu evidenciju i potvrdu humane kampilobakteroze.

References

BARRIOS PR., REUERSEN J., LOWMAN R., BISAILLON JR., MICHEL P., FRIDRIKSDOTTIR V., GUNNARSSON E., STERN N., BERKE M.E., MARTIN W. (2006): Risk factors for *Campylobacter spp.* colonization in broiler flocks in Iceland. Preventive Veterinary Medicine 74(4):264-78

EFSA (2005):Report of the EFSA Panel on Biological Hazards on the request from the Commission related to Campylobacter in animals and foodstuffs. Annex to The EFSA Journal No173, Pp1-105

EFSA (2009): The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal, No223

EFSA (2010): Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in 2010: EU (1):1437(89 pp.). doi:10.2903/j.efsa.2010.1437

HRNJAKOVIĆ CVJETKOVIĆ I., MILOŠEVIĆ V., JERANT PANTIĆ V., STEFAN MIKIĆ S., CVJETKOVIĆ D, RADOVANOV J., KOVAČEVIĆ G. (2011): Najčešće bakterijske zoonoze u ljudi u Vojvodini u periodu 2005-2009. Arhiv veterinarske medicine,4(1):11-8

INSTITUT ZA JAVNO ZDRAVLJE SRBIJE "DR MILAN JOVANOVIĆ BATUT" (2010): Zdravstveno-statistički godišnjak Srbije. ISSN 2217-3714. http://www.batut.org.rs/download/publikacije/pub2010.pdf

KAZWALA R., COLLINS J., HANNAN J.(1992): The establishment and spread of experimental *Campylobacter jejuni* infections in young chickens., Preventive Veterinary Medicine, 13:19-26

OCEPEK M., PATE M., GRUNTAR I., KUŠAR D., GOLOG M., MIĆUNOVIĆ J., ZDOVC I.(2011): *Campylobacter jejuni* in humans, animals, food and environment in Slovenia: Antibiotic resistance and molecular genotyping testing in 2007-2011. Microbiologia Balkanica 2011 7th Balkan Congress of microbiology& 8. Congress of microbiologists of Serbia MIKROMED 2011. Belgrade Serbia 25-29.10.2011., CD rom

PETROVIĆ J., BALTIĆ M., KLJAJIĆ R, KAPETANOV M. (2007a): Rezistencija zoonotskih patogena koji poticu iz mesa živine prema antimikrobnim lekovima. Tehnologija mesa 301:147-52

PETROVIĆ J., Karabasil N., BALTIĆ M., KLJAJIĆ R., DIMITRIJEVIĆ M., KRASIĆ J. (2007b): Ispitivanje osetljivosti na razlicite antimikrobne lekove sojeva salmonella izolovanih u lancu proizvodnje mesa živine i svinja. 1. Međunarodni kongres Tehnologija, kvalitet i bezbednost hrane. I Simpozijum biotehnologije i mikrobiologije hrane, 13-15.10.2007. Novi Sad, str105-11;

PETROVIĆ J., BALTIĆ M., KLJAJIĆ R., KAPETANOV M. (2007c): Rezistencija zoonotskih patogena koji potiču iz mesa živine prema antimikrobnim lekovima.Tehnologija mesa, 49(3-4):147-152

PETROVIĆ J., PETROVIĆ T., KOVAČEVIĆ M. (2008a): Food Safety Trends: Antimicrobial resistance in food borne pathogens, viruses in food and safe disposal of animal wastes. The second joint PSU-UNS international conference on BioScience: Food, agriculture and the environment, 22-24.06.2008, Novi Sad, Serbia, Proceedings, Pp.36-44.

PETROVIĆ J., MILANOV D., RATAJAC R. (2008b): Savremeni trendovi u bezbednosti hrane: Rezistencija zoonotskih patogena prema antimikrobnim lekovima. Veterinarski glasnik, 62(5-6);Pp 257-63.

PETROVIĆ J., BALTIĆ M., KARABASIL N., MILANOV D. (2008c):Fluoroquinolone resistance of *C.jejuni* isolated from carcasses of broilers treated with enrofloxacine. Abstract Book, XII International Congress of Bacteriology and Applied Microbiology, 5-9.08.2008, Istanbul, Pp.262.

STOJANOV I., MILIĆ N., AŠANIN R., VIDIĆ B., GRGIĆ Ž., PRICA N.(2008a): Effects of some immunosuppressive factors on campylobacteriosis outbreaks in poultry. Acta Veterinaria, 58(2-3);167-77

STOJANOV I., TAMBUR Z., KAPETANOV M., PUŠIĆ I., PRODANOV J. (2008b): Campylobacteriosis of Domestic Animals - The Problem of Infection Control.Proceedings, The Second Joint PSU-UNS International Conference on BioScience: Food, Agriculture and the Environment, 22-24.06.2008, Novi Sad, Serbia, pp.72-77

STOJANOV I., STOJANOVIĆ D., MILIĆ N., ŽIVKOV-BALOŠ M., KAPETANOV M. (2011): Influence of Salmonella Infection in Chickens on the Outcome of Campylobacteriosis in Experimental Conditions. Acta Veterinaria, 61(1);57-65

VAN BOVEN M., VELDMAN K., DE JONG M., MEVIUS D. (2003): Rapid selection of quinolone resistance in Campylobacter jejuni but not in Echerichia coli in ndividually housed broilers. J of Antimicrob Chemother, 52;719-23

WHO (2002): Report of a Joint FAO/WHO Expert Consultation . Risk assessment of *Campylobacter* spp. in broiler chickens and Vibrio spp. in seafood. 2002

ZONIOS D., PANAYIOTAKOPOULOS G., KABLESTAS E., TZIMA E., STEFANOU I., ARCHIMANDRITIS A. (2005): Campylobacter fetus bacteraemia in a healthy individual: clinical and therapeutical implications, J Infect, 51(4);329-32

Received 16 October 2012; accepted for publication 20 March 2013

Biotechnology in Animal Husbandry 29 (2), p 233-250 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.3
DOI: 10.2298/BAH1302233N

OBJECTIVES AND APPROACHES IN THE BREEDING OF PERENNIAL LEGUMES FOR USE IN TEMPORARY PASTURELANDS

G. Naydenova[1], Ts. Hristova[2], Y. Aleksiev[2]

[1] Soybean Experimental Station, Pavlikeni 5200, Bulgaria
[2] Research Institute of Mountain Stockbreeding and Agriculture, Troyan 5600, Bulgaria
Corresponding author: gmvg@abv.bg
Review paper

Abstract: Legumes are the major element of grassland ecosystem**,** on which the forage quality depends. Breeding of pasture varieties in perennial legumes firstly aims at achieving tolerance and persistence of the legume component in the pasture. In species having low natural grazing tolerance (lucerne and red clover) it is necessary to conduct breeding for biological, morphological and physiological characteristics, directly related to grazing tolerance. In the species having high grazing tolerance (white clover, birds foot trefoil, sainfoin), the pasture persistence is considered as a function of particular morphological characteristics, adaptive potential and stress tolerance. The indirect breeding for pasture persistence includes also breeding for competitive ability and co-adaptivity with grass pasture species adapted to the region and practices of cultivation, as well as breeding for improved nodulation and nitrogen fixation as an important element of the adaptive ability. The breeding for grazing tolerance and persistence is concentrated also on the mechanisms of self-maintenance of the legumes in the pasture – vegetative reproduction, spread and self-seeding. Many breeding programmes are concentrated also on morphology providing better intake by the animals, on main and specific quality characteristics, on anti-nutrient factors, on adaptation to systems and practices of pasture establishment and use. The breeding strategy using germplasm consolidated through the mechanisms of specific adaptation to unfavourable or specific agro-ecological conditions and influences – i.e. breeding through ecotypic selection is considered to be the most efficient for grazing purposes. The interactions in the system: pasture species – companion species in the pasture herbaceous community – grazing animals (kind, category, grazing style and behaviourial reactions) and the applied regime of use of the pasture are direct and strong. These interactions are of essential importance in genotypic differentiation of the pasture ecotypes. The adaptive variability of the pasture ecotypes is used directly, as well as in recombination breeding.

Key words: forage legumes, breeding for grazing use

Introduction

Legumes are the major element of the grassland ***ecosystem***, on which the forage quality depends. Their fresh mass has high content of readily available protein, minerals, vitamins, neutral detergent fiber (*Lauriault et al., 2005; Carlier et al., 2008*). Their participation in the pasture sward is related firstly with increased intake of DM as result of higher intake rate and a longer time spent grazing (*Penning et al., 1995; Caradus et al., 1995*) and hence, with an increase of the animal productivity (*Fraser et al., 2004*). The positive nutrient effect of legumes is particularly important, consisting in increasing levels of polyunsaturated fatty acids in milk or meat (*Wu et al., 1997; Fraser et al., 2004*) and reducing methane production (*Dewhurst et al., 2009*). The inclusion of a nitrogen-fixing legume component in the sward corresponds to the principles of sustainable agriculture and extensive agricultural production (*Heichel and Henjum, 1991; Morris and Greene, 2001*). Legumes increase the nitrogen utilization in the herbaceous associations firstly through a decrease of the competition for soil nitrate ("nitrate sparing") and secondly – through N transfer (*Temperton, 2007*). They have great importance also for the fact that they contribute to even distribution of the yield from the pasture by seasons (*Sleugh et al., 2000*).

The breeding of legume varieties for grazing use can be defined as breeding for achieving a reasonable balance between persistence, quality, yield and animal safety (*Sewell et al., 2011*). The grazing use necessitates distinct plant type and selection work (*Annicchiarico et al., 2010*). The breeding process for these purposes is slow and expensive, because it requires a complete cycle of testing of perennial herbaceous species and it is conducted together with assessment of the effects of important ecological factors, such as grazing animals and companion herbaceous species in the pasture mixtures. Therefore, the test of the breeding materials requires large amounts and units of them.

Breeding for survival and persistence under grazing

Persistence of the legume plants in the pasture is dynamic and determined by interactions among plant species, soil fertility, pests and pathogens, grazing stock, and seasonal weather conditions (*Widdup and Barrett, 2011*). Grazing, considered as frequent, early, selective or excessive removal of the over-ground mass, as trampling and contamination, appears to be the most important limiting factor of the persistence of species having low natural grazing tolerance (red clover and lucerne). Therefore in these species, in the first place, it is necessary to conduct breeding for biological, morphological and physiological characteristics, which provide survival of the individual plants and of the population of legumes under grazing. The traits, directly related to grazing tolerance, were studied to the greatest

extent in lucerne and they were generalized by *Katepa-Mupondwa et al. (2002),* as follows: deep set crowns, broad crowns, prolific and nonsynchronous budding, extended periods of budding, subsurface budding, maintenance of stubble leaf area under grazing, early fall dormancy, and maintenance of root carbohydrates. In red clover, the plant morphology, associated with grazing tolerance is defined as follows: more profuse and finer stems, a more prostrate and lover growth habit and smaller leaves (*Rumball et al., 2003; Ford and Barrett, 2011; Boller et al., 2012*). In earlier studies the red clover persistence in pastures is considered in an aspect of the interaction of legume genotype with associate grass, season and regime of pasture use (*Pineiro and Harris, 1978; Cosgrove and Brougham, 1985*).

There are opinions that the breeding for grazing tolerance, based on individual traits, is not efficient. In the first place, because grazing tolerance must be considered as a complex trait that encompasses many morphological and physiological plant characteristics and their interactions with the environment (*Katepa-Mupondwa et al., 2002*). Furthermore, it is known that the selection for one or several characteristics, related to grazing tolerance, leads to decreased productivity and loss of other desired qualities (*Smith et al., 2000*). Morphological types, related to grazing tolerance, are different and specific, depending on the kind of the grazing animals and intensity of pasture use (*Annicchiarico et al., 2010; Ford and Barrett, 2011*), which also makes the breeding for individual traits unstable.

In white clover – the legume possessing the highest grazing tolerance – the breeding for pasture persistence is also associated with morphological characteristics - stolon diameter and branching, leaf size and root structure (*Ayres et al., 1996; Jahufer et al., 2002; Sanderson et al., 2003; Annicchiarico and Piano, 2004; Bouton et al., 2005; Jahufer et al., 2008*). The stolon and root morphology and architecture influence the white clover persistence in the pasture through the degree of utilization of water and nutrients. The stolon characteristics condition the processes of clonal persistence (*Bouton et al., 2005*). Moreover, white clover and birdsfoot trefoil form a relatively persistent seedbank. On this ground, breeding for high and ecologically stable seed productivity is conducted, which maintains a seed bank, providing the presence of these species in the pasture (*Pedersson and Brink, 1997; Ayres et al., 2008*). Birdsfoot trefoil is also mentioned in other comparative studies as a species possessing potential as a self-seeding pasture species (*Carr et al., 2005a, 2005b*). In other cases, to achieve sustainable presence, founded also on the basis of the seed ecology, the conducted breeding is for hard seed character. The hard seed character conserves the seed viability after passage through the digestive tract of animals and allows the legume spead by the animals and their natural reseeding (*Doucette et al., 2001; Abbate et al., 2003*).

Under grazing the legume persistence proves to be a more important trait than productivity. Persistence is a complex trait often negatively correlated with other important traits and its phenotyping is laborious (*Herrmann, 2007*). The plant

genetic potential for persistence is connected to a great extent with primary and secondary metabolites relating to stress tolerance and defence (*Widdup and Barrett, 2011*). According to *Taylor* (2008) the persistence, considered within the framework of the life cycle, is a result of the interaction of plant adaptivity and its stress tolerance. The breeding for drought and winter stress tolerance, for tolerance and resistance to soil pathogens is considered to be a means of achieving persistence in red clover (*Taylor, 2008*); white clover (*Annicchiarico, 1997; Collins, 2002, Widdup and Barrett, 2011*), lucerne (*Smith et al., 2000; Bouton, 2012*), sainfoin (*Mowrey and Matches, 1991; Mowrey and Volesky, 1993; Morrill et al., 1998; Gray et al., 2006; Demdoum et al., 2010*). In white clover the breeding for pasture persistence is associated also with breeding for competitive ability, consisting in good growth in conditions of shading, competitive root morphology, and compatibility with adapted grass pasture species to area and practices of cultivation (*Caradus et al., 1995*). According to *Escaray et al.* (2012), rhizobial symbionts can contribute to the adaptation of legumes not only to saline and alkaline soils, but also to a wider range of adverse conditions. In this aspect, improved nodulation and nitrogen fixation also prove to be aims in the indirect breeding for persistence.

In many cases it is assumed that pasture persistence of the species can be increased through a change in their system of reproduction from closed (such one with seeds) to open (with the opportunity also for vegetative reproduction in the sward) (*Taylor, 2008*). In this case, the ideotype of pasture legume is considered to be the white clover, which has excellent grazing tolerance, as well as due to its open system of reproduction through stolons. In wild ecotypes of the seed-propagating species, ability of vegetative reproduction was also found. Creeping forms (spreading by rhizomes or stolons) were found in birdsfoot trefoil (*Beuselinck, 1989; 2004*), lucerne (*Heinrichs, 1973; Piano et al., 1996*) and red clover (*Smith and Bishop, 1993; Brock et al., 2003; Rumball et al., 2003*). However, it should be mentioned that there is a substantial microenvironmental influence on the expression of this ability of vegetative spread in the pasture sward and it proves to be strongly linked to specific ecological conditions, this applying also to the varieties developed on the basis of these ecotypes (*Hyslop et al., 1998; Kallenbach et al., 2001; Beuselinck et al., 2005*).

Interspecific hybridization has been also used as a means of introgression of different types of systems of vegetative reproduction. In red clover the work was done with interspecific hybrids, obtained from crosses with the rhizomatous species *Trifolium medium* (*Merker, 1991; Sawai et al., 1995*). Similarly, through the hybridization with *Trifolium ambiguum* in white clover it was achieved to combine a stoloniferous system with a rhizomatous system of reproduction (*Anderson, 1991*) and the drought tolerance and persistence was increased in large leaved white clover varieties under grazing (*Meredith et al., 1995; Marshall et al., 2001*).

Interspecific hybridization has been also used for introgression of other traits, providing survival and persistence under grazing. Yellow (sickle) lucerne (*Medicago sativa ssp. falcate*), obtained from crosses of *Medicago sativa X Medicago falcate* possesses tolerance to summer drought, winter tolerance and deeper position of root crown. In contrast to subspecies sativa, the plant survival does not depend on duration of the period of carbohydrate recovery in the roots after removal of the over-ground mass (*Berdahl et al., 1989; Bittman et al., 1991*). Through hybridization with *Trifolium uniflorum*, in white clover the depth of development of the root system was increased and hence the drought tolerance was increased (*Pandey, 1987*). The hybrids of *Trifolium repens X Trifolium occidentale* were intended to be used as a source of viral resistance (*Pederson and McLaughlin, 1989*). Nevertheless it should be mentioned that in legumes there are no practically spread varieties, developed through interspecific hybridization (*Abberton, 2007*), as it is for grasses, in this case for Festulolium.

Breeding for traits conditioning grazing suitability

Breeding criteria connected with grazing suitability, are numerous and heterogeneous. Selection for morphological traits, which provide better intake of legumes by animals, should be considered as a kind of breeding for productivity. The examples of such traits in red clover are great leafiness of stems, more profuse and thin stems, lower and flatter growth habit, smaller leaves (*Boller et al., 2012*), in birdsfoot trefoil - fine stem, prostrate growth (*Radović et al., 2008*), in white clover - upright habit with long petioles, large-leaved character (*Widdup and Barrett, 2011; Mihovski and Goranova, 2006*). It should be also considered that the intake of legumes depends very much on species, style of grazing and behavioural reactions of the grazing animals (*Taylor et al., 1987; Marten et al., 1990; Popp et al., 1999*). That is an important reason to include animals in the breeding process.

From the point of view of grazing use, the breeding for main nutritional forage characteristics (crude protein, acid detergent fibre, neutral detergent fibre, ash, lignin, lipid, metabolizable energy and organic matter digestibility), as well as the breeding for specific qualitative characteristics are of importance. The following examples of the latter can be mentioned: the breeding for high levels of the polyphenol oxidase enzyme, protecting proteins and glycerol-based lipid in the rumen (*Winters and Minchin, 2001; Lee et al., 2009; Weiher et al., 2010*); or the breeding for optimal content (2-4%) of condensed tannins, which in a grazing situation have the important functions, such as: to reduce protein fermentation to ammonia in the rumen and methane gas emissions, control of rumen bloat and internal parasite infections (*Arrigo et al., 2009; Escaray et al., 2012*).

In connection with the intake, as well as due to the direct and quick effect of the grazed herbage on the biochemical processes in animal organism, the breeding against antinutrient factors is taken into consideration. Such ones are: content of cyanogenic glucosides in white clover (*Collinge and Hughes, 1982;*

Paplauskienë and Sprainaitis, 2003; Annicchiarico, 2012), content of phytoestrogens, formononetin, biochanin-A and coumestrol in red clover (*Francis and Quinlivan, 1974; Gosden et al., 1979*) and lucerne (*Cantero, 1993*), high levels of condensed tannins (above 6%) in birdsfoot trefoil (*Aerts et al., 1999; Wen et al., 2003*). As a particularly important antinutrient factor is considered to be the bloating potential of legumes, which have very low natural content of condensed tannins – lucerne, red and white clover. In order to overcome this limitation the work is done mostly with lucerne, because its bloating potential is higher than that of clovers, on the one hand, and due the necessity (search) for high and sustainable productivity and reduced cost of pastured forage, on the other hand. The selection for lower initial rate of dry matter disappearance (low initial rates of digestion) has been proposed to develop alfalfa cultivars with reduced bloating potential (*Goplen, 1972; Goplen and Howarth, 1976; Basigalup et al., 2003*). This new strain reduces the incidence and severity of frothy bloat on pasture. Their effectiveness in controlling bloat was related to feeding or grazing management practices, the maturity of the plants and the season of use (*Berg et al., 2000*). The methods of genetic transformation prove to be very successful in overcoming the bloating potential. The newest studies show that in the leaves of genetically modified lucerne and white clover plants condensed tannins can be synthesized and accumulated up to levels preventing the bloat (1.8%) (*Hancock et al., 2012*). There are programmes for breeding of sainfoin varieties suitable for cultivation in mixtures with alfalfa, thus reducing also the bloating problems (*Acharya, 2006; Wang et al., 2007*).

In their turn, the different systems and practices of establishment and use of pastures also necessitate specific breeding objectives. For instance, often legumes become a component of existing pasture swards through underseeding (*Guretzky et al., 2004*). The best result of this method was observed in red clover and birdsfoot trefoil (*George, 1984*). That justifies in red clover to conduct breeding for increased frost-seeded seedling establishment (*Riday 2006, 2008*). Furthermore, the legumes in the pasture are assigned the function to prolong the grazing season, to increase the yield and quality of grazing in the second half of summer and autumn. In this aspect, major breeding criteria prove to be productivity in secondary growth and rate of regrowth – in birdsfoot trefoil (*Blumenthal and McGraw, 1999; Radović et al., 2008; Scheffer-Basso et al., 2011*) and sainfoin (*Martiniello, 2005*), drought tolerance – in white clover (*Caradus et al., 1995*) and sainfoin (*Demdoum et al., 2010*), late beginning of fall dormancy and aftergrass character in combination with winter survival – in red clover and lucerne (*Bouton, 2012*).

Ecotypic selection as efficient breeding approach

Breeding of varieties for grazing use should be considered in the first place as breeding for specific adaptation (*Annicchiarico et al., 2010*). From this point of

view, in the breeding for grazing purposes, the breeding strategy using germplasm consolidated through the mechanisms of specific adaptation – i.e. breeding through ecotypic selection is considered to be the most efficient.

The use of specific adaptations to soil, climatic and topographic conditions is important to legumes, because they are considerably more susceptible to unfavourable conditions than grasses. On the other hand, most often the pastures have an agro-ecological niche in areas with low-fertile lands and in mountainous areas. A great deal of tolerance to constrained environments has been lost in commercial cultivars of legume grass, because breeding emphasis has been centered on dry matter production and persistence under non-restrictive conditions (*Papadopoulos et al., 1999; Vasiljević et al., 2008*). Many studies mention the advantages of the naturally formed populations of legumes, when searching for preadaptive and acclimatization potential to a stress abiotic influence. The genotypic characteristics achieved through ecotypic selection are: cold tolerance (*Finne, 2002*), tolerance to soils poor in phosphorus (*Spencer, 1980*), to soils of high concentrations of aluminium and manganese (*Mitev and Goranova, 2008*), drought tolerance (*Ayres et al., 1996*), hard seed character, providing self-reseeding (*Asci et al., 2011*). The ecotypes are also used as a source of other characteristics of importance to grazing use, such as: persistence, combined with productivity (*Piano and Annicchiarico, 1995;Goranova et al., 2005; Radović et al., 2008*), nutritional quality (*Goranova and Mihovsky, 2005*; *Vučković et al., 2007*), persistence (*Lugic et al., 2004*), good yield in the later stages of growth cycle (*Naydenova, 2008*). As a base of these facts, it could be mentioned that the high degree of intra-population variability and allogamy of perennial legumes allow a rapid genetic change in the genotype of the populations under natural or artificial breeding pressure (*Collins, 2002*). This adaptive variability of the ecotypes, being important to the breeding for grazing purposes, can be used directly, as well as it can be transferred into a cultural germplasm, using conventional crossing methods (*Garcia De Los Santos et al., 2001; Boller et al., 2012*). Up to now, the recombination breeding has been used most successively in white clover, to combine the high productivity of large leaved forms and the stoloniferous characteristics of wild-growing medium and small leaved ecotypes, which provide persistence and drought tolerance (*Jahufer et al., 1999*). In red clover there are also many contemporary varieties directly developed from ecotypes or after hybridization of ecotypes (*Lehmann et al., 1998; Boller, 2000*).

The genotypic result of the interaction between ecological conditions and mode of use are the cultivated ecotypes that have a long history of cultivation in a definite area, under a specific agronomic practice. The interactions in the system: pasture species – companion species in the pasture herbaceous community – grazing animals (kind, category, grazing style and behaviourial reactions) and the applied regime of use of the pasture are direct and strong. These interactions are of essential importance in the consolidation of the so-called pasture ecotypes. The

pasture ecotypes can also originate from varieties with a broad area of spread. *Bouton and Gates* (2003), *Moutray* (2000), as well as *Smith et al.* (1989) described procedures for breeding of pasture lucerne varieties through submitting populations of adapted varieties to continuous grazing. In this case, pasture ecotypes are developed through recurrent phenotypic selection. With regard to duration of use and yield the varieties bred in this way, due to good ecological adaptation, are equal or exceed the widespread varieties also in other regimes of use.

The interspecific interactions in the pasture mixture have a significant influence on the morphology (*Pineiro and Harris, 1978*), productivity, quality (*Wen et al., 2003*) and survival (*Guretzky et al., 2004*) of legume components. In this respect, the breeding strategy, based on the ecotypic selection, is considered to be very suitable, because the ecotypes develop in natural plant communities and their genotype is also a result of coevolution. The choice of components from jointly existing populations, with high "general ecological combination ability" is of crucial importance to establishment of productive pasture mixtures, in which the dynamic stability of the sward is maintained (*Hill, 1990*). *Collins and Rhodes* (1989), *Turkington* (1996) reported such results for white clover and perennial ryegrass. Similar data in breeding of pasture lucerne varieties were presented by *Berdahl et al.* (1986). According to them, the populations with natural adaptation to grazing, as well as to species and genotype of grass components in the mixture have the highest value.

Conclusion

Genetic providing of the functions, which are assigned to legumes in the pasture, requires from the breeding for grazing purposes to search for or to develop germplasm, in which persistence, forage quality, productivity and animal safety are positively related. The pasture persistence is also examined in the relation with grazing tolerance, plant morphology, adaptive potential and stress tolerance. The indirect breeding for pasture persistence includes also breeding for competitive ability and coadaptivity towards other pasture species and genotypes, as well as breeding for improved nitrogen fixation as a particularly important element of the adaptive ability. The breeding for grazing tolerance and persistence is concentrated also on the mechanisms of self-maintenance of the legumes in the pasture – vegetative reproduction, spread and self-seeding. Many breeding programmes are directed also to morphology providing better intake by the animals, to main and specific quality characteristics, to anti-nutrient factors, to adaptation to systems and practices of pasture establishment and use. The adaptive variability of the pasture ecotypes of perennial legumes are of prime importance in the breeding of pasture varieties.

Ciljevi i pristupi u proizvodnji višegodišnjih leguminoza za upotrebu na privremenim pasnjacima

G. Naydenova, Ts. Hristova, Y. Aleksiev

Rezime

Leguminoze su glavni element ekosistema travnjaka od čega zavisi kvalitet krmnih biljaka. Oplemenjivanje pašnjačkih sorti višegodišnjih leguminoza prvo ima za cilj postizanje tolerancije i upornosti komponente leguminoze u u pašnjaku. Kod vrsta koje imaju nisku prirodnu toleranciju ispaše (lucerka i crvena detelina), neophodno je sprovesti uzgoj za biološke, morfološke i fiziološke karakteristike, u direktnoj vezi sa tolerancijom ispaše. U vrstama koje imaju visoku toleranciju ispaše (bela detelina, žuti zvezdan, esparzeta), perzistencija se smatra funkcijom pojedinih morfoloških karakteristika, adaptivni potencijalom i tolerancijom na stres. Indirektno gajenje na perzistenciju pašnjaka uključuje i oplemenjivanje na konkurentsku sposobnost i koadaptivnost vrsta trave sa pašnjacima prilagođeno regionu i praksi kultivacije, kao i uzgoja na poboljšanju nodulaciju i fiksaciju azota kao važan element adaptivne sposobnosti. Oplemenjivanje na toleranciju ispaše i istrajnost je takođe koncentrisano na mehanizmima samostalnog održavanja mahunarki na pašnjacima - vegetativnog razmnožavanja, širi i samo-setve. Mnogi programi za uzgoj su takođe usmerena na morfologiju kojom se obezbeđuje bolji unos od strane životinja, na magistralne i specifične karakteristike kvaliteta, na faktor anti-nutrijenata, na prilagođavanje sistemu i praksi osnivanja i korišćenja pašnjaka. Strategije oplemenjivanja korišćenjem germplazme konsolidovana kroz specifične mehanizme adaptacije na nepovoljne ili određene agroekološke uslove i uticaje - tj kultivacije kroz ekotipičnu selekcije se smatra da je najefikasnija za svrhu ispaše. Interakcije u sistemu: pašnjačke vrste - prateće vrste u pašnjačkoj zajednici zeljastih biljaka - ispaša životinja (vrsta, kategorija, stil ispaše i bihaviurlne reakcije) i primenjeni režim korišćenja pašnjaka je direktan i jak. Ove interakcije su od suštinskog značaja u genotipskim diferencijacijama ekotipova pašnjaka. Adaptivna varijabilnost ekotipova pašnjaka se direktno koristi, kao i u rekombinantnom oplemenjivanju.

References

ABBATE V., GRESTA F., OCCHIPINTI A. (2003): An assessment of self-reseeding forage legume ecotypes collected in the native pasture of the Ragusa plateau (Sicily). Tecnica Agricola, 55, 3-20.
ABBERTON M. (2007): Interspecific hybridization in the genus Trifolium. Plant Breeding, 126, 337-342.
ACHARYA S. (2006): New Forage Options for Western Canada. WCDS Advances in Dairy Technology, 18, 79-89.
AERTS R., BARRY T., MCNABB W. (1999): Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. Agriculture, Ecosystems & Environment, 75, 1–12.
ANDERSON J. A., TAYLOR N. L., WILLIAMS E. G. (1991): Cytology and fertility of the interspecific hybrid *Trifolium ambiguum* × *T. repens* and backcross populations. Crop Science, 31, 683–687.
ANNICCHIARICO P. (1997): Indirect selection for persistence and seed yield in large-leaved white clover. Plant Breeding, 116, 192-194.
ANNICCHIARICO P. (2012): Agronomic value and morphophysiological diversity of non-Ladino Italian white clover wild populations compared with variety germplasm. Crop and Pasture Science, 63, 377–388.
ANNICCHIARICO P., PIANO E. (2004): Indirect selection for root development of white clover and implications for drought tolerance. Journal Agronomy and Crop Science, 190, 28–34.
ANNICCHIARICO P., SCOTTI C., CARELLI M., PECETTI L. (2010): Questions and avenues for lucerne improvement. Czech Journal of Genetics and Plant Breeding, 46 (1), 1–13.
ARRIGO Y., DOHME F. (2009): Sainfoin versus alfalfa as supplements for grazing dairy cows. Revue suisse d'agriculturev., 41(5), 283-288.
ASCI O., ACAR Z., AYAN I., BASARAN U., MUT H. (2011): Effect of pretreatments on seed germination rate of red clover (*Trifolium pratense L.*) populations. African Journal of Agricultural Research, 6(13), 3055-3060.
AYRES J., CARADUS J., LANE L., MURISON R. (1996): White clover breeding for dryland sheep and cattle pastures in Australia. In Woodfield (ed) White clover: New Zealand's competitive edge pp. 155-158.
AYRES J., KELMAN W., WIEDEMANN S., LANE L., MCCORKELL B. (2008): Developing birdsfoot trefoil (Lotus corniculatus L.) varieties for permanent pasture applications in low latitude regions of eastern Australia. Australian Journal of Experimental Agriculture, 48, 488–498.
BASIGALUP, D., CASTELL C., GIAVENO C. (2003): Response to selection for lower initial rate of dry matter disappearance in the development of a bloat-tolerant

non-dormant alfalfa population (Medicago sativa L.). Journal of Genetic and Breeding, 57(1), 31-37.
BERDAHL J., WILTON A., FRANK A. (1989): Survival and agronomic performance of 25 alfalfa cultivars and strains interseeded into rangeland. Journal of Range Management, 42, 312–316.
BERDAHL, J., WILTON A., LORENZ R., FRANK A. (1986): Alfalfa survival and vigor in rangeland grazed by sheep. Journal of Range Management, 39, 59-62.
BERG B., MAJAK W., MCALLISTER T., HALL J., MCCARTNEY D., COULMAN B., GOPLEN B., ACHARYA S., TAIT R., CHENG K. (2000): Bloat in cattle grazing alfalfa cultivars selected for a low initial rate of digestion: A review. Canadian Journal of Plant Science, 80, 493–502.
BEUSELINCK P. (1989): Lotus spp. expedition in Morocco. Lotus Newsletter, 20, 31.
BEUSELINCK P. (2004) :Registration of 'ARS-2424' birdsfoot trefoil germplasm. Crop Science, 44, 2277–2278.
BEUSELINCK R., BRUMMER E., VIANDS D., ASAY K., SMITH R., STEINER J., BRAUER D. (2005): Genotype and environment affect rhizome growth of birdsfoot trefoil. Crop Science, 45,1736–1740.
BITTMAN S., WADDINGTON J., MCCARTNEY D. (1991): Performance of alfalfa strains grown in mixture with smooth bromegrass as affected by management. Canadian Journal of Plant Science, 71, 1029–1037.
BLUMENTHAL M., MCGRAW R. (1999): Lotus adaptation, use, and management. The science and technology of Lotus. CSSA Special Publication, 28, 97-119.
BOLLER B., (2000): Corvus and Pica, zwei neue "sozialere" matenkleesorten. Agrarforschung, 3, 484-489.
BOLLER B., TANNER P., SCHUBIGER F. (2012): Pastor – a new red clover suitable for grazing. Agrarforschung Schweiz, 3(1), 20-27.

BOUTON J., GATES R. (2003): Grazing-tolerant alfalfa cultivars perform well under rotational stocking and hay management. Agronomy Journal, 95(6), 1461–1464.
BOUTON J. (2012): Breeding lucerne for persistence. Crop & Pasture science, 63, 95–106.
BOUTON J., WOODFIELD D., HOVELAND C., MCCANN M., CARADUS J. (2005): Enhanced survival and animal performance from ecotype derived white clover cultivars. Crop Science, 45, 1596–1602.
BROCK J. HYSLOP M., WIDDUP K. (2003): A review of red and white clovers in the dry land environment. In: Legumes for dryland pastures. Proceedings of a New Zealand Grassland Association (Inc.), 2003, Grassland Research and Practice Series, 11, 101-107.

CANTERO BONILLA M. (1993): Effect of upland lucerne grazing in Tierra de Campos on physiology and morphology aspects of reproduction system of Manchega breed ewes. Tesis (Doctor en Veterinaria). Il., graf.; Bibliografia pp. 163-189.
CARADUS J., WOODFIELD D., STEWART A. (1995): Overview and vision for white clover. Agronomy Society of New Zealand. Special Publication, 11/ Grassland Research and Practice series, 6.
CARLIER L., VLAHOVA M., TODOROV M., MIHOVSKY TS. (2008): Importance of grasslands. Journal of Mountain Agriculture on the Balkans, 11(3), 534-555.
CARR P., POLAND W., TISOR L., (2005A): Natural reseeding by forage legumes following wheat in Western North Dakota. Agronomy Journal, 97(4),1270-1277.
CARR P., POLAND W. AND TISOR L., (2005B): Forage legume regeneration from the soil seed bank in Western North Dakota. Agronomy Journal, 97(2), 505-513.
COLLINGE D., HUGHES M. (1982): Developmental and physiological studies on the cyanogenic glucosides of white clover (*Trifolium repens L.*). Journal of Experimental Botany, 33(1), 154-161.
COLLINS R., RHODES I. (1989): Yield of white clover populations in mixture with contrasting perennial ryegrasses, Grass and Forage Science, 44(1), 111-115.
COLLINS R. (2002): Effects of drought stress and winer stress on the persistence of white clover. Interregional and cooperative research and development network on pastures and fodder crop production. Lowland grasslands subnetwork. Food and agriculture organization of the United Nations Rome, 2002.
COSGROVE G., BROUGHAM R. (1985): Grazing management influences on seasonality and performance of ryegrass and redcloverin a mixture. In: Proceedings of the New Zealand grassland association, 46, 71-76.
DEMDOUM S., MUÑOZ F., DELGADO I. (2010): Forage production of a collection of sainfoin over a three year period. In: The contributions of grasslands to the conservation of Mediterranean biodiversity. Options Méditerranéennes, 92,101-104.
DEWHURST R., DELABY L., MOLONEY A., BOLAND T., LEWIS E. (2009): Nutritive value of forage legumes used for grazing and silage. Irish Journal of Agricultural and Food Research, 48, 167–187.
DOUCETTE K., WITTENBERG K., MCCAUGHEY W. (2001): Seed recovery and germination of reseeded species fed to Cattle. Journal of Range Management, 54, 575–581.
ESCARAY F., MENENDEZ A., GARRIZ A., PIECKENSTAIN F., ESTRELLA M., CASTAGNO L., CARRASCO P., SANJUAN J., RUIZ O. (2012): Ecological and agronomic importance of the plant genus Lotus. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. Plant Science, 182, 121– 133.

FINNE M. (2002): Genetic variation in a Norwegian germplasm collection of white clover (*Trifolium repens L.*). Interregional and cooperative research and development network on pastures and fodder crop production. Lowland grasslands subnetwork. Food and agriculture organization of the United Nations Rome, 2002.
FORD J., BARRETT B. (2011): Improving red clover persistence under grazing. In: Proceedings of the New Zealand Grassland Association, 73,119-124.
FRANCIS C., QUINLIVAN B. (1974): Selection for formononetin content in red clover (*Trifolium pratense L.*). Plant introduction, Breeding and Seed Production, pp. 84-88.
FRASER M., SPEIJERS M., THEOBALD V., FYCHAN R., JONES R. (2004): Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial ryegrass swards. Grass and Forage Science, 59, 345–356.
GARCIA DE LOS SANTOS G., STEINER J., BEUSELINCK P. (2001): Adaptive ecology of Lotus corniculatus L. Genotipes: I.I. Crossing ability, Crop Science, 41, 564–570.
GEORGE J. (1984): Grass sward improvement by frost-seeding with legumes. In: Proceedings of the American forage and grassland conference, Houston, TX, 1984, pp. 265-269.
GOPLEN B. (1972): Breeding for low soluble fraction I (nonbloating) alfalfa. In: Report of the twenty third alfalfa improvement conference, Ottawa, Ontario, Canada, 1972, pp. 20-21.
GOPLEN, B., HOWARTH R. (1976): Breeding a non-bloating alfalfa cultivar. In: Report of the twenty fifth alfalfa improvement conference, Ithaca, New York, USA, 1976, pp. 49.
GORANOVA G., MIHOVSKY Ts., GEORGIEV V. (2005): Breeding evaluation of diploid red clover accessions (*Tr. pratense L.*). Plant Science, 42, 160-164.
GORANOVA G., MIHOVSKY Ts. (2005): Phenotypic evaluation of variability in bioproductive and quality traits of European and Asian accessions of red clover (*Tr. pratense L.*). Ecology and Future, 4, 88-93.
GOSDEN A., DAVIES W., JONES R. (1979): Selection for low and high formononetin: a phyto-oestrogen of red clover (*Trifolium pratense*). Biuletyn instytutu hodowli i aklimatyzacji roslin, 135(1), 328-333.
GRAY F., SHIGAKI T., KOCH D., DELANEY R., GRAY A., MAJERUS M., CASH D, DITTERLINE R., WICHMAN D. (2006): Registration of 'Shoshone' Sainfoin. Crop Science, 46, 988.
GURETZKY J., MOORE K., KNAPP A., BRUMMER E. (2004): Emergence and survival of legumes seeded into pastures varying in landscape position. Crop Science, 44, 227–233.
HANCOCK K., COLLETTE V., FRASER K., GREIG M., XUE H., RICHARDSON K., JONES C., RASMUSSEN S. (2012): Expression of the R2R3–MYB transcription factor tamyb14 from T. arvense activates proanthocyanidin

biosynthesis in the legumes *Trifolium repens* and *Medicago sativa*. Plant Physiology, 159, 1204–1220.
HEICHEL G., HENJUM K. (1991): Dinitrogen fixation, N transfer and productivity of forage legume-grass communities. Crop Science, 31, 202–208.
HEINRICHS D. (1973): Time factor in expression of the creeping-rooted character in alfalfa. Canadian Journal of Plant Science, 53, 511-514.
HERRMANN D., BOLLER B., STUDER B., WIDMER F., KÖLLIKER R. (2007): Improving persistence in red clover: insights from QTL analysis and comparative phenotypic evaluation. Crop Science, 48, 269–277.
HILL J. (1990): The three C's – competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. Theoretical and Applied Genetics, 79(2),168-176.
HYSLOP M., KEMP P., HODGSON J. (1998): Preliminary investigations into persistence of a vegetatively reproductive red clover cv. Astred in mixeds wards. In: Proceeding Annual Conference Agronomy Society of New Zealand, 28: 117-122.
JAHUFER M., COOPER M., AYRES J., BRAY R. (2002): Identification of research to improve the efficiency of conventional breeding strategies for white clover in Australia. Australian Journal of Agricultural Research, 53, 239-257.
JAHUFER M., COOPER M., BRAY R., AYRES J. (1999): Evaluation of white clover (*Trifolium repens L*.) populations for summer moisture stress adaptation in Australia. Australian Journal of Agricultural Research, 50(4), 561-574.
JAHUFER M., NICHOLS S., CRUSH R., OUYANG L., DUNN A., FORD J., CARE D., GRIFFITHS A., JONES C.S., JONES C.G. (2008): Genotypic variation for root trait morphology in a white clover mapping population grown in sand. Crop Science, 48, 487–494.
KALLENBACH R., MCGRAW R., BEUSELINCK P., ROBERTS C. (2001): Summer and autumn growth of rhizomatous birdsfoot trefoil. Agronomy Journal, 41, 149–156.
KATEPA-MUPONDWA F., SINGH A., SMITH S. JR., MCCAUGHEY W. (2002): Grazing tolerance of alfalfa (Medicago spp.) under continuous and rotational stocking systems in pure stands and in mixture with meadow bromegrass (*Bromus riparius Rehm. syn. B. biebersteinii Roem & Schult*). Canadian Journal of Plant Science, 82, 337–347.
LAURIAULT L., KIRKSEY R., DONART G., SAWYER J., VAN LEEUWEN D. (2005): Pasture and stocker cattle performance on furrow-irrigated alfalfa-tall wheatgrass pastures, southern High Plains, USA. Crop Science, 45, 305–315.
LEE M., TWEED J., MINCHIN F., WINTERS A. (2009): Red clover polyphenol oxidase: Activation, activity and efficacy under grazing. Animal Feed Science and Technology, 149(3), 250-264.
LEHMANN J., BRINER H., MOSIMANN E. (1998): Rotklee und wiesenschwingelsorten in Prufung. Agrarforschung, 5, 177-180.

LUGIC Z., IGNJATOVIC S., LAZAREVIC D., RADOVIC J., DINIC B. (2004): Investigation of some important characteristics of different genotypes of white clover (*Trifolium repens L.*) in agroecological conditions of Serbia. Journal of Mountain Agriculture on the Balkans, 7(5), 518-525.

MARSHALL A., ABBERTON M., MICHAELSON-YEATES T., RHODES I., WILLIAMS, T. (2001): Drought tolerance of interspecific hybrids between *T. repens and T. ambiguum*. In: Gomide, Mattos, Carneiro da Silva (eds) Grassland ecosystems: an outlook into the 21st century. Proceedings 19th International Grassland Congress, Sao Paulo, Brazil, 2001, pp. 528-529.

MARTEN G., JORDAN R., RISTAU E. (1990): Performance and adverse response of sheep during grazing of four legumes. Crop Science, 30(4), 860-866.

MARTINIELLO P. (2005): Recurrent phenotypic selection of legumes and grass perennial forage crops using Mediterranean autochthonous germplasm. Journal of Genetics and Breeding, 59, 3–4.

MEREDITH M., MICHAELSON-YEATES T., OUGHAM H., THOMAS H. (1995): *Trifolium ambiguum* as a source of variation in the breeding of white clover. Euphytica, 82, 185-191.

MERKER A. (1991): Hybrids between *Trifolium medium* and *Trifolium pratense*. Hereditas, 101, 267-266.

MIHOVSKY TS., GORANOVA G. (2006): Comparative evaluation of different varieties of white clover (*Trifolium repens L.*) under the conditions of the foothill region of central Balkan mountain. Plant Science (Bg), 43(1), 57-61.

MITEV D., GORANOVA G. (2008): Sustainable developmentof a mixed sward of red fecue, Kentucky bluegrass and Lucerne on the slopes of the Central Balkan mountains. Journal of Mountain Agriculture on the Balkans, 11(2), 249-258.

MORRILL W., DITTERLINE R., CASH S. (1998): Insect pests and associated root pathogens of sainfoin in western USA. Field Crops Research, 59(2), 129-134.

MORRIS J., GREENE S. (2001): Defining a multiple-use germplasm collection for the genus Trifolium. Crop Science, 41, 893-901.

MOUTRAY J. (2000): The future of alfalfa as a grazing crop: Grazing tolerance. In: M. Phillips et al. (eds). Proc. Am. Forage and Grassl. Council and 37th North Am. Alfalfa Improvement Conf., Madison, WI., 2000, pp. 345–350.

MOWREY D., MATCHES A. (1991): Persistence of sainfoin under different grazing regimes. Agronomy Journal, 83(4), 714-716.

MOWREY D., VOLESKY J. (1993): Feasibility of grazing sainfoin on the southern Great Plains. Journal of Range Management, 46(6), 539-542.

NAYDENOVA G. (2008): Productive, biological and morphological parameters of breeding populations of red clover. Plant Science, 45, 230-235.

PANDEY K., GRANT J., WILLIAMS E. (1987): Interspecific hybridisation between *Trifolium repens and T. uniflorum.* Australian Journal of Botany, 35, 171–182.

PAPADOPOULOS Y., KELMAN W., BEUSELINCK P. (1999): Traditional breeding of *Lotus* species. CSSA Special Publication, 28, 187–198.
PAPLAUSKIENË V., SPRAINAITIS A. (2003): Variability of cyanogenic glucoside in white clover plants. Biolologija, 1, 85-88.
PEDERSON G., MCLAUGHLIN M. (1989): Resistance to viruses in Trifolium interspecific hybrids related to white clover. Plant Disease, 73, 997–999.
PEDERSSON G., BRINK G. (1997): Seed production of pasture-collected white clover populations vs.cultivars. In: M. Williams (ed.) Proc. Am. Forage Grassl.Counc il Ft. Worth, TX,1997 pp. 279-279.
PENNING P., PARSONS A., ORR R., HARVEY A., CHAMPION R. (1995): Intake and behaviour responses by sheep, in different physiological states, when grazing monocultures of grass or white clover. Applied Animal Behaviour Science, 45, 63–78.
PIANO E., ANNICCHIARICO P. (1995): Persistence of Ladino white clover ecotypes and its relationship with other agronomic traits. Grass & Forage Science, 50(3), 195-198.
PIANO E., VALENTINI P., PECETTI L., ROMANI M. (1996): Evaluation of a lucerne germplasm collection in relation to traits conferring grazing tolerance. Euphytica, 89(2), 279-288.
PINEIRO J., HARRIS V. (1978): Performance of mixtures of ryegrass cultivars and prairie grass with red clover cultivars under two grazing frequencies. II. Shoot populations and natural reseeding of prairie grass. New Zealand Journal of Agricultural Research, 21, 665-673.
POPP J., MCCAUGHEY W., THOMAS L., COHEN R. (1999): Nutrient selection by cattle from grass and grass/legume pastures. Canadian Journal of Animal Science, 79, 391-395.
RADOVIĆ J., LUGIĆ Z., SOKOLOVIĆ D., ŠTRBANOVIĆ R., VASIĆ T. (2008): Breeding of white clover (*Trifolium repens L.*) and birdsfoot trefoil (*Lotus corniculatus L.*) in Serbia. In: Grain Legumes Technology Transfer Platform (GL-TTP) Workshop, 2, Novi Sad, Serbia, pp. 27-28.
RIDAY H. (2006): Breeding for improved frost-seeded red clover seedling establishment. In: Mercer C. (ed) Proceedings of Advances in pasture plant breeding, Christchurch, New Zealand, 2006, pp. 141-144.
RIDAY H. (2008): Heritability of frost-seeded red clover establishment. Euphytica, 163(1), 81-87.
RUMBALL W., KEOGH R. MILLER J. (2003): 'Crossway' and 'Grasslands Broadway' red clovers (Trifolium pratense L.). New Zealand Journal of Agricultural Research, 46, 57-59.
SANDERSON M., BYERS R., SKINNER R., ELWINGER G. (2003): Growth and complexity of white clover stolons in response to biotic and abiotic stress. Crop Science, 43, 2197–2205.

SAWAI A., YAMAGUCHI H., UCHIYAMA K. (1995): Fertility and morphology of the hromosome-doubled hybrid *T. medium* × *T. pratense* (red clover) and backcross progeny. J. Jpn. Soc. Grassl.Sci., 41(2), 122–127.
SCHEFFER-BASSO S., BRUSTOLIN R., DALL'AGNOL M. (2011): Performance of *Lotus corniculatus* L. genotypes submitted to cutting interval: subsidies to a breeding program. Revista Brasileira de Zootecnia, 40(8), http://dx.doi.org/10.1590/S1516-35982011000800004.
SEWELL J., HILL R., REICH J. (2011): Persistence of Grazing Tolerant Lucernes under Australian. Conditions. Pasture Persistence. Grassland Research and Practice Series, 15, 187-190.
SLEUGH B., MOORE K., GEORGE J., BRUMMER E. (2000): Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. Agronomy Journal, 92(1), 24–29.
SMITH R., BISHOP D. (1993): Astred - a stoloniferous red clover. In: Proceeding of international grassland congress, Palmerston, North New Zealand, 1993, 17, 421-423.
SMITH S., BOUTON J., HOVELAND C. (1989): Alfalfa Persistence and Regrowth Potential under Continuous Grazing. Agronomy Journal, 81(6), 960-965.
SMITH S., BOUTON J., SINGH A., MCCAUGHEY W. (2000): Development and evaluation of grazing-tolerant alfalfa cultivars: A review. Canadian Journal of Plant Science, 80(3), 503-512.
SPENCER K., GOVAARS A., HELY F. (1980): Early phosphorus nutrition of eight forms of two clover species, *Trifolium ambiguum and T. repens*. New Zealand Journal of Agricultural Research, 23(4), 457-475.
TAYLOR J., ROBINSON G., HEDGES D., WHALLEY R. (1987): Camping and faeces distribution by Merino sheep. Applied Animal Behaviour Science, 17(3-4), 273-288.
TAYLOR N. (2008): A century of clover breeding developments in the United States. Crop Science, 48, 1–13.
TEMPERTON V. (2007): Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. Oecologia, 151, 190–205.
TURKINGTON R.(1996): Intergenotypic interactions in plant mixtures. Euphytica, 92, 105-119.
VASILJEVIĆ S., LUGIĆ Z., ŠURLAN-MOMIROVIĆ G., IVANOVIĆ M. (2008): Characteristics of special importance in red clover (*Trifolium pratensis L.*) breeding. In: Grain Legumes Technology Transfer Platform (GL-TTP) Workshop, 2, Novi Sad, Serbia, 2008, pp. 86-87.
VUČKOVIĆ S., STOJANOVIĆ I., PRODANOVIĆ S., ĆUPINA B., ŽIVANOVIĆ T., VOJIN S., JELAČIĆ S. (2007): Morphological and nutritional properties of birdsfoot trefoil (*Lotus corniculatus L.*) autochthonus populations in Serbia and Bosnia and Herzegovina. Genetic Resources and Crop Evolution, 54, 421-428.

WANG Y., BARBIERI L., BERG B., MCALLISTER T. (2007): Effects of mixing sainfoin with alfalfa on ensiling, ruminal fermentation and total tract digestion of silage. Animal Feed Science and Technology, 135, 296–314.
WEIHER N., KRAWUTSCHE M., GIERUS M., TAUBE F. (2010): Influence of genotype and mechanical stress on the specific polyphenol oxidase activity in pure red clover swards. Grassland Science in Europe, 15, 419-421.
WEN L., ROBERTS C., WILLIAMS J., KALLENBACH R., BEUSELINCK P., MCGRAW R. (2003): Condensed tannin concentration of rhizomatous and nonrhizomatous birdsfoot trefoil in grazed mixtures and monocultures. Crop Science, 43, 302–306.
WIDDUP K., BARRETT B. (2011): Achieving persistence and productivity in white clover pasture persistence. Grassland Research and Practice Series, 15, 173-180.
WINTERS A., MINCHIN F. (2001): Red clover and the future for pasture legumes as an alternative protein source for ruminants. IGER Innovations, pp. 30-33.
WU Z., SATTER L., KANNEGANTI V., PARIZA M. (1997): Paddocks containing red clover compared to all grass paddocks support high cla levels in milk. In: USDFRC Research Summary, Agricultural Research Service, USDA, 1997, pp. 94-95.

Received 22 April 2013; accepted for publication 15 June 2013

Biotechnology in Animal Husbandry 29 (2), p 251-257 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 637.5'62
DOI: 10.2298/BAH1302251P

THE INFLUENCE OF THE BEEF BULL BREED ON CARCASS WEIGHT AND YIELD OF MALE OFFSPRING

R. Petraškienė[1], N. Pečiulaitienė[2], V. Jukna[2], E. Meškinytė-Kaušilienė[2], J. Klementavičiūtė[2], V. Valaitienė[2]

[1]Lithuanian cattle breeders association**,** Kalvarijos 128, Kaunas LT-46403, Lithuania
[2] Lithuanian Health Sciences University, Veterinary Academy, Laboratory of Meat Characteristics and Quality Assessment, Tilžės 18, Kaunas LT-47181, Lithuania
*Corresponding author: *nijole@lva.lt*
** Original scientific paper

Abstract: The aim of this study was to investigate of beef breeds - Hereford (HE), Charolais (CH) Limousine (LI), Simmental (SM), Belgian Blue (BB) and Salers (SA) - of the bulls, which are used for dairy cows and heifers insemination, influence on male sex offspring carcass weight and yield. In this article, for analysis were used of all in 7 months in one slaughterhouse slaughtered bulls 12-30 months of age data. Of these 689 crossbreeds, whose cow was dairy and bull was beef breed. For this study was used only crossbreeds (689 bulls) data. This is the thirty-six (36) bull offspring from 546 farms. Bulls were divided into groups according to age during slaughter (12-18 months, 18-24 months, 24-30 months). Studies have shown, that the father's breed at different growth periods had a different effect on carcass traits of crossbreeds. Therefore, the crossbreeding use on purpose to improve dairy breeds offspring carcass characteristics, it is necessary to carefully choose the right breed for crossbreeding, bearing in mind, what age of the offspring will be slaughtered.

Key words: beef cattle, bulls, offspring, carcass yield

Introduction

In Lithuania most part of the beef is obtained from dairy cattle. Meat composed small part of purebred beef cattle and their crossbreeds. In order to improve meat quality obtained from dairy cattle, crossbreeding is widely carried out of dairy cows in a lower quality of the breeding value with beef breeds bulls.

When animals grow, due to differences in growing intensive individual tissues and organs, carcass yield is increasing. The more muscle and fat tissue in carcass, the better it is (*Wajda et al., 2002; Berg et al., 2003; Alberti et al., 2005*). In order that the bulls to grow quickly and well develop their muscles, they must be

reared intensively. The bulls fairly well fed fastest growing from birth to 12 months of age. The higher weight is of bull and the heavier and better quality is carcass. Carcass yield is carcass weight and of mass before slaughter ratio as a percentage. This is an important indicator of cattle production. Cattle carcass yield and morphological composition depends on the breed, sex, of feeding different periods of growth, of the individual traits of animals, of body condition and age *(Ingr, et al., 1989; Groth et al., 1999, Miller et al., 2002, Jukna et al., 2008*). The largest carcass yield are of beef cattle (67-70 percent). Intensively grown of many dairy breed bulls weigh 450-500 kg and thier carcass yield composed about 54-55 percent. The bulls carcass yield are higher than heifers and heifers - than the cows. The biggest yield of the carcass is from grown-up and good condition animals (*Crews et al., 2003; Pečiulaitis et al., 2007; Jukna et al., 2010*). During the all growing period, abundantly full-fledged rations of fed cattle carcass yield is higher. It has very depends on the animal body condition. Morphological composition of meat depends on individual tissues ratio, the most important are muscle, fat and bone tissues (*Jukna et al., 2006*).

The aim of this study was to investigate of beef breeds - Hereford (HE), Charolais (CH) Limousine (LI), Simmental (SM), Belgian Blue (BB) and Salers (SA) - of the bulls, which are used for dairy cows and heifers insemination, influence on male sex offspring carcass weight and yield.

Material and methods

In this article, for analysis data were used for slaughtered bulls 12-30 months of age during the period of 7 months, in the same conditions. Total of 2233 bulls, which pedigree could be accurately determined, were used. Of these 689 crossbreeds, whose cow was dairy and bull was beef breed. For this study was used only crossbreeds (689 bulls) data. This is the thirty-six (36) bull offspring from 546 farms.

Bulls were divided into groups according to age during slaughter (12-18 months, 18-24 months, 24-30 months). The Belgian Blue breed bulls offspring have not been from 24-30 months age category because the offspring of this breed are usually slaughtered younger (before reaching your desired weight).

Table 1. Breeds used in the study and number of animals

Breed	Number of bull-parent	Number of slaughtered bulls
Hereford	6	85
Charolais	9	196
Limousine	10	268
Beef Simmental	2	28
Belgian Blue	6	30
Salers	3	82
Total	**36**	**689**

During slaughter was determined the animal's mass before slaughter, animal weighing, warm carcass mass and carcass yield.

Carcass yield calculated by the following formula: H = (S * 100) / G, where H - carcass yield,% S - warm carcass weight, kg G - animal mass until slaughter, kg.

The mean (X), the standard error (mx) were calculated for each trait.

The reliability (p) of the difference between the arithmetic means of the two groups was determined by Student's (t) criteria.

Data processed with statistical package R, version 2.01. (*Gentlemen, Ihaka, 1997)*.

Results and discussion

Studies have shown, that breed of bull‘s has affected offspring carcass mass and yield. From the data presented in Figure 1 seen, that the bulls grown on 12 to 18 months age carcass mass largest 253.6±2.3 kg was obtained of the Belgian Blue breeds bulls, whereas the least 224.0±2.1 kg Hereford bulls offspring ($p<0.025$). The difference amounted to 29.6 kg or 13.2 percent. Comparing the bulls groups from 18 to 24 months age difference of carcass mass of heaviness between the groups was determined the least and amounted to only 6.9 percent (as well as between the Belgian Blue and Hereford bulls offspring, $p>0.05$).

The most notable carcass mass differences were obtained between the groups of bulls in the age period from 24 to 30 months. The largest difference was determined between Charolais and Hereford bulls offspring groups (Belgian Blue breeds bulls offspring were not available in this category). Charolais bulls offspring carcass were 62.2 kg or 29.4 percent heavier than Hereford breeds bulls offspring, ($p<0.01$). Our research obtained data about beef breed's bull influence of offspring carcass mass and quality coincident with the data of other researchers (*Serra et al., 2004; Ozluturk et al., 2004*).

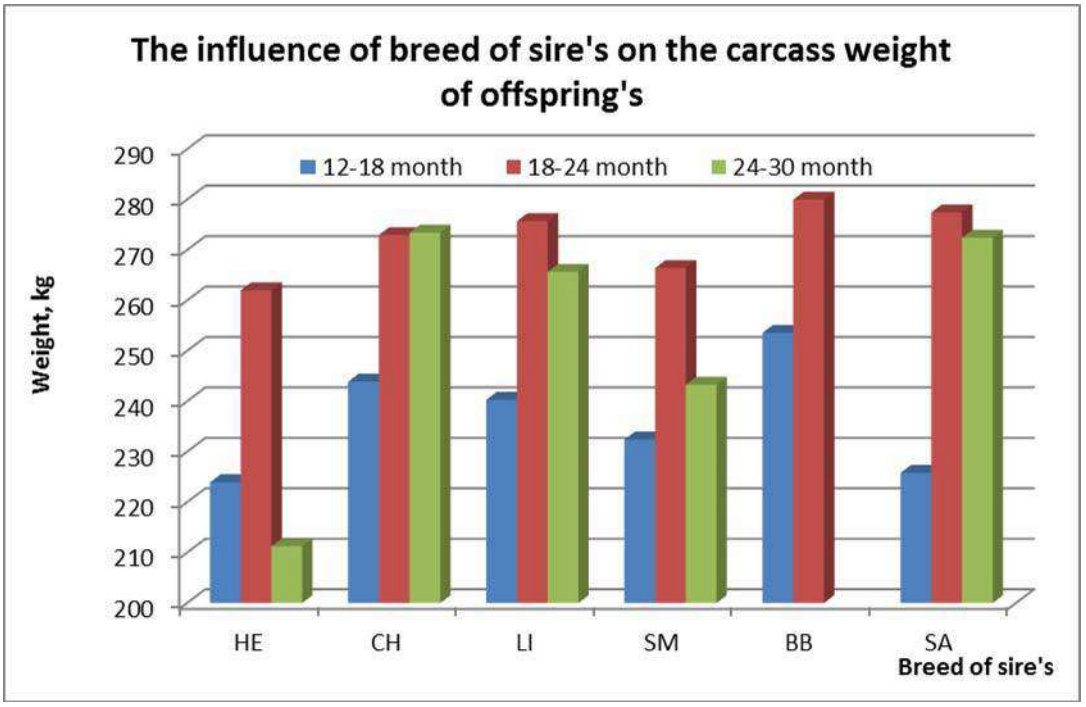


Figure1. The influence of sire breed on the carcass weight of their offspring

From the data presented in Figure 2 seen, that beef breed's bulls influence to offspring carcass yield was also established significant. The bulls have reached from 12 to 18 months age of carcass yield greater was obtained 2.8 percent of Limousine breeds offspring group than in the Belgian Blue and Simmental breeds, $p<0.01$. The highest differences of carcass yield percent were established from 18 to 24 months in the bulls age group between the Limousine and Hereford breed bulls groups. The difference amounted to 2.3 percent, $p<0.005$. In other bulls of the same age groups, carcass yield percent obtained the similar. Compared with other age bulls groups, the highest carcass yield percent were found in the age group from 24 to 30 months of Limousine breed and the lowest Simmental. The difference between the Limousine and Simmental crossbreeds were composed 5.1 percent, $p<0.005$. For crossbreeding using of Hereford, Charolais, Limousine and Salers breeds bulls, the best carcass yield of offspring was obtained slaughter from 18 to 24 months age. While, using for crossbreeding with Belgian Blue breed bulls, offspring carcass yield the best then some offspring reaches from 24 to 30 months age. In this study we determined a significant bull's breed effect on carcass traits of offspring, and this consistent with the data of other researchers (*Koch et. al., 1989; Jukna Č., Jukna V., 2002; Serra et al., 2004; Levachin et al., 2008*).

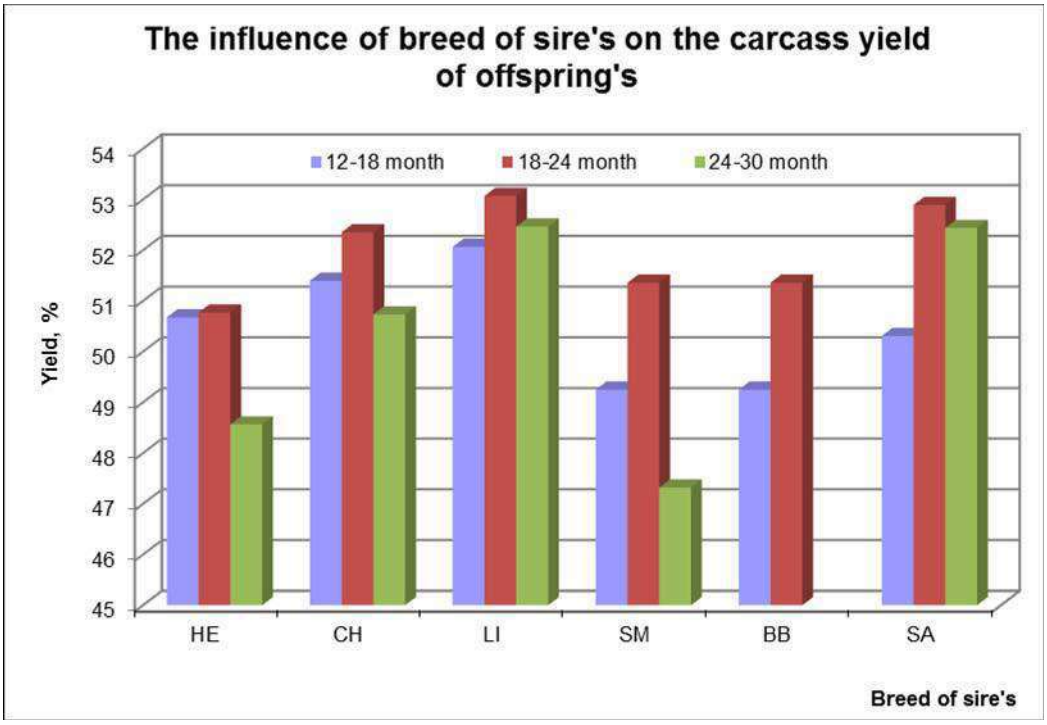


Figure2. The influence of sire breed on the carcass yield of their offspring

Conclusions

To sum up the research data can draw the following conclusions: that the father's breed at different growth periods had a different effect on carcass traits of crossbreeds. Therefore, the crossbreeding use on purpose to improve dairy breeds offspring carcass characteristics, it is necessary to carefully choose the right breed for crossbreeding, bearing in mind, what age of the offspring will be slaughtered.

Uticaj rase priplodnih bikova na težinu trupa i prinos mesa kod muških potomoka

R. Petraškienė, N. Pečiulaitienė, V. Jukna, E. Meškinytė-Kaušilienė, J. Klementavičiūtė, V. Valaitienė

Rezime

Cilj ovog istraživanja bio je da se ispita rasa bikova - hereford (HE), šarole (CH) limuzina (LI), simental (SM), belgijska plava (BB) i salers (SA) - koje se koriste za odgoj mlečnih krava i junica u osemenjavanju, uticaj na muške potomke, odnosno težinu trupa i prinos mesa/randman. U ovom radu, za analizu su korišćeni podaci za zaklana goveda starosti 12-30 meseci u periodu od 7 meseci, dobijeni u jednoj klanici. U ispitivanje je uključeno 689 meleza, čije majke su bile mlečne krava a očevi, bikovi tovnih rasa. U ovom istraživanju su korišćeni samo podaci za meleze (689 junadi), odnosno potomstvo trideset šest (36) bikova sa 546 farme. Bikovi su bili podeljeni u grupe prema uzrastu prilikom klanja (12-18 meseci, 18-24 meseci, 24-30 meseci).

Istraživanja su pokazala, da je očeva rasa u različitim periodima rasta imali drugačiji uticaj na osobine trupa meleza. Dakle, upotreba ukrštanja za ciljano poboljšanje karakteristika trupa potomstva mlečnih rasa, potrebno je pažljivo odabrati pravu rasu za ukrštanje, imajući u vidu starost odn. uzrast potomaka pri klanju.

References

ALBERTI P., RIPOLL G., GOYACHE F., LAHOZ F., OLEETA J. L., PANEA B., SANUDO C. (2005): Carcass characterization of seven Spanish beef breeds slaughtered at two commercial weights. Animal Science. 71, 514-521.

BERG R. T. BUTTERFIELD R. M. (2003): Growth patterns of bovine muscle, fat and bone. Animal Science. 27, 611-619.

CREWS D. H., POLLAK E. J., WAEBER R., QUAAS R. L., LIPSEY R. J. (2003): Genetic parameters for carcass traits and their live animal indicators in Simmental cattle. Animal Science. 81, 1427-1433.

FREITAS A. F., WILCOX C. J., COSTA C. N. (1998): Breed Group Effects on Milk Production of Brazilian Crossbred Dairy Cows. Dairy Science. 81, 2306-2311

GENTLEMEN R., IHAKA R. (1997): Notes on R: A programming environment for data analysis and graphics. Department of statistics university of Auckland.

GROTH Z., WIELGOSZ-GROTH., KIJAK Z., PAGORZELSKA J., WRONSKI M. (1999): Comparison of meat quality in yong Black and White breed bulls and their hybrids with beef breeds. Animal and Feed Science. 8, 145-156.

INGR I., PAUL A., STEINBAUSEROVA M. (1989): Vyskyt DFD masa u bucku z beznych porazek. Zivocisna Vyroba. 34, (7) 619-626.

JUKNA Č., JUKNA V. (2002): Priešskerdiminės masės įtaka galvijų skerdenų ir mėsos kokybei. Žemės ūkio mokslai. 4, 28-32.
JUKNA Č., JUKNA V., PEČIULAITIENĖ N. (2006): Lietuvos juodmargių bulių įtaka palikuonių penėjimosi ir mėsinėms savybėms. Veterinarija ir zootechnika. 36 (58), 27 –29.
JUKNA Č., JUKNA V., PEČIULAITIENĖ N., KLEMENTAVIČIŪTĖ J. (2008): Galvijų raumenų elektros laidumas ir jo ryšys su mėsos kokybe. Veterinarija ir zootechnika. 41 (63), 60–63.
JUKNA Č., JUKNA V., PEČIULAITIENĖ N., MEŠKINYTĖ-KAUŠILIENĖ E. (2010): The influence of bull's genotype to production crossing effectiveness. Baltic Animal Breeding. XV Conference. Riga 31st May - 1st June.
KOCH R. M., DIKEMAN M. E., LIPSEY R. J., ALLEN D. M., CROUSE J. D. (1989): Characterization of biological types of cattle - cycle II: III. carcass composition, quality and palatability. Animal Science. 43, 48-62.
LEVACHIN V.Y., SYRAZETNYKOV F. H., POPOV V. V., SALO A. V., TYTOV M. G., AHMETOVA F. F. (2008): Efektyvnost skreščyvanyja bestyževskovo skota sčerefardskym y lymuzynskym skotom pryproyzvodstve govedyny Zootechnyje. 6, 18–20.
MILLER M. F., KERTH C., R., WISE J. W., LANSDELL J. L., STOWEL J. E., RMSEY C. E. (2002): Slaughter plan location. USDA quality grade, external fat thickness and aging time effects on sensory characteristics of beef loin strip steak. Animal Science. 75, 662–667.
OZLUTURK A., TUZEMEN N., YANAR M., ESENBUGA N., DURSUN E. (2004): Fattening performance, carcass traits and meat quality characteristics of calves sired by Chalolais, Simmental and Eastern Anatolian Red sires mated to Eastern Anatolian Red dams. Meat science. 67, 463–470.
PEČIULAITIS A., PEČIULAITIENĖ N., JUKNA V. (2007): Mėsos rinkos dalyvių sąžiningumas – sėkmingos mėsinės galvijininkystės plėtros garantas. VšĮ „Terra Publica“ 6 (08), 14–17.
SERRA X., GIL M., GISPERT M., GUERRERO L., OLIVER M. A., SANUDO C., CAMPO M., PANEA B., OLLETA L., QUINTANILLA R., PIEDRAFITA J. (2004): Characterization of young bulls of the *Bruna dels Pirineus* cattle breed (selected from old Brown Swiss) in relation to carcass, meat quality and biochemical traits. Animal Science. 66, 425–436.
WAJDA S., DASZKIEWICZ T. (2002): Slaughter value and meat quality in heifers of different EUROP adipose classes. Animal Science. 20, 235–242.

Received 8 January 2013; accepted for publication 20 March 2013

Biotechnology in Animal Husbandry 29 (2), p 259-267 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.09'234
DOI: 10.2298/BAH1302259S

CAUSES FOR CULLING FIRST CALVING COWS ON FARMS WITH DIFFERENT LEVELS OF PRODUCTION

P. Stojić[1], R. Beskorovajni[1], V. Pantelić[2], Ž. Novaković[2], S. Bojković Kovačević[3], D. Stanojević[4]

[1] Institute PKB Agroekonomik, Zrenjaninski put bb, 11213 Padinska Skela, Republic of Serbia
[2] Institute for Animal Husbandry, Autoput 16, P.Box 23, 11080 Zemun, Republic of Serbia
[3] PKB Center for Cattle Production, Zrenjaninski put bb, 11213 Padinska Skela, Republic of Serbia
[4] Faculty of Agriculture, Nemanjina 6, 11080 Zemun-Belgrade, Republic of Serbia
Corresponding author: pStojić@sezampro.rs
Original scientific paper

Abstract: It is general knowledge that management influences results in cattle production to the highest extent, and that the culling of cows is a very good indicator of the success of farm management. A comparison of results of culling for first calving cows on farms with various levels of production in 2011 established differences both for the number of culled animals and the reasons for culling. On farms with higher levels of production, the share of first calving cows in overall culling was 25.9% or 4.5% less than on farms with a lower level of production, i.e. 4.8% less died, and 0.7% first calving cows had to be slaughtered, while 5.6% more first calving cows were culled for economic reasons. At both levels of production, dominant reasons for culling were diseases of the legs and hoofs, which can be linked to the tie stall system (more pronounced on farms with higher production) and metabolic disorders (more dominant on farms with lower production). Reproduction was a more considerable problem on farms with higher milk production, while culling due to selection was more pronounced on farms with lower production. In early lactation of first calving cows, regardless of the level of production, dominant reasons for culling on farms are leg and hoof problems and metabolic disorders (total: 55% i.e. 55.9%). When reasons for culling of first calving cows after 100 days of lactation are investigated, on farms with high production the significance of diseases of legs and hoofs remains almost unchanged, but culling due to reproduction grows to 28%. On farms with lower production, culling due to leg and hoof diseases is considerably reduced after 100 days of lactation, however culling due to selection is tripled (62%).

Key words: Holstein, first calving cows, level of production, culling

Introduction

Under conditions of high milk production, management strives to decrease differences between cows in the herds, i.e. to homogenize the herd as much as possible. This is most often realized by applying a comprehensive rearing technology, using quality genetics and planned culling, which contributes to selection efficiency by a more reliable evaluation of heritability *(Djedović et al., 2002 and Stojić et al., 2000).*

As for the culling cows, depending on the level of production, dominant reasons for culling also change. According to research by numerous authors, total culling, including also deaths, is 32-36% (*Stojić et al., 2012; Chiumia, 2011; Pinedo et al., 2010; Seegers et al., 1998 and Dürr, 1997),* while *Maher et al., (2008)* established total culling at a level of 21.3%. In high producing herds, according to research by *Nienartowicz-Zdrojewska et al., (2009),* cows were most frequently culled because of problems with reproduction, mastitis and leg and hoof diseases. The relationship between low production, metabolic disorders and low reproductive efficiency was noted by *Beaudeau et al., (2000).* However, relevant to the free and tie stall system, *Beaudeau et al., (1993),* established that the rearing system influences culling caused by mastitis and distortions, but not culling caused by metabolic disorders or diseases of the locomotion apparatus.

By analyzing culling in Quebec, Canada, in the 1981-1994 period, *Dürr, (1997),* established that voluntary culling of cows due to low production, age, constitution, is decreasing more rapidly, while forced (involuntary) culling is increasing, and that according to *Fetrow et al., (2006),* this has to be controlled and gradually decrease. *Pinedo et al., (2010),* established that low production and mastitis, as reasons for planned culling, had a 2.5 fold lower share in culling in relation to reproductive problems and injuries. *Stojić et al., (2012)* established that culling due to leg and hoof problems was more dominant than culling for reasons of selection (28.4% : 23.27%). The most frequent reasons stated for unplanned i.e. culling not due to selection are reproduction (*Ansari-Lari et al., 2012; Stojić et al., 2012; Chiumia, 2011; Pinedo et al., 2010; Fetrow et al., 2006; Seegers et al. 1998 and Allaire et al., 1976*), followed by mastitis (*Chiumia, 2011 and Fetrow et al., 2006*), and leg and hoof diseases (*Stojić et al., 2012; Chiumia, 2011 and Fetrow et al., 2006*). The causes of culling of first calving heifers on farms were also studied by *Novaković et al. (2009)* and *Petrović M.D. et al. (2004).*

For first calving cows, it was established that first calving cows that became pregnant later are culled earlier, due to a higher risk of low production, as well as cows with a weaker expression of characteristics of type (*Dürr, 1997*). In the overall structure of culled cows, first calving cows accounted for 1/5 (*Pinedo et al., 2010 and Maher et al., 2008*) to 1/3 (*Stojić et al., 2012 and Chiumia, 2011*).

The most frequent reasons for culling were selection and low production (20-29.2%), followed by reproductive problems (13-27%) (*Stojić et al., 2012; Seegers et al., 1998 and Allaire et al., (1976*). In the tie stall system, 26.7% of first calving cows were culled due to leg and hoof diseases, and 13.5% due to metabolic disorders (*Stojić et al., 2012).* According to research by *Dürr, (1997),* on the average, first calving cows were culled 215.6 days after calving.

Materials and Methods

Results for cow culling on seven large farms with a tie stall system located in the northeastern part of the Belgrade region were investigated. In 2011, average of 8837 dairy cows was reared on these farms, with cow numbers by farms of 1080 to 1524. They realized an average annual production (daily milk production/number of cows x 365 days) of 7587 liters of milk. The analysis of culling first calving cows at different levels of milk production was done on results for culling from two farms each with the highest and the lowest annual production.

On all four farms, cows are reared in a tie stall system in semi-open barns (closed during the winter with bales of hay, during the summer acting as overhangs, with only a minority of barns that are fully enclosed), housing 120-130 cows. Cows are milked using machines and each barn has a milk line, vacuum line and its own lacto-freeze. Cows are grouped by production and fed mixed meals with individual addition of minor quantities of concentrated feeds. In essence, meals consist of alfalfa hay, alfalfa haylage, whole plant maize silage, complete concentrate mixtures, soy meal, molasses and other additions.

According to reasons for culling, all culled animals were divided into 8 groups, while according to the time of culling they were divided into two groups: up to 100 and over 100 days. Data was analyzed using standard mathematical-statistical procedures.

Results and Discussion

The two farms with the highest milk production had an annual production per cow of 8175 liters i.e. 8061 liters, while the two farms with the lowest production productions of 7013 liters and 7321 liters (Table 1). On all farms, rearing conditions were almost identical (facilities, milking equipment), the same bulls were used for artificial insemination, the same operative meals and complete concentrate mixtures were used and the conditions for production of roughages were the same. For this reason, it can be said that to the most extent, differences in production, are in fact a consequence of farm management.

Table 1. Annual milk production per cow on the observed farms

Level of production	Farms	Annual Production			
		ANC	MILK	Fat	Prot
High	H1	1524	8175	3.49	3.31
	H2	1106	8061	3.31	3.29
Low	L1	1345	7013	3.33	3.30
	L2	1080	7321	3.33	3.29
Average of 7 farms		8837	7587	3.36	3.29

Abbreviations: **ANC** – Average number of cows; **MILK** – Annual milk production per cow, kg; **Fat** – Average fat content,%; **Prot** – Average protein content,%

Research results indicate that on farms with a high level of production, the share of culled first calving cows in total culling was 4.5% lower, amounting to 25.9% (Table 2). When the structure of culling is investigated relevant to the basic reasons for culling, it can be established that farms with better production have 5.6% more culling of first calving cows for economic reasons (ER) and 0.7% more because of forced slaughter (FS), i.e. of the total number of culled first calving cows, 7% less died (D). It is to be expected that the number of cows culled as a consequence of death and forced slaughter must be reduced, if we strive for higher milk production, all the more so, because the economic effect is also better if more cows are culled for economic reasons.

Table 2. Number of culled first calving cows by primal reasons of culling

Level of production	Farms	TNCC	NCFCC	Primal reasons of culling		
				D	FS	ER
High	H1	528	136 (25.8%)	17 (12.5%)	3 (2.2%)	116 (85.3%)
	H2	422	110 (26.1%)	22 (20.0%)	3 (2.7%)	85 (77.3%)
	H1+H2	*950*	*246 (25.9%)*	*39 (15.9%)*	*6 (2.4%)*	*201 (81.7%)*
Low	L1	434	119 (27.4%)	33 (27.7%)	7 (5.9%)	79 (66.4%)
	L2	391	132 (33.8%)	19 (14.4%)	1 (0.8%)	112 (84.8%)
	L1+L2	*825*	*251 (30.4%)*	*52 (20.7%)*	*8 (3.2)*	*191 (76.1%)*

Abbreviations: **TNCC** – Total number of culled cows; **NCFCC** – Number of culled first calving cows; **D** – Death; **FS** – Forced slaughter; **ER** – Sale for economic reasons

Similar results for culling of first calving cows in relation to the total number of cows were established by *Stojić et al., (2012); Chiumia, (2011) and Seegers et al.,(1998),* while considerably lower results were established by *Pinedo et al., (2010) and Maher et al., (2008).*

Table 3. Reasons of culling on farms with different levels of production

Reasons of culling	Farm H1		Farm H2		High production level (H1+H2)		Farm L1		Farm L2		Low production level (L1+L2)	
	n	%	n	%	n	%	n	%	n	%	n	%
Reproductive diseases	40	29.4	9	8.2	49	19.9	1	0.8	4	3.0	5	2.0
Difficult calving and abortion	4	2.9	3	2.7	7	2.8	1	0.8	4	3.0	5	2.0
Metabolic disorders	12	8.8	15	13.6	27	11.0	29	24.4	13	9.8	42	16.7
Diseases of legs and hooves	15	11.0	65	59.1	80	32.5	15	12.6	41	31.1	56	22.3
Mastitis	19	14.0	2	1.8	21	8.5	1	0.8	0	0.0	1	0.4
Udder damage	9	6.6	0	0.0	9	3.7	0	0.0	0	0.0	0	0.0
Other diseases	14	10.3	13	11.8	27	11.0	21	17.6	9	6.8	30	12.0
Selection reasons	23	16.9	3	2.7	26	10.6	51	42.9	61	46.2	112	44.6

Table 3 shows that on farms with better milk production first calving cows were dominantly culled due to leg and hoof diseases (32.5%), followed by reproduction problems (19.9%), metabolic disorders (11%), as well as for selection reasons (10.6%). On farms with lower production, dominant reasons for culling were selection (44.6%), leg diseases (22.3%), and metabolic disorders (16.7%).

Table 4. Reasons of culling on farms with different levels of production by duration of lactation

Reasons of culling	High production level (H1+H2)				Low production level (L1+L2)			
	≤100 days in lactation		≥101 days in lactation		≤100 days in lactation		≥101 days in lactation	
	n	%	n	%	n	%	n	%
Reproductive diseases	5	5.6	44	28.0	0	0.0	5	3.5
Difficult calving and abortion	2	2.2	5	3.2	5	4.6	0	0.0
Metabolic disorders	19	21.3	8	5.1	31	28.4	11	7.7
Diseases of legs and hooves	30	33.7	50	31.8	30	27.5	26	18.3
Mastitis	9	10.1	12	7.6	1	0.9	0	0.0
Udder damage	2	2.2	7	4.5	0	0.0	0	0.0
Other diseases	11	12.4	16	10.2	18	16.5	12	8.5
Selection reasons	11	12.4	15	9.6	24	22.0	88	62.0

In the first 100 days post calving (Table 4), on farms with higher milk production most first calving cows were culled die to leg and hoof problems (33.7%), metabolic disorders (21.3%) and mastitis (10.1%). 12.4% first calving cows were culled because of selection. The significance of leg and hoof diseases remained almost at the same level for culling after 100 days post calving (31.8%),

however the number of cows culled due to reproduction (28%) significantly increased.

On farms with a lower level of production, during the first 100 days, most first calving cows were culled due to metabolic disorders (28.4%), which are in fact a problem of inadequate nutrition, with nutrition being the most important key to good management. This was followed by leg and hoof problems (27.5%), and culling due to selection (22%). As for culling after 100 days of lactation, culling due to leg and hoof diseases (18.3%), was significantly reduced, but culling because of selection almost tripled (62%). It is very difficult to provide a realistic explanation for this phenomenon, all the more so since reproductive problems are not dominant in the later phase of lactation, and leg and hoof problems also decrease.

Table 5. Longevity of culled first calving cows on farms with different levels of production

Items	High production level (H1+H2)				Low production level (L1+L2)			
	n	x	SD	CV	n	x	SD	CV
Days in milking	246	248.13	197.83	79.73	251	251.42	233.40	92.83
Milk production per milking day, kg	246	16.91	8.67	51.29	250	15.55	8.95	57.55
Milk production per day of life, kg	246	4.49	3.57	79.48	251	3.77	3.28	87.12

Differences for culling due to selection can maybe be explained by differences in production between first calving cows (Table 5), although they are not high. Maybe the sequence of significance of the diagnosis stated as the reason of culling is more important for this phenomenon, because it depends on management. For this reason the system for making final diagnoses for cow culling should be unified. Thus, farms could be compared more easily, but existing problems in production could also be better perceived.

Considerably lower values for the culling of cows due to leg and foot diseases were established by *Stojić et al., (2012); Chiumia, (2011) and Fetrow et al. (2006)*, as well as for total culling and culling of first calving cows. Other authors mainly established higher numbers of culling, both total and of first calving cows, resulting from reproductive diseases (*Ansari-Lari et al., 2012; Chiumia, 2011; Fetrow et al., 2006, Seegers et al., 1998 and Allaire et al., 1976*).

Conclusion

After a comparison of results for culling of first calving cows on farms with different levels of production in 2011 it can be said that there were differences

for the numbers of culled cows, but also for reasons for culling. On farms with higher levels of production, the share of first calving cows in total culling was 25.9%, or 4.5% less than on farms with lower levels of production, i.e. there were 4.8% less deaths and 0.7% less forced slaughter among first calving cows, while 5.6% more first calving cows were culled for economic reasons.

For both levels of production, dominant reasons for culling were leg and hoof diseases (more pronounced on farms with higher production), and metabolic disorders (more dominant on farms with lower production). Reproduction was a more dominant problem on farms with higher milk production. The most dominant reason for culling on farms with low production is culling due to selection (44.6%).

In early lactation of first calving cows and on farms with higher and with lower production, the dominant reasons for culling are leg and hoof problems and metabolic disorders (total 55% i.e. 55.9%). When investigating reasons for culling first calving cows after 100 days of lactation, on farms with high production, the importance of leg and hoof diseases remained almost unchanged, but culling due to reproduction increased to 28%. On farms with lower production, culling due to leg and hoof diseases is considerably lower after 100 days of lactation, but the number of first calving cows culled due to selection reasons is tripled (62%).

In the coming period, it would be desirable to unify the system for making final diagnoses for culling cows.

Uzroci izlučenja prvotelki na farmama sa različitim nivoom proizvodnje

P. Stojić, R. Beskorovajni, V. Pantelić, Ž. Novaković, S. Bojković Kovačević, D. Stanojević

Rezime

Opšte je poznato da menadžment u najvećoj meri utiče na rezultate u govedarskoj proizvodnji, a izlučenja krava su vrlo kvalitetan pokazatelj uspešnosti upravljanja farmama. Poredeći rezultate izlučenja prvotelki na farmama sa različitim nivoom proizvodnje u toku 2011. god. ustanovljene su razlike i u broju izlučenih grla, ali i u razlozima izlučenja. Na farmama sa višim nivoom proizvodnje učešće prvotelki u ukupnim izlučenjima bilo je 25.9% što je za 4.5% manje nego na farmama sa nižim nivoom proizvodnje, odnosno uginulo je za 4,8% manje i prinudno je zaklano za 0,7% manje prvotelki, a iz ekonomskih razloga izlučeno za 5.6% prvotelki više.

Na oba nivoa proizvodnje dominantni razlozi izlučenja su bili oboljenja nogu i papaka što se može dovesti u vezu sa vezanim sistemom držanja (izraženije na farmama sa većom proizvodnjom) i metabolički poremećaji (dominantnije izraženi na farmama sa nižom proizvodnjom). Reprodukcija je bila znatniji problem na farmama sa većom proizvodnjom mleka, odnosno selekcijska izlučenja na farmama sa nižom proizvodnjom. U ranoj laktaciji prvotelki, bez obzira na nivo proizvodnje, na farmama kao razlozi izlučenja dominiraju problemi sa nogama i papcima i metabolički poremećaji (ukupno 55% tj. 55.9%). Kada se posmatraju razlozi izlučenja prvotelki nakon 100 dana laktacije, na farmama sa visokom proizvodnjom značaj obolenja nogu i papaka je gotovo nepromenjen, ali izlučenja usled reprodukcije rastu na 28%. Na farmama sa nižom proizvodnjom, izlučenja usled oboljenja nogu i papaka su znatno smanjena nakon 100 dana laktacije, ali je zato utrostručen broj izlučenih iz selekcijskih razloga (62%).

References

ALLAIRE F.R., STERWERF H.E., LUDWICK T.M. (1976): Variations in Removal Reasons and Culling Rates with Age for Dairy Females. J. Dairy Sci., 60:254-267

ANSARI-LARI M., MOHEBBI-FANI M., ROWSHAN-GHASRODASHTI A. (2012): Causes of culling in dairy cows and its relation to age at culling and interval from calving in Shiraz, Southern Iran. Veterinary Research Forum, 3 (4): 233 - 237

BEAUDEAU F., SEEGERS H., DUCROCQ V., FOURICHON C., BAREILLE N. (2000): Effect of health disorders on culling in dairy cows: A review and a critical discussion. Ann. Zootech. 49: 293–311

BEAUDEAU F., HENKEN A., FOURICHON C., FRANKENA K., SEEGERS H. (1993): Associations between health disorders and culling of dairy cows: A review. Livestock Production Science, 35 (3): 213-236

CHIUMIA D. (2011): Vulnerability in Holstein-Friesian dairy cows: Risk factors for culling and effect of temperament on oestrus. M. Sc. thesis. The University of Edinburgh

DJEDOVIĆ R., LATINOVIĆ D., STOJIĆ P., BOGDANOVIĆ V., TRIFUNOVIĆ G. (2002): Naslednost osobina mlečnosti krava u zavisnosti od nivoa proizvodnje. Biotehnologija u stočarstvu, 18 (5-6):17-22

DÜRR J.W. (1997): Genetic and phenotypic studies on culling in quebec holstein cows. Ph.D. thesis. Department of Animal Science, McGill University, Montreal, Canada

FETROW J., NORDLUND K.V., NORMAN H.D. (2006): Invited Review: Culling: Nomenclature, Definitions, and Recommendations. J. Dairy Sci. 89:1896–1905

MAHER P., GOOD M., MORE S.J. (2008): Trends in cow numbers and culling

rate in the Irish cattle population, 2003 to 2006. Irish Veterinary Journal, 61 (7): 455-463
MOUSSAVI A.H. (2008): Days in milk at culling Holstein dairy cows. J. Anim. Vet. Adv., 7 (1): 89-93
NIENARTOWICZ-ZDROJEWSKA ANNA, DYMARSKI IRENEUSZ, SOBEK Z., WOLC ANNA (2009): Culling reasons as related to lifetime dairy performance in Polish Friesian (Black-and-White) cows on Pawłowice farm in the years 1909-2006. Animal Science Papers and Reports, 27 (3): 173-180
NOVAKOVIĆ Ž., ALEKSIĆ S., SRETENOVIĆ LJ., PETROVIĆ M.M., PANTELIĆ V., OSTOJIĆ-ANDRIĆ D. (2009): Longevity of high-yielding cows. Biotechnology in Animal Husbandry 25 (5-6): 645-654
PETROVIĆ M.D., SKALICKI Z., GUTIĆ M., BOGDANOVIĆ V. (2004): Uticaj paragenetskih faktora na osobine dugovečnosti krava simentalske rase. Biotechnology in Animal Husbandry, 20(3-4):23-29
PINEDO P.J., DE VRIES A, WEBB D.W. (2010): Dynamics of culling risk with disposal codes reported by Dairy Herd Improvement dairy herds. J. Dairy Sci. 93: 2250–2261
SEEGERS H., BEAUDEAU F., FOURICHON C., BAREILLE N. (1998): Reasons for culling in French Holstein cows. Preventive Veterinary Medicine, 36:257-271
STOJIĆ P., BOJKOVIĆ-KOVAČEVIĆ S., BESKOROVAJNI R., JEREMIC I., PANTELIĆ V. (2012): Causes of cow culling in the tie stall system. Biotechnology in Animal Husbandry 28 (4): 697-704
STOJIĆ P., DJEDOVIĆ RADICA, LATINOVIĆ D., NIKOLIĆ R. (2000): Heritabilnost osobina mlečnosti u različitim uslovima proizvodnje mleka goveda. Journal of Scientific Agricultural Research, 61 (3): 15-22

Received 7 May 2013; accepted for publication 15 June 2013

Biotechnology in Animal Husbandry 29 (2), p 269-275 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 619:616.24'632
DOI: 10.2298/BAH1302269B

MASTITIS THERAPY-DIRECT AND INDIRECT COSTS

S. Boboš[1], M. Radinović[1], B.Vidić[2], M. Pajić[1], V. Vidić[2], A. Galfi[1]

[1]Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
[2]Scientific Veterinary Institute "Novi Sad", Rumenacki put 20, 21000 Novi Sad, Republic of Serbia
Corresponding author: bobos@polj.uns.ac.rs
Original scientific paper

Abstract: One of the most important problems in milk production, causing great economic loses is certainly mastitis. In order to minimize economic losses from mastitis dairy farms introduce different mastitis management programs. These programs include mastitis therapy and prevention. In mastitis control prevention is most important and when mastitis occurs cost of therapy and milk discharge is very important. In our study we examined cost of mastitis treatment and milk loss in different mastitis management programs. We concluded that most costly are mastitis caused by specific pathogens. Cost of milk loss is 2.4 times bigger than cost of drug consumption. Applying of tit-dipping has great importance in reduction of mastitis caused by specific pathogens and less importance for conditional saprophytes. In total, cost of mastitis treatment on whole farm was almost the same for all mastitis management programs, while the effect of the program on farm C was the most expensive in the cows with the finding of specific pathogens.

Key words: mastitis, therapy cost, pathogens, saprophytes

Introduction

Dairy production on modern farms is very intensive because of genetic predisposition of animals and optimal conditions of feed and husbandry. Health disorders who are common in dairy cows affect production and quality of milk and lastly on economic results. Mastitis is one of the most costly health problems in the dairy industry. Nationally, mastitis is estimated to cost dairy producers approximately 6% of the value of production in United States *(Wells and Ott, 1998)*. Reduced milk production is the major cost associated with subclinical mastitis and a substantial cost associated with clinical mastitis (*Eberhart et al., 1987*). A review by *Schepers and Dijkhuizen* (*1991*) indicated that mastitis caused a 40 to 50% decrease in the economic net margin per cow, with the largest part of this loss due to a 5 to 7% decrease in milk yield per lactation. Estimates of milk yield loss range from 100 to 500 kg/cow per lactation (*Erb et al., 1985; Firat,*

1993; Hortet and Seegers, 1998). When clinical mastitis occurs, additional costs result from discard of abnormal milk, drugs, and veterinary services. Antibiotics are commonly used to treat clinical mastitis episodes *(Ziv, 1992)*. Administration of antibiotics usually requires a withdrawal time, during which milk is unmarketable and must be discarded or fed to calves (*Plummer et al., 1984*). This cost can be substantial and is used as justification for avoiding antibiotic use. Those results suggested that antibiotic therapy was beneficial in terms of cow health and welfare, but economic factors were not reported. The recovery of milk yield, the amount of unmarketable milk, and drug costs all may have been affected by treatment protocols. Mastitis reduces dairy farm profitability, with losses stemming from milk production decreases and discarded milk and costs of treatment and culling (*Gröhn et al., 2005*). The specific inflammatory response from a mastitis incident is dependent on the bacterial species involved (*Bannerman, 2009*). Depending on the pathogen involved, the effect may vary, so studies determining which pathogens have the greatest impact on cow health, production, and profitability are valuable (*Gröhn et al., 2004*). In our study we examined impact of different types of bacterial infection of udder and different management on losses from mastitis.

Materials and Methods

Study was conducted on three dairy farms with approximately same number of cows during one year period. All farms had different management in treating mastitis problem. First farm, (farm A) was using tit-dipping and treating of clinical mastitis in lactation and subclinical mastitis in dry period. Second farm, (farm B) was using treating of clinical mastitis in lactation and subclinical mastitis in dry period but without tit-dipping. Third farm, (farm C) was using tit-dipping and treating of clinical mastitis in lactation and blanket-therapy in dry period. Mastitis detection in cows was conducted by determining sings of mastitis, such as milk from one or more glands was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the gland, or generalized illness. From cows with sings of mastitis milk samples were taken with applying asepsis. Samples were then stored in refrigerator and transported in laboratory for detection of microorganisms. Each sample was inoculated on plates with substrates. Substrates are marked with number of samples and put in thermostat on incubation at 37 º C. Incubation lasted for 48 hours, with one reading after 24 hours and one at the end of incubation.

When reading the plates a special attention was given to the presence of microbial growth, size, shape, color and arrangement of colony-forming and particularly in the presence of hemolysis. For the differentiation of certain types of bacteria special tests are used. After differentiation was done all bacteria were divided in two groups. First group was specific pathogens containing

Staphylococcus aureus, *Streptococcus agalactiae* and *Escherichia coli.* Another group was conditional saprophytes containing *Micrococcus* spp, coagulase negative *staphylococcus* and *Corynebacterium bovi*s.

Data on drugs consumption and milk loss per mastitis treating were gathered on farms by interviewing veterinarians. To calculate cost of drugs for treating mastitis average price of milk was used.

Statistical analysis

Correlation coefficient between number of mastitis cases and cost of therapy and milk loss was calculated using program "Statistica version 10".

Results and Discussion

Data collected on farms in experiment showed appearance of mastitis on all three farms during whole year. Calculation of cost of mastitis treatment on farms showed differences between farms. Costs of mastitis treatment and milk loss due to antibiotic presence on all three farms are shown in tables.

Table 1. Cost of mastitis treatment and milk loss on farm A

bacteriological findings	Farm A					
	number of cows		drug consumption calculated in milk		milk loss due to antibiotic presence	
	number	%	by cow	total	by cow	total
negative	58	23,39	17,82	1034	37,79	2191
specific pathogens	68	27,42	86,86	5907	209,19	14274
conditional saprophytes	122	49,9	11,63	1419	31,74	3827

Data showed in table 1 indicate that drug consumption and milk loss were biggest when mastitis was caused by specific pathogens on farm A. By comparing milk loss due to antibiotic presence and drug consumption calculated in milk, on farm A average ratio is 2.41. Total milk loss and drug consumption for all mastitis types is 28 652 kg.

Table 2. Cost of mastitis treatment and milk loss on farm B

bacteriological findings	Farm B					
	number of cows		drug consumption calculated in milk		milk loss due to antibiotic presence	
	number	%	by cow	total	by cow	total
negative	48	19,36	6,87	330	16,29	782
specific pathogens	102	41,12	69,02	7040	164,81	16811
conditional saprophytes	98	39,52	10,10	990	27,55	2700

Data showed in table 2 indicate that drug consumption and milk loss were biggest when mastitis was caused by specific pathogens on farm B. By comparing milk loss due to antibiotic presence and drug consumption calculated in milk, on farm B average ratio is 2,49. Total milk loss and drug consumption for all mastitis types is 28 653 kg.

Table 3. Cost of mastitis treatment and milk loss on farm C

bacteriological findings	Farm C					
	number of cows		drug consumption calculated in milk		milk loss due to antibiotic presence	
	number	%	by cow	total	by cow	total
negative	78	31,45	33,98	2651	81,35	6345
specific pathogens	31	12,50	106,45	3300	229,82	7124
conditional saprophytes	139	56,05	17,33	2409	49,09	6823

Data showed in table 3 indicate that drug consumption and milk loss were biggest when mastitis was caused by specific pathogens on farm C. By comparing milk loss due to antibiotic presence and drug consumption calculated in milk, on farm C average ratio is 2.45. Total milk loss and drug consumption for all mastitis types is 28 652 kg.

Correlation coefficient between number of mastitis cases and cost of therapy and milk loss was 0.14, so positive correlation was calculated.

When a cow contracts mastitis, the dairy farmer needs to decide whether treatment is warranted, and if so, what treatment is most appropriate. Ideally, these decisions are made based on the organism causing mastitis. In determining how to treat a cow, one common way of grouping these organisms is to separate them into gram-positive and gram-negative. These two groups of organisms cause mastitis of different symptoms and severity, and this classification can form the basis of on-farm treatment protocols (*Hertl et al., 2010*). In our study we have divided organisms on specific pathogens and conditional saprophytes in order to describe their ability to cause mastitis rather than their staining characteristics. On all three farms cost of treating mastitis and milk loss were highest when specific pathogens were isolated from milk. Also on all farms milk loss due to antibiotics was bigger than cost of antibiotics for treating. Average ratio between these two costs on farm A was 2.41, so on every kg of milk given for antibiotics 2.41 kg were wasted because of antibiotic residuals. On farm B average ratio was 2.49, and on farm C average ratio was 2.45. On all three farms it was approximately the same ratio between cost of antibiotics and milk loss due to antibiotic residuals. Similar results were obtained by *Shim et al 2004*. who claim that costs for milk loss is several times bigger than cost for antibiotic treatment. If we compare cost for treatment

between farms we can see from tables that cost per one mastitis case is greater in farms A and C who used tit-dipping than in farm B because of counting in costs for tit-dipping, but farms A and C had lesser number of mastitis especially those caused by specific pathogens. Applying of tit-dipping has decreased number of pathogens much more than number of conditional saprophytes, these findings are accordant to results given by *Bobos, (1991).* Correlation between number of mastitis cases and cost of therapy and milk loss for all three farms was positive and points that number of cases on big farms is more important than type of mastitis. By counting costs of mastitis in total-including specific pathogens, conditional saprophytes and negative findings on all three farms total count is approximately the same. This result indicates that in economic aspect all three management of mastitis on farms give almost the same effect.

Conclusion

On all three farms biggest milk loss and cost of drugs consumption was in mastitis caused by specific pathogens. Ratio between milk loss and cost of drugs consumption calculated in milk was almost the same on all farms. Total costs of mastitis on all three farms were approximately the same regardless to different mastitis management programs.

Acknowledgment

Research was financed by the Provincial Secretariat of Science and Technological Development, Republic of Serbia, project number 114-451-2230/2011 and grant TR31084 and grant III 43007 from Serbian Ministry of Education, Science and Technological Developmment.

Terapija mastitisa -direktni i indirektni troškovi

S. Boboš, M. Radinović, B.Vidić, M.Pajić, V. Vidić, A. Galfi

Rezime

Jedan od najvažnijih problema u proizvodnji mleka, koji izaziva velike ekonomske gubitke svakako je mastitis. Da bi se smanjili ekonomski gubici zbog mastitisa farme muznih krava uvode različite programe kontrole mastitisa. Ovi program uključuju terapiju i prevenciju mastitisa. U kontoli mastitisa prevencija je najvažnija i kada se pojavi mastitis veoma je značajan trošak terapije i odbacivanja mleka. U našoj studiji ispitali smo trošak terapije mastitisa i odbacivanja mleka u

različitim programima kontrole mastitisa. Zaključili smo da je najveći trošak zbog mastitisa izazvaniih sa specifičnim patogenima. Trošak odbacivanja mleka je u proseku 2,4 puta veći od troška lekova. Primena potapanja sisa ima veliki značaj na smanjenje mastitisa izazvanih sa specifičnim patogenima i manji značajna smanjenje mastitisa izazvanih sa uslovno saprofitima. U ukupnom zbiru trošak terapije mastitisa i odbčenog mleka je približno isti u svim programima kontrole mastitisa, dok je efekat primene programa na farmi C bio najskuplji u krava sa nalazom specifičnih patogena.

References

BANNERMAN D. D. (2009): Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. J. Anim. Sci. 87(13 Suppl.):10–25.

BOBOŠ S. (1991): Uticaj različitih programa suzbijanja mastitisa na proizvodnju mleka i zdravstveno stanje vimena, Doktorska disertacija, Beograd.

EBERHART R. J., HARMON R. J., JASPER D. E., NATZKE R. P., NICKERSON S. C., RENEAU J. K., ROW E. H., SMITH K. L. and SPENCER S. B. (1987): Current Concepts of Bovine Mastitis. 3rd ed. Natl.Mastitis Council, Inc., Arlington, VA.

ERB H. N., SMITH R. D., OLTENACU, P. A., GUARD C. L., HILLMAN R. B., POWERS P. A., SMITH M. C., WHITE M. E. (1985): Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows. J. Dairy Sci. 68:3337–3349.

FIRAT M. Z. (1993): An investigation into the effects of clinical mastitis on milk yield in dairy cows. Livest. Prod. Sci. 36:311–321.

GRÖHN YT, GONZÁLEZ RN, WILSON DJ, HERTL JA, BENNETT G, SCHULTE H, et al. (2005): Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. Prev. Vet. Med.;71:105–125

GRÖHN YT, WILSON DJ, GONZÁLEZ RN, HERTL JA, SCHULTE H, BENNETT G, et al. (2004): Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87:3358–3374.

HERTL JA, GRÖHN YT, LEACH JDG, BAR D, BENNETT GJ, GONZÁLEZ RN, et al. (2010). Effects of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows. J. Dairy Sci. 93:1551–1560.

HORTET P., SEEGERS H. (1998): Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Prev. Vet. Med. 37:1–20.

PLUMMER D., McKEAN J., KILMER L., STEVERMER E., OWINGS W. (1984): Residue avoidance program: Drug withdrawal times for dairy cows. RAP-D3. Iowa State University Cooperative Extension Service, Ames.

SCHEPERS J. A., DIJKHUIZEN A. A.(1991): The economics of mastitis and mastitis control in dairy cattle: Acritical analysis of estimates published since 1970. Prev. Vet. Med. 10:213–224.
SHIM E. H., SHANKS R. D., MORIN D. E (2004): Milk Loss and Treatment Costs Associated with Two Treatment Protocols for Clinical Mastitis in Dairy Cows, J. Dairy Sci. 87:2702–2708.
WELLS S. J., OTT S. L.(1998): What is the current milk quality in the U.S.? Pages 10–18 in 1998 National Mastitis Council Annual Meeting Proceedings. National Mastitis Council, Madison, WI.
ZIV G. (1992): Treatment of peracute and acute mastitis. Vet. Clin. North Am. Food Anim. Pract. 8:1–15.

Received 13 June 2013; accepted for publication 26 June 2013

Biotechnology in Animal Husbandry 29 (2), p 277-286 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.08'638
DOI: 10.2298/BAH1302277I

EFFECT OF MATING METHOD, SEX AND BIRTH TYPE ON GROWTH OF LAMBS

Z. Ilić[1], A. Jevtić-Vukmirović[2], M. P. Petrović[3], V. Caro Petrović[3], B. Milošević[1], Z. Spasić[1], B. Ristanović[1]

[1]Faculty of Agriculture, Lesak
[2]High School Agricultural Food, Prokuplje, Serbia
[3]Institute for Animal Husbandry, Belgrade-Zemun
Corresponding author: zoran.ilic@pr.ac.rs
Original scientific paper

Abstract: Estrus synchronization methods was use to control the reproductive traits of sheep, as well as bringing more females at the same stage of estrus and ovulation. According to the points mentioned above, the aim of the present study was to investigate and compare mating method and influence of fixed factors on birth and weaning weight of lambs. Statistical analysis showed that exist difference in the body weights between genotypes of lambs. In the first group, the difference in weight of lambs at birth, regardless of mating method was not significant (P> 0.05), while in the second group, the difference was slightly significant (P <0.05). As for the birth type,sex and within the same genotype, there was a statistical significance (P <0.05) between singles obtained naturally, between the triplets obtained naturally and between triplets received hormonal method. All the differences between body weight at 30 days (mating method, sex and birth type under the same genotype) were statistically significant (P <0.05).The determined differences in the body weights at 60 days (sex, mating method and type of birth under the same genotype) were statistically significant (P <0.05). The results showed that the differences (mating method, sex and type of birth under the same genotype) were statistically significant (P <0.05), except in between body weight at 90 days in twins among two genotypes obtained by natural method, which were not statistically significant (P> 0.05).

Key words: sheep, mating method, body weight, lamb

Introduction

In order to boost the production of sheep and technological process of organizing principles in industry, science has made efforts to successfully manage the process of breeding ewes. Today, in many countries, including ours, estrus synchronization methods was use to control the reproductive traits of sheep, as well as bringing more females at the same stage of estrus and ovulation. This method

enabled the two or three lambs a year for two years, with the aim of increasing meat production. *Petrovic (2007*) point out that the use of proper hygiene in the reproduction of sheep, breeding age, environmental conditions, qualitative and quantitative nutrition, prevention and treatment are the key factors. The same authors argue that, the recognition of estrus and time of admission is the essential part of the concept, and therefore the pregnancy of animals. For more than half a century attempts have been made to synchronize the period of sexual receptivity, or estrus, in farm animals. Synchronization of estrus can save labor and is a key component in artificial insemination (AI) programs. Synchronizing estrus during the normal breeding season provides a means for producers to schedule the lambing period, concentrating labor needs into a short frame of time. Estrous synchronization also can help in improving and the uniformity of the lamb crop for market, *Knights et al,(2001).* Estrus synchronization (ES) in goats and sheep is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous progesterone or by inducing premature luteolysis. The latter approach is not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadotropin can be used to induce and synchronize estrus in anovular does and ewes, *S. Wildeus (1999*). Successful estrus synchronization programs have a key role in lambing rate efficiency and profitability of sheep holders in semi-intensive production systems (*Knights et al., 2001).* Using different intravaginal devices impregnated with P4 or synthetic progestogens has been extended all over the world. Controlled internal drug release (CIDR) device impregnated with 300 mg P4 and intravaginal sponges containing with 60 mg medroxyprogesterone acetate (MAP) or 40 mg fluoroprogesterone acetate (FGA) are two main intravaginal devices that are used vastly for ewes estrus synchronization (*Ungerfeld and Rubianes,* 2002). PGF2α and/or its analogue are luteolytic factors and according to its role in regression mechanism of corpus luteum (*Turk et al., 2008*), double injections (9 to 11 days interval) of PGF2α is common for ewes' estrus synchronization (*Atamani and Akoz, 2006; Wildeus 2000*). Also, *Dixon et al*. (*2006*) used progesterone and/or its analogue treatment and reported greater estrus observation in progesterone-PGF2α combination treated ewes than those received PGF2α alone. Combination of PMSG and P4 impregnated intravaginal devices causes readily estrus synchronization (*Romano 2004*). PMSG treatment reduces the interval between the onset of estrus and ovulation (*Dogan and Nur, 2006*), also in no PMSG received ewes, ovulations occurs later (*Barrett et al., 2004).* One dose of PMSG can stimulate follicular development and higher ovulation rate in ewes (*Koyuncu and Ozis Alticekic, 2010).* Due to the importance of PMSG in sheep reproduction efficiency and injection of 500 IU PMSG with 40 mg of FGA for estrus synchronization in breeding season, 87% of treated ewes exhibited overt sign of estrus when compared with 48% in the control group (Langford, 1982) or CIDR removal until the estrus onset was shorter in progesterone-PGF2α. Obtained results by *Dogan and Nur (2006*) demonstrated

that the interval between Sponge progesterone - PGF2α combination treated ewes than those that received progesterone alone. One dose of PMSG can stimulate follicular development and higher ovulation rate in ewes (*Koyuncu and Ozis Alticekic, 2010*). Estrous synchronization is a valuable management tool which has been successfully employed to enhance reproductive efficiency, particularly in ruminants (*Kusina et al., 2000*). In small ruminants, estrous synchronization is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin F2α or by extending the cycle artificially with exogenous progesterone or more potent progestagens (*Jainudeen et al., 2000; Kusina et al., 2000*). As prostaglandin treatment is limited to the breeding season, different protocols of estrous synchronization using progestin's have been introduced (*Ainsworth and Wolynetz, 1982; Godfrey et al., 1997; Rosado et al., 1998).* Estrus synchronization by CIDR method was as effective as Sponge (MAP) and PGF2α treatment for inducing estrus. Also, CIDR treated ewes exhibited overt signs of estrus earlier than other treatment groups, *Naderipour et al, (2012).* According to the points mentioned above and the importance of estrus synchronization to optimize ewes reproduction efficiency, the aim of the present study was to investigate and compare mating method and influence of fixed factors on birth and weaning weight of lambs.

Materials and Methods

Investigation of the effect of estrus synchronization was performed on private farm in Vrnjacka Banja.

- The first group of 60 local Pirot ewes mated with ram of Wurttemberg breed of sheep.
- Second group of 60 local Pirot ewes were mated with ram of Improved Pirot breed of sheep.

Within each group 30 sheep were subjected to natural mating and the rest 30 sheep within each group have been utilized for estrus synchronization. The first subgroup of both groups performed the synchronization and the second subgroup of both groups performed the natural mating. One ram was mated for every ten ewes.

Table 1. Experimental scheme of mating

Group	First (1)		Second (2)	
Ram	Wuerttemberg (W)		Pirotski (P)	
Number of animals in group	60		60	
Subgroups	Natural	Synchronize	Natural	Synchronize
No. of animals	30	30	30	30
No. of rams	3	3	3	3
Used product	-	Veramix	-	Veramix

For induction of estrus synchronization and in sheep experimental group applied the technique of intra vaginal sponges with progestin's (Veramix, Pharmacia & Upjohn, Belgium) Applicator coated Vaseline on the outside and the inside part, frimly held and introduced into the vagina to the cervix. Rod applicator sponge is pushed forward and then pilled out a tube applicator from the vagina, extracting rod that holds the sponge that carried control setting using a plastic end that protrudes from the vagina. Synchronization of sheep had done in the month of April. Sponges were removed from the vagina 12 days after the application. The first sign of estrus were reported 35 hous after the extraction. The experimental sheep lambing commenced on the 30th of August those that are conceptualized by, or on the 16th September who became pregnant in the next estrus. Before the synchronization of estrus a dose of an anthelmintic treatment and vaccination of sheep against anaerobic bacteria was performed. Determination of the production result based on the body weight have been made for both groups. Recording of body weight started from newborn lambs (birth weight-BW), lambs body weight at 30 days, (BW30), 60 days (BW60) and 90 days (BW90). Statistical analysis was performed by GLM procedure of SPSS package, using the next model:

$Yijklm = \mu + Gi + Mj + Bk + Sl + \varepsilon ijklm,$

where

Yijklm = body weight of mth lamb of lth sex, kjth birth type, jth mating method, ith genotype

μ = overall population mean

Gi= effect of genotype(fixed effect –2 classes)

Mj= effect of mating method (fixed effect –2 classes)

Bk = effect of birth type (fixed effect –3 classes)

Sl = effect of sex (fixed effect –2 classes)

εijklm = residual error

Results and Discussion

Table 2. The mean value of lambs body weight from birth to 90 days- Wurttemberg, ram, kg

Mating method	Birth type	Sex	BWB		BW30		BW60		BW90	
			Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
S	Single	male	3,40	0.04	11,51	0.04	15,63	0.16	22,15	0.18
		female	3,11	0.02	10,72	0.12	14,62	0.11	20,28	0.17
	average		3,26	0.04	11,13	0.10	15,15	0.14	21,26	0.23
	Twins	male	3,09	0.15	10,40	0.12	14,84	0.24	20,28	0.24
		female	2,91	0.09	10,14	0.15	14,28	0.27	19,53	0.39
	average		3,00	0.08	10,27	0.10	14,56	0.19	19,90	0.25
	Triplets	male	2,65	0.11	10,13	0.15	14,40	0.23	19,57	0.39
		female	2,38	0.14	9,31	0.17	12,77	0.66	17,59	0.54
	average		2,55	0.09	9,82	0.18	13,79	0.39	18,83	0.46
Synchronization- average			3,06	0.06	10,27	0.04	14,75	0.15	20,48	0.23
N	Single	male	3,66	0.05	11,74	0.14	16,53	0.34	22,86	0.40
		female	3,37	0.24	11,19	0.08	16,08	0.45	22,08	0.46
	average		3,55	0.04	11.53	0.11	16,36	0.27	22,57	0.31
	Twins	male	3,40	0.10	10,98	0.28	15,93	0.17	21,89	0.21
		female	3,15	0.10	10,97	0.08	15,64	0.23	21,52	0.23
	average		3,24	0.08	10.97	0.10	15,74	0.16	21,64	0.17
	Triplets	male	2,76	0.09	10,54	0.23	14,70	0.60	20,19	0.75
		female	2,59	0.23	9,83	0.33	14,65	0.36	20,13	0.41
	average		2,68	0.11	10.22	0.22	14,67	0.35	20,17	0.43
Natural- average			3,27	0.06	11.09	0.11	15,82	0.18	21,79	0.23
Overall average			3,17	0.04	10.90	0.08	15,30	0.13	21,16	0.18

S- synchronization, N-natural

On table 2 showed that the birth weight of single born male lambs and female from natural mating were heavier than the single born male and female lambs from synchronized mating for the same difference of 0.26 kg. The male and female lambs born twin from natural mating were heavier for 0.31 kg and 0.24 kg. The male and female lambs born triplet were heavier in natural mating with a difference of 0.11 kg for male lambs and 0.21 kg for female in synchronized mating.

Table 3. The mean value of lambs body weight from birth to 90 days-Pirot ram, kg.

Mating method	Birth type	Sex	BWB		BW30		BW60		BW90	
			Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
S	Single	male	3,38	0.04	11,13	0.07	15,71	0.10	21,61	0.17
		female	3,09	0.03	10,44	0.09	14,73	0.06	20,12	0.12
	average		3,23	0.04	10,77	0.09	15,20	0.12	20,83	0.19
	Twins	male	3,23	0.19	10,55	0.21	14,90	0.18	20,25	0.31
		female	2,86	0.08	9,98	0.09	14,50	0.19	19,63	0.21
	average		3,01	0.10	10,21	0.13	14,66	0.14	19,88	0.19
	Triplets	male	2,51	0.05	9,92	0.13	14,12	0.24	18,75	0.27
		female	2,27	0.08	9,52	0.17	13,30	0.23	17,75	0.46
	average		2,39	0.07	9,72	0.13	13,71	0.24	18,25	0.33
Synchronization- total			3,04	0.06	10,47	0.09	14,83	0.12	20,19	0.19
N	Single	male	3,57	0.02	11,44	0.18	16,20	0.17	22,70	0.23
		female	3,46	0.06	10,91	0.14	15,49	0.32	21,36	0.40
	average		3,51	0.03	11,17	0.13	15,85	0.19	22,03	0.27
	Twins	male	3,30	0.06	11,08	0.09	15,65	0.27	21,66	0.29
		female	3,07	0.10	10,37	0.14	15,45	0.24	21,04	0.24
	average		3,20	0.06	10,76	0.11	15,56	0.18	21,38	0.20
	Triplets	male	2,70	0.05	9,66	0.12	15,33	0.19	20,28	0.22
		female	2,53	0.08	9,12	0.10	12,01	0.29	17,17	0.32
	average		2,59	0.07	9,30	0.19	13,12	0.10	18,21	0.20
Natural- average			3,29	0.05	10.83	0.10	15,51	0.17	21,43	0.21
Overall average			3,17	0.04	10,66	0.07	15,19	0.11	20,84	0.16

S- synchronization, N-natural

From table 2, can also be seen, that the body weight of male lambs and female lambs from natural mating at 30 days were heavier in all birth type with differences of: 0.23 kg; 0.47 kg, 0.58 kg; 0.83 kg, 0.41 kg; 0.52 kg. At 60 days, lambs from natural mating were heavier in both sexes as well as in all birth types the differences were: 0.9 kg ;1.46 kg, 1.09 kg;1.36 kg, 0.3 kg;1.88 kg. Same at 90 days, lambs from natural mating were dominant in weight in all ages and in both sexes. The differences were as follows : 0.71 kg;1.8 kg, 1.61 kg;1.99 kg, 0.67 kg;2.54 kg.

As shown in table 3, the birth weight of male and female lambs from natural mating were heavier than synchronized in all birth type.. The differences were: 0.19 kg;0.37 kg, 0.07 kg;0.21 kg, 0.19 kg;0.26 kg respectively. *Yilmaz and Altin*, (*2011)* birth type and the sex of the lambs were found to significantly affect birth weight, which is an important factor in lamb production which was in accordance with ours. As can be seen on tables 2 and 3 indicated that generally synchronized mating affected the body weight of lambs from birth to 90 days.

Özbey and Esen (2000) quoted that estrus synchronization usually increases the number, but reduces the birth weight of lambs, which is related with the results we obtained. The average birth weight in our study was (3.17 kg) was lower than the average found by other studies performed by some authors on different genotypes Chios × Akkaraman F1, 4.72 kg; Kıvırcık × Akkaraman F1, 4.75 kg; Chios × *(*Kıvırcık × Morkaraman*)* F1, 4.45 kg; Kıvırcık × (Chios × Morkaraman) F1, 4.25 kg; crossbred lambs (*Akcapinar et al .; 2000, Ozbey and Esen 2000*); Kıvırcık lambs under half-intensive conditions, 3.69 kg (*Altine et al., 1998,);* and Akkaramans, Merino, and their crosses under village conditions, 3.74 kg (*Thieme et al., 1999)* but almost similar with the result (3.18 kg) obtained by *Yilmaz and Altin, (2011)*.

At 30 days, the lambs born singles and twins were heavier from natural mating but born triplets were heavier from synchronized in both sexes. Differences in weight: 0.31 kg; 0.47 kg, 0.53 kg; 0.39 kg, 0.26 kg; 0.4 kg.

The lambs at 60 and 90 days, female born triplets were heavier from synchronized but lambs born singles and twins in both sexes and the male born triplets were heavier from natural mating. The weight differences were as follows: 0.49 kg;0.76 kg, 0.75 kg;0.95 kg, 1.21 kg;1.29 kg and 1.09 kg;1.24 kg, 1.41 kg;1.41 kg (same differences in both sexes), 1.53 kg;0.58 kg. Our results were similar with results of *Petrovic et al.(2011*).

Statistical analysis of our results showed that exist difference in the body weights between groups of lambs depending of investigated factors. In the first group, the difference in weight of lambs at birth, with regard of mating method was not significant (P> 0.05), while in the second group, the difference was statistically significant (P <0.05). As for the birth type, and within the same group, there was a statistical significance (P<0.05) between singles obtained naturally, between the triplets obtained naturally and between triplets received hormonal method.

All the differences between male body weight at 30 days (mating method, sex and birth type under the same genotype) were statistically significant (P <0.05).

The determined differences in the body weights at 60 days (between genotypes, mating method, sex and type of birth under the same genotype) were statistically significant (P <0.05).

The results showed that the differences (mating method, sex and type of birth under the same genotype) were statistically significant (P <0.05), except in between body weight at 90 days in twins among two genotypes obtained by natural method, which were not statistically significant (P> 0.05).

Conclusion

Based on the results obtained can be concluded that sex had a significant effect on body weight of lambs in all ages regardless of mating method. The estrus

synchronization can increase the number of offspring, but reduces the birth weight of lambs. Generally, natural mating shows dominance in body growth in all ages of lambs.

Acknowledgment

Research was financed by the Ministry of Education and Science of Republic of Serbia under the projects TR31001 and TR31053.

Uticaj metoda oplodnje genotipa, pola i tipa rođenja na porast jagnjadi

Z. Ilić, A Jevtić-Vukmirović, M P. Petrović, V. Caro Petrović, B. Milošević, Z. Spasić, B. Ristanović

Rezime

Metod sinhronizacije estrusa se koristi da kontroliše reproduktivne osobine ovaca. Cilj ovog rada bio je da se ispita i uporedi način parenja i uticaj osnovnih fiksnih faktora na masu jagnjadi. Statistička analiza je pokazala da je razlika telesne mase između grupa jagnjadi na rođenju bila statistički značajna. U prvoj grupi, razlika u masi jagnjadi na rođenju, zavisno od načina oplodnje nije bila značajna (P> 0,05), dok je u drugoj grupi, razlika statistički značajna (P <0,05). Što se tiče tipa rođenja, a u okviru istog genotipa, postoji statistička značajnost (P <0,05) između jedinaca dobijenih prirodno, između prirodno dobijenih trojki i između trojke dobijenih hormonskim metodom. Sve razlike između masa tela muških grla sa 30 dana (načinu oplodnje, tipa rođenja i u okviru istog genotipa) su statistički značajne (P <0,05). Utvrđene razlike u telesnoj masi sa 60 dana su takođe statistički značajne (P <0,05). Rezultati su pokazali da su razlike između genotipova pod uticajem fiksnih faktora statistički značajne (P <0,05), osim između telesne mase sa 90 dana kod blizanaca (P> 0,05).

References

AINSWORTH, L. AND M. S. WOLYNETZ. (1982): Synchronization of estrus and reproductive performance of ewes treated with synthetic progestagens administered by subcutaneous ear implant or by intravaginal sponge pessary. J. Anim. Sci. 54: 1120-1127.

AKCAPINAR, H., OZBEYAZ C, UNAL, N., AVCI. M. (2000): The possibilities of developing dam and sire lines using Akkaraman, Sakız and Kıvırcık Sheep breeds for lamb production I. Fertility in Akkaraman sheep, survival rate and growth characteristics of Sakız × Akkaraman F1 and Kıvırcık × Akkaraman F1 lambs. Türk Veterinerlik ve Hayvancılık Dergisi.24:71-79.

ALTINEL, A., EVRIM, M., OZCAN, M., BASPINAR, H., DELIGOZOĞLU, F.(1998): Studies on possibility of producing high quality lambs by crossbreeding among Sakiz, Kivircik and German Black Headed Mutton sheep. Turk J. Vet. Anim. Sci. 22: 257- 265.

ATAMANI M.B, AKOZ M. (2006): GnRH-PGF2α and PGF2α-PGF2α synchronization in Akkarman cross-breed sheep in the breeding season. Bull Vet Inst Pulawy. 50: 101-104.

BARRETT D.M, BARTLEWSKI P.M, BATISTA-ARTEAGA M, SYMINGTON A, RAWLINGS N.C. (2004): Ultrasound and endocrine evaluation of the ovarian response to a single dose of 500 IU of eCG following a 12-day treatment with progestogen-releasing intravaginal sponges in the breeding and nonbreeding seasons in ewes. Theriogenology. 61: 311-327.

DIXON A.B, KNIGHTS M, PATE J.L, LEWIS P.E, INSKEEP E.K. (2006): Reproductive performance of ewes after 5-day treatment with intravaginal inserts containing progesterone in combination with injection of prostaglandinF2a. Reprod Domest Anim. 41: 142-148.

DOGAN I, NUR Z. (2006): Different estrous induction methods during the non-breeding season in Kivircik ewes. Vet Med Czech. 51: 133-138.

GODFREY, R. W., GRAY M. L. COLLINS J. R. (1997): A comparison of two methods of estrous synchronization of hair sheep in the tropics. Anim. Reprod. Sci. 47:99-106.

JAINUDEEN, M. R., H. WAHID AND E. S. E. HAFEZ. (2000): Ovulation induction, embryo production and transfer. In: reproduction in farm Animals, seventh ed (Ed. B. Hafz and E. S. E. Hafez). Lippincott Williams & Wilkins, Philadelphia, pp. 405-409.

KNIGHTS M, HOEHN T, LEWIS P.E, INSKEEP E.K. (2001): Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. J Anim Sci. 79: 1120-113

KOYUNCU M , OZIS ALTICEKIC S. (2010): Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. Asian-Aust. J. Anim. Sci. 23: 308-311.

KUSINA, N. T., TARWIREI, F. HAMUDIKUWANDA, H. AGUMBA G. MUKWENA J. (2000): A comparison of the effects of progesterone sponges and ear implants, PGF2α, and their combination on efficacy of estrus synchronization and fertility of Mashona goat does. Theriogenology 53:1567-1580.

NADERIPOUR H, YADI J, SHAD A.G.K , SIRJANI M.A. (2012): The effects of three methods of synchronization on estrus induction and hormonal profile in Kalkuhi ewes: A comparison study. African Journal of Biotechnology. 11:530-533.

ÖZBEY, O.AND ESEN, F. (2000): The effects of oestrus synchronisation and flushing on birth weight of lambs, growth and survival rates in Awassi ewes. Journal Veteriner Fakültesi Dergisi, Uludağ Üniversitesi.19:119-127.

PETROVIC P.M .(2007): Sustainable sheepbreeding (in serbian). Institute for Animal Husbandry, Belgrade, 256 p.p.

PETROVIĆ, P. M., RUŽIĆ MUSLIĆ D, CARO PETROVIĆ V, MAKSIMOVIĆ N. (2011): Influence of environmental factors on birth weight variability of indigenous Serbian breeds of sheep. African Journal of Biotechnology. 10: 4673-4676.

ROMANO, J. E. (2004): Synchronization of estrus using CIDR, FGA or MAP intravaginal pessaries during the breeding season in Nubian goats. Small Ruminant Research. 55:15-19.

ROSADO, J., SILVA E, GALINA M. A. (1998): Reproductive management of hair sheep with progesterone and gonadotropins in the tropics. Small Rumin. Res. 27:237-242.

SPSS for Windows, Rel. 20.0. 2012. Chicago: SPSS Inc.

WILDEUS S. (2000): Current concepts in synchronization of estrus: Sheep and goats. Sheep and Goat Journal of Animal Science. 77: 1-14.

THIEME O., KARAZEYBEK M., AZMAN A.M., UĞURLU A.(1999) : Performance of village sheep flocks in Central Anatolia. I. Growth of lambs. Turk J. Vet. Anim. Sci. 23: 467-474.Turk J. Vet. Anim. Sci. 24: 71-79.

TURK G, GUR S, SONMEZ M, BOZKURT T, AKSU EH, AKSOY H. (2008): Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with progestagen-PMSG-PGF2alpha combination. Reprod Dome st Anim. 43: 308-313.

UNGERFELD R, RUBIANES E. (2002): Short term primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCGestrous induction in anestrus ewes. Small Ruminant Res. 46: 63-66.

WILDEUS S. (1999): Current concepts in synchronization of estrus: sheep and goats. Proc. Am. Soc. Anim Sci. 38: 1-14.

YILMAZ M, ALTIN T.(2011): Growth characteristics in lambs of estrus synchronized ewes in grower conditions. Turk. J. Vet. Anim. Sci. 35(6): 421-429.

Received 13 June 2013; accepted for publication 26 June 2013

Biotechnology in Animal Husbandry 29 (2), p 287-297 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.31'638
DOI: 10.2298/BAH1302287C

THE LINEAR RELATIONSHIP BETWEEN GROWTH TRAITS OF SHARPLANINA LAMBS IN EXTENSIVE FARMING PRACTICES

V. Caro Petrović[1], M.P. Petrović[1], M.M.Petrovic[1], Z. Ilić[2], J. Stojković[2], D. Ružić Muslić[1], N. Maksimovic[1]

1Institute for Animal Husbandry, Belgrade-Zemun
2Faculty for Agriculture, Lesak
Coressponding author: violycaro@yahoo.com
Original scientific paper

Abstract: The fastest phase of growth, observed in young animals, is often assumed to be linear, and linear regressions or ratios between BW gain and time are used to model growth. However, growth curves, due to their flexibility, are likely to be more suitable to describe even early growth. The research was performed in the region of Sharplanina Mountain in the population of the local Šarplanina breed of sheep. The following traits of lambs were considered: BWB, BW30, BW60 and BW90. Statistical analysis was conducted using the Pearson's correlation and multivariate linear regression model. This involved computing all possible and the best subset regression equation. Each equation was then assessed by its coefficient of determination (R2) and the constant based on the number of variable that used for the prediction. Results showed that weight of lambs from birth to weaning increased by about six times. Specifically lambs achieved an average total gain of 17.66 kg, or 196 g per day. There were a very significant correlation ($P<0.01$) between BWB and BW30, BW60 and BW90. Likewise, shown a very significant correlation ($P<0.01$) between BW30-BW60, BW30-BW90 and BW60-BW90. Also shown the coefficient of multiple determinations (R-squared R2) was 0.507 which means that 50.7% of the variance BW90, determined variance of the predictor variables represented in the model. Adjusted coefficient of multiple determination (adjusted R2) is 0.506 which means that 50.6% of the variance BW90, determined variance of the predictor variables that are in the model. Any increase in weight of lambs during the observed period of age is associated with an increase of dependent variable BW90. In particular, any increase in BW30 to 1 kg, is associated with an increase in BW90 to 1.928 kg.

Key words: lamb, growth, correlation, linear regression

Introduction

Growth traits of lambs are of primary importance in the production of lamb meat. It is known as mentioned by *Petrović (2000)* that there was correlation between growth traits. To define breeding program is necessary to know the strength of linear correlation among growth traits of lambs. The fit of a growth function, and hence the variables estimated, will depend on the number and timing of available BW observations. The fastest phase of growth, observed in young animals, is often assumed to be linear, and linear regressions or ratios between BW gain and time are used to model growth. However, growth curves, due to their flexibility, are likely to be more suitable to describe even early growth. The ability to change the shape of the growth curve by breeding may be an attractive prospect for livestock producers (e.g., to increase early growth but restrict mature size, and hence maintenance requirements). To determine the genetic flexibility of the shape of growth curves, genetic parameters must be calculated for the underlying curve variables (*Lambe et al., 2006*).The response to selection for a trait is dependent on the selection intensity, heritability, and linear relationship between growth traits (*Snyman et al. 1997, Olivier et al. 2001).* Genetic correlations among ewe traits were generally positive and moderate to high in magnitude. Also, selection on any of the component traits should not adversely affect other component traits, *Bromley et al., (2001).* Improvements in total weight of weaned lambs through selection on weaning weight and litter size at birth and weaning *(Bradford et al., 1999; Olivier et al. (2001, Snowder, 2002, Cloete et al., 2004)* have been reported. Riggio et al. (2008) estimated the genetic parameters of the body weight and suggested that taking live body weight of lambs into account as a selection criterion would increase selection accuracy. Such appropriate selective procedure requires accurate estimates of (co)variance components and genetic parameters. Genetic parameters for growth traits of different sheep breeds have been reported (Safari et al., 2005; Miraei-Ashtiani et al., 2007; Rashidi et al., 2008; Gowane et al., 2010; Mohammadi et al., 2010, Caro Petrović et al.,2012).The aim of this paper is to determine linear relationship and correlation between growth traits of lambs from Sharplanina breed of sheep.

Material and methods

The research was performed in the region of Sharplanina Mountain in the population of the local Sharplanina breed of sheep. All tested lambs had the same conditions of housing care and nutrition.

The following traits of lambs were considered: body weight at birth (BWB), body weight at 30 days (BW30), body weight at 60 days (BW60) body weight at 90 days (BW90). Animals were managed following traditional extensive

farming practices. During spring-summer season, natural pasture was the main source of feed without any additives. During the autumn-winter season, the sheep were fed hay and concentrate. Lambing season was from January to March, and lambs were kept with their mothers while in the special box received high-quality hay and a concentrate with 18% of protein. Body weights of lambs were weighed at birth and once a month until weaning at 3 months of age. Statistical analysis was conducted using the software program SPSS (2012). Procedure was applied using the Pearson correlation and multivariate linear regressions model. This involved computing all possible and the best subset regression equation. Each equation was then assessed by its coefficient of determination (R2) and the constant based on the number of variable that used for the prediction.

Pearson coefficients of correlations are calculated using of the next formula:

$$rxy = Sxy / Sx \times Sy$$

Where:

rxy= Coefficient of correlations
Sxy= Covariance
Sx and Sy= Standard deviations

Basic regressions model takes the form:

$Y = \alpha + \beta 1x1 + \beta 2x2 + ... + \beta 1x1 + \beta nxn$

where

Y= the expected value of the dependent variable
α= Value of the dependent variable when not yet begun to act independently variable
β= the rate and direction of change of dependent variable in the value of independent variable

Results and discussion

Results of means and standard errors for body weight of lambs are given in Table 1.

From the said table, it can be observed that the weight of lambs from birth to weaning increased by about six times. Specifically lambs achieved an average total gain of 17.66 kg, or 196 g per day. Compared with the results of other scholars (*Petrović et al, 2011, Mekić et al, 2005*), we can determine that the obtained values of growth alike with the other Pramenka sheep populations in the Balkan regions.

Table 1. Body weight of lambs from birth to weaning, kg

Traits	N	Minimum	Maximum	Mean	S.E.
BWB	300	3.00	4.00	3.74	±0.01
BW30	300	8.50	12.00	9.92	±0.03
BW60	300	14.00	18.00	16.08	±0.06
BW90	300	19.10	25.00	21.40	±0.10
Valid N (listwise)	300				

To persuade the strength of linear relationship or association, growth traits of lambs in Table 2, presented the values of Pearson correlation and covariance. Covariance in probability theory and statistics is a measure of strength of the relationship between two variables. However, as an absolute measure of the degree of association is not suited for the assessment, and resort to the correlation coefficient as a relative measure, which in this paper done.

Table 2. Correlation and covariance of the growth traits of lambs from birth to weaning

Traits		BW1	BW30	BW60	BW90
BWB	Pearson Correlation	1	.560**	.179**	.359**
	Sig. (2-tailed)		.000	.002	.000
	Sum of Squares and Cross-products	19.737	28.359	15.338	49.216
	Covariance	.066	.095	.051	.165
	N	300	300	300	300
BW30	Pearson Correlation	.560**	1	.618**	.712**
	Sig. (2-tailed)	.000		.000	.000
	Sum of Squares and Cross-products	28.359	130.082	135.871	250.790
	Covariance	.095	.435	.454	.839
	N	300	300	300	300
BW60	Pearson Correlation	.179**	.618**	1	.466**
	Sig. (2-tailed)	.002	.000		.000
	Sum of Squares and Cross-products	15.338	135.871	371.189	276.964
	Covariance	.051	.454	1.241	.926
	N	300	300	300	300
BW90	Pearson Correlation	.359**	.712**	.466**	1
	Sig. (2-tailed)	.000	.000	.000	
	Sum of Squares and Cross-products	49.216	250.790	276.964	952.941
	Covariance	.165	.839	.926	3.187
	N	300	300	300	300
**. Correlation is significant at the 0.01 level (2-tailed).					

Based on the results of the above (table 2), have attained that there were a very significant correlation (P <0.01) between BWB and BW30, BW60 and BW90. Also, there were very significant correlation (P <0.01) between BW30-BW60, BW30-BW90 and BW60-BW90.

Correlation was highest between the weight of lambs at 30 days and body weight at 90 days (0.712) and the lowest between body weight at birth and weight of lambs at 60 days (0.179).

Correlation estimate of BW with other body weight traits was in agreement with estimate of *Mohammadi et al., (2010)* in Sanjabi sheep and *Mohammadi et al. (2011)* in Zandi sheep.

Positive correlations were reported by *Duguma et al. (2002)* in Tygerhoek Merino sheep, *Gowane et al. (2010)* in Malpura sheep. Correlation between BWB and BW90 in the present study (0.359) was consistent with the results of *Vaez-Torshizi et al. (1992), Eftekhari-Shahroudi et al. (2002), Neser et al. (2001), and Baneh et al. (2010).*

Positive correlations among body weight traits indicated that there was no genetic, phenotypic and environmental antagonist relationship among considered traits. selection for any of these body weights is likely to result in positive response in terms of genetic and phenotypic values.

Weight of lambs at 90 days from the aspect of research and practice is paramount, as it is the age of the market. To determine the size of the expected change of dependent variable Y (weight at 90 days- BW90) for each unit change in an independent variable X (BW60, BW30, BWB), performed a multivariate regression analysis, the results of which are presented in the Tables 3,4,5 and 6.

Table 3. Regression model summary

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.712a	.507	.506	1.25510
a. Predictors: (Constant), BW30				

From the table 3, we can see that the method of "stepwise" had been formed models in one step, which shows that the coefficient of multiple regressions (R) is 0.712. It is a measure of correlation between the score values of masses from 90 and a set of predictor variables that are in the final model.

It is also displayed that the coefficient of multiple determination (R-squared R2) was 0.507 which means that 50.7% of the variance BW90, determined variance of the predictor variables represented in the model.

Adjusted coefficient of multiple determination (adjusted R2) is 0.506 which means that 50.6% of the variance BW90, determined variance of the predictor variables that were in the model.

Regression as a model for predicting the physical development of lambs is used by many other researchers (*Afolayan et al., 2006; Kunene et al., 2007; Hamito, 2009).*

Results of analysis of variance are shown in table 4.

Table 4. Results of analysis of variance

ANOVAa						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	483.506	1	483.506	306.933	.000b
	Residual	469.434	298	1.575		
	Total	952.941	299			
a. Dependent Variable: BW90						
b. Predictors: (Constant), BW30						

From Table 4 have shown that the value of F-test is 306.933 (P = 0.000), thus confirming that the multiple correlation coefficient in the final model statistically significant. In other words, the regression model significantly predicts the value of the criterion variables.

The final results of multiple or multiple regression are shown in Table 5.

Table 5. Results obtained regression coefficient

Coefficients						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.265	1.095		2.068	.039
	BW30	1.928	.110	.712	17.520	.000
a. Dependent Variable: BW90						

From Table 5 can visualize that in the final model-1, in addition to the regression constants are also predictors BW30. Any increase in weight of lambs during the observed period of age is associated with an increase of the dependent variable BW90. In particular, any increase in BW30 to 1 kg, is associated with an increase in BW90 to 1.928 kg. Standardized coefficients in the table indicate the size of the standard deviation of changes in BW90 if the value of the predictor variables increased by one standard deviation.

Based on the results of multiple regressions, it can be ceased that there is a significant linear correlation of medium intensity between the BW90 and body weight from birth to 60 days of observation.

The data show that about 50% of the variability of BW90, can be explained by variations BWB, BW30, BW60 and 50% of the variability is determined by other factors, particularly genetics and environment. Lewis and *Brotherstone (2002)* and *Fischer et al. (2004),* stated that regression method had a significant contribution in the prediction and assessment of growth of lambs. Some scholars (*Bassano et al., 2001; Adeyinka and Mohammed, 2006; Thiruvenkadan, 2005; Kunene et al., 2007; Hamito, 2009*) suggested that the number and particular type of traits required in a model depended on the breed, age of animals, season.

Table 6.Values of variables that are excluded from the model

Excluded Variables						
Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	BWB	-.058b	-1.182	.238	-.068	.687
	BW60	.041b	.789	.430	.046	.618
a. Dependent Variable: BW90						
b. Predictors in the Model: (Constant), BW30						

Variables BWB and BW60 did not qualify for inclusion in the model because the condition P <0.05 (Table 6).

From the table (6) can be seen that the value of Beta In, show how to make the coefficients of variables excluded if they were included in the model. Additionally, was also given the values and partial correlation coefficients. Based on the foregoing it can be affirmed that there is no significant linear relationship between BW90, BWB and BW60 (P> 0,05). This observation can be associated with the criteria in selection. Genetic parameters for growth traits of different sheep breeds have been reported (*Safari et al., 2005; Miraei-Ashtiani et al., 2007; Rashidi et al., 2008; Gowane et al., 2010; Mohammadi et al., 2010, Caro Petrović et al.,2012).*

Conclusion

Based on the research conducted and the results obtained, we can conclude that weight of lambs from birth to weaning increased by 17.66 kg, or 196 g per day.

There were a very significant correlation between BWB and BW30, BW60 and BW90. Likewise, a very significant correlation obtained between BW30-BW60, BW30-BW90 and BW60-BW90.

The coefficient of multiple determinations means that 50.7% of the variance BW90, determined variance of the predictor variables represented in the model.
Adjusted coefficient of multiple determination means that 50.6% of the variance BW90, determined variance of the predictor variables that are in the model.
Any increase in weight of lambs during the observed period of age is associated with an increase of BW90 dependent variable. In particular, any increase in BW30 to 1 kg, is associated with an increase in BW90 to 1,928 kg.

Linearna povezanost osobina porasta jagnjadi šarplaninske rase u ekstenzivnim uslovima gajenja

V. Caro Petrović, M.P. Petrović, M.M Petrovic, Z. Ilic, J. Stojković, D.Ruzic Muslic, N. Maksimovic

Rezime

Istraživanje je sprovedeno u regionu Šar planine u populaciji lokalne šarplaninske rase ovaca. Sledeće osobine jagnjad su posmatrane: masa pri rođenju, sa 30,60 i 90 dana uzrasta (BWB, BW30, BW60 i BW90). Statistička analiza je sprovedena primenom SPSS paketa. Izračunate su Pearson korelacije i multivarijantna linearna regresija. To je značilo računanje svih mogućih i najboljih podskupova jednačina regresije. Svaka jednačina je ocenjena kao i njen koeficijent determinacije (R2). Rezultati su pokazali da je masa jagnjadi od rođenja do odbijanja povećana za oko šest puta. Konkretno jagnjad su ostvarila prosečan ukupan prirast od 17,66 kg, ili 196 g dnevno. Utvrđena je vrlo značajna korelacija (P <0,01) između BWB i BW30, BW60 i BW90. Isto tako, pokazala se veoma značajna korelacija (P <0,01) između BW30-BW60, BW30-BW90 i BW60-BW90. Takođe, dobijeni koeficijent višestruke determinacije (R-skuared R2) znači da je 50,7% varijanse BW90, determinisano varijansom prediktorskih varijabli predstavljenih u modelu. Korigovani koeficijent višestruke determinacije (R2 prilagođeni) je 0.506, što znači da je 50,6% varijanse BW90, uslovljeno varijansama prediktorskih varijabli koje su u modelu. Svako povećanje mase jagnjadi u toku posmatranog perioda života je povezano sa povećanjem zavisno promenljive BW90. Praktično, svako povećanje BW30 za 1 kg, je povezano sa povećanjem BW90 za 1.928 kg.

References

ADEYINKA I.A. AND MOHAMMED I.D. (2006): Relationship of live weight and linear body measurement in two breeds of goat of Northern Nigeria. Journal of Animal and Veterinary Advances, 5:891-893.

AFOLOYAN R.A., ADEYINKA I.A., LAKPINI C.A.M.(2006): The estimation of live weight from body measurement in Yankasa sheep. Czech J. Anim. Sci. 51: 343-348.

BANEH H., HAFEZIAN S.H., RASHIDI A., GHOLIZADEH M. (2010): Estimation of genetic parameters of body weight traits in Ghezel sheep. Asian-Aust. J. Anim. Sci. 23: 149-153.

BASSANO B, BERGERO D., PERACINO A. (2001): Accuracy of body weight prediction in Alpine Ibex (Capra ibex, L. 1758) using morphometry. Journal of Animal Physiology and Animal Nutrition, 87: 79-85.

BRADFORD G.E. SAKUL H, DALLY M.R. (1999): Selection for weaning weight or litter size in range sheep. 11. Correlated responses and effect on productivity. Sheep Goat Res. Journal 15: 138-147.

BROMLEY C.M., VAN VLECK L.D., SNOWDER G.D. (2001): Genetic correlations for litter weight weaned with growth, prolificacy and wool traits in Columbia, Polypay, Ramboillet and Targhee sheep. Journal of Animal Science 79: 339-346.

CLOETE S.W.P., GILMOIR A.R., OLIVIER J.J., VAN WYK J.B. (2004): Genetic and Phenotypic trends and parameters in reproduction, greasy fleece weight and live weight in Merino lines divergently selected for multiple rearing ability. Aust. J. Exp. Agric. 44: 745-754.

DUGUMA G., SCHOEMAN S.J., CLOETE S.W.P., JORDAN G.F.(2002): Genetic parameter estimates of early growth traits in the Tygerhoek Merino flock. South African Journal of Animal Science 32, 66-75.

EFTEKHARI-SHAHROUDI E., BAHRINI M.R., VEN DOULK D., DANESH MESGARAN M. (2002):The factor affecting some economical traits in Kermani sheep. Iran. J. Agr. Sci. 33: 395-402.

FISHER T.M., VAN DER WERF J.H.F., BANKS R.G., BALL A.J. (2004): Description of lamb growth using random regression on field data. Livestock Production Science 89, 175-185.

GOWANE G.R., CHPRA A., PRAKASH V., ARORA A.L.(2010): Estimates of (co) variance components and genetic parameters for body weights and first greasy fleece weight in Malpura sheep. Livestock Science 131, 94-101.

HAMITO D. (2009): Estimation of weight and age of sheep and goats. Ethiopia sheep and goat productivity improvement program (ESGPIP). Technical Bulletin No. 23. P 11.

KUNENE N., NESAMVUNI E.A. , FOSSEY A.(2007): Characterization of Zulu (Nguni) sheep using linear body measurements and some environmental factors affecting these measurements. S.Afr. J. Anim. Sci. 37 (1), 11–20.
KUNENE N., NESAMVUNI E.A., FOSSEY A.(2007): Characterization of Zulu (Nguni) sheep using linear body measurements and some environmental factors affecting these measurements. South African Journal of Animal Science. 37: 11-20.
LAMBE N.R., NAVAJAS E.A., SIMM G., BUNGER L. (2006): A genetic investigation of various growth models to describe growth of lambs of two contrasting breeds. Journal of Animal Science vol. 84 no.10 pp 2642-2654.
LEWIS R.M. AND BROTHERSTONE S. (2002): A genetic evaluation of growth in sheep using random regression techniques. Animal Science 74, 60-70.
MEKIĆ C., PETROVIĆ M.P., TRIFUNOVIČ G.,ŽUJOVIĆ M., PERIŠIĆ D. (2005): Milk production of Svrljig stock of domestic tuff sheep. Biotechnology in Animal Husbandry 21 (5-6), p 85-88.
MIRAEI-ASTIANI S.R., SEYEDALIAN S.A.R., MORADI SHAHRBABAK M. (2007): Variance components and heritabilities for body weight traits in Sangsari sheep using univariate and multivariate animal models. Small Ruminant Research 73, 109-114
MOHAMMADI K., RASHIDI A., MOKHTARI M.S., BEIGI NASSIRI M.T. (2011): The estimation of (co)variance components for growth traits and Kleiber ratios in Zandi sheep. Small Ruminant 99, 116-121.
MOHAMMADI Y. , RASHIDI A., MOKHTARI M.S., ESMAILIZADEH A.K.(2010): Quantitative genetic analysis of growth traits and Kleiber ratios in Sanjabi sheep. Small Ruminant Research 93, 88-93
NESER F.W., ERASMUS G.J., VAN WYK J.B. (2001): Genetic parameter estimates for pre-weaning weight traits in Dorper sheep. Small Ruminant Res. 40: 197-202.
OLIVIER W.J., SNYMAN M.A., OLIVIER J.J., VAN WYK J.B., ERASMUS G.J.(2001): Direct and correlated responses to selection for total weight of lamb weaned in Merino sheep. South African Journal of Animal Science 31: 115-121.

PETROVIĆ M.P. (2000): Genetika i oplemenjivanje ovaca. Beograd: Naučna knjiga.
PETROVIĆ V.C., PETROVIĆ M.P., PETROVIĆ M.M., ILIĆ Z.,MAKSIMOVIĆ N., RUŽIĆ-MUSLIĆ D., STOLIĆ N.(2012): Estimation of phenotypic and genetic trends of the growth traits in Lipska and Svrljig sheep. Biotechnology in Animal Husbandry 28 (4), p 743-749.
PETROVIČ M.P., RUŽIĆ-MUSLIĆ D., PETROVIĆ V.C., MAKSIMOVIĆ N. (2011): Influence of environmental factors on birth weight variability of indigenous Serbian breeds of sheep. African Journal of Biotechnology vol.10 (22), pp.4673-4676.

RASHIDI A., MOKHTARI M.S., SAFI JAHANSHAHI A., MOHAMMAD ABADI M.R.(2008): Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. Small Ruminant Research 74, 165-171.
RIGGIO V., FINOCCHIARO R., BISHOP S.C.(2008): Genetic parameters for early lamb survival and growth in Scottish Blackface sheep. Journal of Animal Science 86, 1758-1764.
SAFARI E., FOGARTY, N.M., GILMOUR A.R. (2005): A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 92, 271-289.
SAFARI, E., FOGARTY, N.M., GILMOUR, A.R. (2005): A review of genetic parameters estimates for wool, growth, meat and reproduction traits in sheep. Livestock Production Science 92, 271-289.
SNOWDER G.D.(2002): Composite trait selection for improving lamb production. Sheep and Goat Res. Journal 17: 42-49.
SNYMAN M.A., OLIVIER J.J., ERASMUS G.J., VAN WYK J.B.(1997): Genetic parameter estimates for total weight of lamb weaned in Afrino and Merino sheep. Livest. Prod. Sci. 48, 111–116.
VAEZ TORSHIZI R., EMAM JOMEH N.,NIK KHAH A., HEJAZI M.(1992): A study of pre-weaning traits in a Baluchi sheep flock. Iranian Journal of Agricultural Science 23, 33-42.

Received 27 May 2013; accepted for publication 26 June. 2013

Biotechnology in Animal Husbandry 29 (2), p 299-310 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'643
DOI: 10.2298/BAH1302299S

THE EFFECT OF BREED, BOAR AND SEASON ON SOME PROPERTIES OF SPERM

R. Savić[1], M. Petrović[1], D. Radojković[1], Č. Radović[2], N. Parunović[3]

[1]University of Belgrade – Faculty of agriculture, 11080, Belgrade- Zemun, Republic of Serbia
[2]Institute for Animal Husbandry, 11080, Belgrade- Zemun, Republic of Serbia
[3]Institute of Meat hygiene and Technology, 11000, Belgrade, Republic of Serbia
Corresponding author: savic@agrif.bg.ac.rs
Original scientific paper

Abstract: The main objective of this study was to assess the phenotypic variability of the ejaculate volume (VOL) and progressive sperm motility (MO) under the influence of breed, boar within breed and season. The study included 555 ejaculates of Danish Landrace (DL), Danish Large White (DLW) and Danish Duroc (DD) boars. The effect of boar was analyzed within the investigated breeds. The impact of the season was investigated as the influence of the month in which the ejaculate was taken. The model included a linear regression effect of boar age when the semen was taken. Data analysis was performed using the statistical package SAS 9.1.3 (SAS Inst. Inc.., 2002-2003). Impact assessment was carried out using the GLM procedure. The impact of all factors analyzed in both sperm traits was significant to highly significant, and the observed factors largely explain the phenotypic variability VOL compared to MO. The highest values were found in VOL of DL boar (164.96 ml ± 3.58), and the MO of boar breed DLW (85.45 ± 0.94%). Boars DD had VOL and MO below the averages of all analyzed breeds. Between boars within studied genotypes differences were established ($p < 0.05$, $p < 0.05$, $p < 0.01$ $p < 0.001$) and a boar of DLW breed compared to all tested boars had the highest volume of ejaculate sperm with progressive motility of 90.47%. Volume, or moTility varied ($p < 0.01$ or $p < 0.05$) influenced by season, and in September and October boars had ejaculates with the VOL values above average and the lowest MO. Since most VOL and MO above the annual average in June, it was possible to get the highest number of doses per ejaculate with optimal reproductive/fertile ability.

Key words: sperm, boars, breed, season, volume, motility

Introduction

When speaking of the reproductive efficiency in swine production, one of the critical points is the knowledge of boar fertility. Success in implementing the technology of artificial insemination depends on the identification and selection of boars whose reproductive performance, evaluated on the basis of libido, performance in insemination and semen quality, are above average (*Okere et al., 2005*).

Fertility of boars can be seen through various quantitative and qualitative characteristics of sperm that determine its fertile ability. To produce high quality sperm of high genetic merit is the most important goal for artificial insemination centres (*Frangež et al., 2005*). Control of qualitative and quantitative traits of boar semen has great economic importance for pig breeders (*Smital, 2010*).

Ejaculate volume and progressive motility of sperm are important characteristics that determine the number of doses produced per ejaculate and reproductive ability of sperm. Estimated sperm motility is the most important parameter of sperm (*Kunowska-Slósarz and Makowsai 2011*). Precise determination of volume, concentration and the percentage of live sperm is very important for the assessment of the maximum dilution of sperm that can be used for artificial insemination, and the number of sows that can be inseminated (*Kanokwan, 2011).*

Production of sperm varies throughout the year and depends on various endogenous and exogenous factors (*Kondracki et al., 2009*). The variability of these traits is influenced by breed, season, age of boar when the ejaculate was taken, the frequency of use of boars and other factors (*Stančić et al., 2003 Frangež et al., 2005; Okere et al., 2005; Wolf and Smital, 2009 ; Smital and Wolf, 2009b; Smital, 2010).* Research results of *Kunowska-Slósarz and Makowska* (2011) showed a significant effect of season and breed on the volume, concentration and number of live sperm in boar ejaculates.

The aim of this study was to determine how breed, season and age of boars affect the phenotypic variability of ejaculate volume and progressive motility of sperm.

Material and methods

The study included a total of 555 ejaculates from 10 boars. Table 1 shows the structure of the data set according to analyzed breeds.

Table 1. Structure of the data set per breeds

Variable	Breeds			All breeds
	DL	DLW	DD	
Number of ejaculates	150	311	94	555
Average number of ejaculates per boar	75.00	51.83	47.00	55.50

DL-Danish Landrace, DLW- Danish Large White, DD- Danish Duroc

The data on the volume of ejaculate (VOL, ml) and progressive sperm motility (MO, %) of boars was used. Ejaculate volume was measured with an accuracy of ± 10 ml, and assessment of progressive sperm motility was performed by a subjective method, observing native sperm sample under a microscope with a standard magnification (visual score). When it comes to the MO, there were no limits, so the research included all MO values from 0 to 100%. Boars with less than five ejaculates were excluded from the analysis, the average number of ejaculates per boar was 55.50. The effect of boar was analyzed within the investigated breeds. The impact of the season was seen as the influence of the month in which the sperm is taken.

Data analysis was performed using the statistical package SAS 9.1.3 (*SAS Inst. Inc.., 2002-2003)*. Impact assessment was carried out using the GLM procedure. We used the following mathematical-statistical model:

$$Y_{ijkl} = \mu + G_i + G(B)_{ij} + S_k + b(x_{ijkl} - \bar{x}_{ijkl}) + \varepsilon_{ijkl},$$

where: Y_{ijkl}- observed sperm trait, μ- general population average, G_i- the effect of breed (i=1, 2, 3), $G(B)_{ij}$ – the effect of boar within the breed, S_k – the effect of season (k=1, 2, 3,...,12*)*, $b(x_{ijkl} - \bar{x}_{ijkl})$ – linear regression effect of the age of boar when ejaculate was taken and ε_{ijkl}- random error. Testing (comparing) of differences in LSMean values was done using the t- test.

Results and Discussion

Table 2 presents the basic statistical parameters of analyzed sperm characteristics. The average volume of examined ejaculate was 156.11 ml, and the average progressive motility of spermatozoa was 84.22%. Ejaculate volume showed a greater absolute and relative variation (59.59 ml and 38.17%) compared with progressive sperm motility (15.30 ml and 18.17%). Among the observed characteristics of sperm correlation coefficient showed a weak positive correlation

(0.365 ***). The average age of the boar when the ejaculate was taken was 628.58 days.

Table 2. Basic statistical parameters

Traits	$\bar{X}$	SD	CV	r
VOL, ml	156.11	59.59	38.17	0.365***
MO, %	84.22	15.30	18.17	
ABE, day	628.58	249.30	39.66	-

$\bar{X}$- Mean; SD- Standard deviation; CV- Coefficient of Variations; r - Correlation Coefficents; VOL- Volume of ejaculates; MO- Progressive motility; ABE- Average of age of the boar with ejaculate

The volume of ejaculate in this study was lower in comparison to the survey by *Kunowska-Slósarz and Makowska (2011),* and the progressive motility was higher. Established progressive motility (84.22%) was higher compared to the survey by *Stančić et al.* (2003), who obtained value of 78%, *Frangež et al. (2005)* who found values of 70.17 to 78.04% depending on the frequency of taking sperm. The reason for higher values of MO in this study may be due to the subjectivity in the visual assessment of sperm motility in the ejaculate.

The statistical significance of the factors included in the model are shown in Table 3. All analyzed factors showed significant to highly significant impact on the observed properties of the sperm. The influence of breed on the VOL and MO was highly significant (F = 49.46 ***, F = 15.26***), while the season had highly statistically significant, and significant effect on sperm traits (F = 2.54** and F = 1.99 *). Ejaculate volume and progressive motility of spermatozoa in the ejaculate varied among boars within the same breed (p <0.001).

Table 3. Effect of factors included in the model

Factor	Semen traits			
	VOL		MO	
	F value	R^2	F value	R^2
G (Breed)	49.46***	0.511	15.26***	0.254
G(B) (Boars within breed)	12.53***		7.33***	
S (Season)	2.54**		1.99*	
b (Age of boar)	0.0985***		0.0071*	

VOL- Volume of ejaculates; MO- Progressive motility; R^2- Coefficient of determination

Linear regression effect of boars age on the VOL was highly significant (b = 0.0985 ***) and regression coefficients showed that with increasing age, ejaculate and VOL increased also. VOL increase with increasing age of boars may be explained by the increase in mass and size of the testes, resulting in increased

production of sperm. From the biological point of view, one of the reasons for increasing sperm production is to increase the number of Sertoli cells in the testes (*Kanokwan, 2011*). The regression coefficient for MO had a lower value (b = 0.0071 *), which indicates that as the age increases also MO increases, but compared to VOL, the dependence is significant at the level of 95%. There is a tendency of increase of ejaculate volume and progressive sperm motility with increasing age of the boar, which is similar to the conclusions made by *Stančić et al. (2003), Wolf and Smital (2009a), Wolf and Smital (2009b), Smital (2009), Banaszewska and Kondracki (2012).*

The relevance of the observed factors indicate the need for continuing study of characteristics of boar sperm in selected pig populations, and taking appropriate steps to improve the properties or eliminating boars whose phenotypic traits of sperm are significantly below average. The calculated coefficients of determination showed that the analyzed effects to a greater extent explain the variability in ejaculate volume (R^2 = 0.511) compared to the progressive sperm motility (R^2 = 0.254).

This research is partially consistent with the research of *Šerniene et al. (2002),* as the above authors found that the seasons and the interaction of some factors had a significant effect on sperm motility. In contrast, breed and age of boars in studies of *Šerniene et al. (2002)* did not affect the variability of the characteristics of sperm. The results of our research on the impact of breed and season on VOL are similar to the research by *Okere et al. (2005),* and when it comes to the variability of MO, there are differences because seasonal effects in the study of these authors were not significant. Comparing with the research of *Kondracki et al. (2009),* the results of our study are similar, given that these authors found seasonal effects on the variability of the analyzed sperm characteristics. Age of boars showed a significant effect on sperm parameters (*Smital, 2009*), so our results are consistent with the research of this author. This research is consistent with the results of *Smital (2010)* in which all studied effects showed a significant effect on all traits of sperm ($p < 0.001$).

In Table 4, LSMean values of boar semen according to the breeds included in the study, are presented. The values of VOL and MO in fertile breeds (DL and DLW) were above average. The largest volume of ejaculate was found in boars of breed DL (164.96 ml), and the lowest in breed DD (102.55 ml). The differences in the mean values of VOL between boar breeds DD and DL (62.41 ml), DD and DLW (61.34 ml) were statistically highly significant ($p < 0.001$).

Boars of breed DLW had the highest progressive sperm motility (85.45 ± 0.94%). In contrast to these, the least progressive motility of spermatozoa in the ejaculate had DD boars (74.03 ± 1.83%). The difference between the mean values of MO in boars DLW and DL (1.22%) was not statistically significant ($p > 0,05$). Boars of breeds DLW and DL had the higher mean sperm motility (+11.42% and

+10.20%) compared to boars of DD breed the differences were very highly significant

Table 4. Effect of breeds on semen traits (LSMean±SE)

Breeds	LSMean±SE	
	VOL (ml)	MO (%)
DL	164.96±3.58^{A}	84.23±1.14^{A}
DLW	163.89±2.95^{A}	85.45±0.94^{A}
DD	102.55±5.77^{B}	74.03±1.83^{B}
All breeds	151.84±2.15	82.92±0.68

DL- Danish Landrace; DLW- Danish Large White; DD- Danish Duroc; VO- Volume of ejaculates; MO- Progressive motility; A, B- Significant at level p<0.001

Contrary to the present study, *Banaszewska and Kondracki (2012)* found higher values of ejaculate volume in the studied breeds of boars (from 162.75 ± 50.52 to 268.79 ± 88.06 ml). The low value of the ejaculate volume of Duroc breed boars (102.55 ± 5.77 ml) found in this study is consistent with research by *Stančić et al. (2003:* 191 mL), *Wolf and Smital (2009b; 200 ml),* and *Banaszewska and Kondracki (2012; 162.75 ml).* They found lower values of ejaculate volume in boars of Duroc breed compared to other breeds, but in our study, the mean value is lower. Unlike research by *Okere et al. (2005),* which determined the Yorkshire boars superiority compared to Landrace boars, in our study, differences in phenotypic values of VOL and MO between DL and DLW were not significant (p> 0,05). The results of this study are similar to research of *Smital and Wolf (2009)* who found slight difference between the breeds, where Czech Landrace had a larger volume of ejaculate, but all other traits of sperm had higher values in the Czech LW boars. The differences in sperm motility ($p \leq 0.01$) in Polish Landrace compared to other tested breeds were determined in the study by *Kunowska-Slósarz and Makowska (2011).*

Table 5 shows LSMean values of sperm traits per boars, and the significance of differences between boars within studied genotypes.

The volume of ejaculate of two DL boars was above the average of all tested boars, while the difference in MO between them was significant (p <0,05). The greatest VOL (from 115.39 to 196.36 ml) and MO (from 67.39 to 92.52%) variation interval was within the breed DLW. The differences between boars DLW were significant to highly significant, indicating that in the selected pig populations it is necessary to monitor properties of sperm, in order to receive per each ejaculate a maximum doses for insemination of optimal reproductive ability.

Table 5. Effect of boars within the breed on semen traits (LSMean±SE)

Breeds	Identification number of boars	LSMean±SE	
		VOL (ml)	MO (%)
DL	8330	169.83±4.60	86.75±1.46^{Aa}
	8416	160.09±5.44	81.70±1.73^{Bb}
DLW	899	160.96±8.26$^{A, a, Aa}$	85.42±2.62 $^{A, Aa}$
	926	158.60±5.60$^{A, a}$	92.52±1.90 $^{A, Bb}$
	936	165.82±4.86$^{A, Aa}$	87.38±1.54 $^{A, Aa}$
	1017	196.36±4.42^{B}	90.47±1.40^{A}
	14448	115.39±11.50 $^{C, b}$	67.39±3.65^{B}
	35571	186.19±7.08 $^{A, b, Bb}$	89.53±2.25^{A}
DD	1251	125.91±10.41^{A}	76.74±3.31
	1314	79.20±4.90^{B}	71.33±1.55

DL- Danish Landrace; DLW- Danish Large White; DD- Danish Duroc; VO- Volume of ejaculates; MO- Progressive motility; A, B, C- Significant at level p<0.001; a, b- Significant at level p<0.01; Aa, Bb- Significant at level p<0.05

Boar with identification number 1017 had the highest volume of ejaculate (196.36 ml) with high progressive motility (90.47%). Comparison with the boar number 926, which had the ejaculates with the highest value of MO (92.52%), the differences in mean values of MO (2.05%) was not significant, but the VOL of ejaculates in boar 1017 was significantly higher (+37.76 ml, p <0.001). Given the high mobility of sperm and determined differences of ejaculates, the boar with identification number 1017 can get the greatest number of doses for artificial insemination of optimal fertile ability. Boars DD had values below the average sperm characteristics, and differences in the VOL were highly significant (p <0.001).

Effect of season on semen characteristics observed is shown in Table 6. If the value of ejaculate volume during the year is analyzed, the highest value was in the month of June (166.03 ± 6.60 ml), while the lowest VOL was during the month of May (137.97 ± 6.74 ml). Lower values of VOL, below the annual average (151.84 ± 02.15 ml) can be observed in January-May Low values during winter may be due to the negative impact of low temperatures in the facilities where boars are kept. At the same time the ejaculate mobility was above average.

September and October are characterized with semen ejaculate volumes above average, but the lowest progressive motility. In September and October spematozoid progressive motility was the lowest (78.47 and 78.63%), which can be explained as a result of the high temperatures during the summer period and the occurrence of chronic stress caused by the stress factor during the autumn period. In the subsequent months a trend of gradual increase can be observed and overcoming of the consequences of heat stress.

Table 6. Effect of season on semen traits (LSMean±SE)

SEZ	LSMean±SE	
	VOL (ml)	MO (%)
1	141.59±6.54	84.92±2.08
2	146.67±6.42	85.31±2.04
3	139.97±6.17	85.81±1.96
4	141.28±6.59	85.28±2.09
5	137.97±6.74	84.54±2.14
6	166.03±6.60	84.76±2.09
7	157.12±6.87	80.60±2.18
8	156.28±7.80	80.28±2.48
9	165.80±6.39	78.47±2.03
10	160.47±6.08	78.63±1.93
11	156.18±5.49	81.36±1.74
12	152.68±6.04	85.09±1.92
Average	151.84±2.15	82.92±0.68

SEZ- Season; VOL- Volume of ejaculates; MO- Progressive motility

The results of comparisons between months are shown in Table 7. VOL was the highest during the month of June, and the differences with respect to all months with lower values of VOL (January-May) were significant to highly significant. Since the highest VOL and MO are above the annual average in June, it was possible to get the highest number of doses per ejaculate with optimal reproductive ability. In comparison to other months the differences were not statistically significant. Comparing the months September and October, when the lowest values of MO were determined, with a period of higher values of MO (December-June), the differences were significant to highly significant.

If VOL is compared, the results of this study are similar to investigations by *Okere et al. (2005), Kondracki et al. (2009), Wolf and Smital (2009),* which have established in the spring, the lowest volume of ejaculate in the investigated breeds. Comparing the results of *Kunowska-Slósarz and Makowska (2011),* the results of this study are partly similar, given that in the study of these authors in the period January-June, VOL value below the annual average and above-average values from August to December were established, while for MO, the lowest values were established in the October-December period and June-July period and a maximum in January-May and August-September.

Table 7. Results of the t- test for LSMean values for seasonal effect

SEZ	1	2	3	4	5	6	7	8	9	10	11	12
1	-	ns	ns	ns	ns	**	ns	ns	**	*	ns	ns
2	ns	-	ns	ns	ns	*	ns	ns	*	ns	ns	ns
3	ns		-	ns	ns	**	ns	ns	**	*	*	ns
4	ns	ns	ns	-	ns	**	ns	ns	**	*	ns	*
5	ns	ns	ns	ns	-	**	*	ns	**	*	*	ns
6	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	ns
7	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns	ns
8	ns	ns	ns	ns	ns	ns	ns	-	ns	ns	ns	ns
9	*	*	**	*	*	*	ns	ns	-	ns	ns	ns
10	*	*	**	*	*	*	ns	ns	ns	-	ns	ns
11	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-	ns
12	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns	-

SEZ- Season; *ns*- p>0.05; *-p<0.05; **-p<0.01; Testing the difference of the LSMean values between months for trait VOL shown above, and for trait MO shown below the diagonal

In the study by *Šerniene et al. (2002)*, the highest motility was found during autumn and winter (84.55 and 85.79%) and lowest during summer (78.48%), so that the results of this study are partially similar to the results of the present study. Reduced sperm motility in this study in September and October, is partially similar to the results of research by *Stančić et al. (2003)* since in the study of these authors progressive motility was the lowest in the October-December period (75%), and highest in July-September (86%).

Conclusion

Ejaculate volume and sperm progressive motility are important characteristics that determine the number of doses produced per ejaculate and reproductive ability of sperm. Both sperm characteristics vary significantly to the highly significantly under the influence of breed, boar within breed and season. Linear regression coefficients indicate that as the age of the boar when the ejaculate is taken increases, VOL ($b = 0.0985$, $p < 0.001$) and MO ($b = 0.0071$, $p < 0,05$) increase. The analyzed factors largely explain the variability of VOL compared to MO.

There are differences between the breeds in terms of phenotypic values of both sperm traits analyzed and also significant differences ($p < 0.001$) between fertile breed (DL and DLW) compared to the very meaty breed DD were established.

Both observed properties of sperm varied under the influence of boar within studied genotypes, and there were differences (P> 0.05, p <0.05, p <0.01 p <0.001), indicating that in the selected swine populations sperm characteristics should be monitored in order to get as many insemination doses with optimal reproductive ability per ejaculate.

Effect of season on the VOL was highly significant (p <0.01), while the MO statistically significantly varied under the influence of the same factor. In September and October, semen samples had values of VOL above average, but the lowest progressive motility. Low values of MO in this period are due to the high temperatures during the summer period and the occurrence of chronic stress. This indicates the need for adequate microclimate conditions in the facilities where boars are kept, so that the temperature fluctuations during the year could be reduced to a minimum.

Acknowledgment

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

Uticaj rase, nerasta i sezone na neke osobine sperme

R. Savić, M. Petrović, D. Radojković, Č. Radović, N. Parunović

Rezime

Osnovni cilj rada bio je da se oceni fenotipska varijabilnost volumena ejakulata (VOL) i progresivne pokretljivosti spermatozoida (MO) pod uticajem rase, nerasta unutar rase i sezone. Istraživanjem je bilo obuhvaćeno 555 ejakulata nerasta rase danski landras (DL), danski jorkšir (DLW) i danski durok (DD). Uticaj nerasta analiziran je unutar ispitivanih rasa. Uticaj sezone ispitivan je kao uticaj meseca u kojem je uzet ejakulat. U model je uključen i linearni regresijski uticaj starosti nerasta prilikom uzimanja ejakulata. Obrada podataka izvršena je pomoću statističkog paketa *SAS 9.1.3 (SAS Inst. Inc., 2002-2003)*. Ocena uticaja je izvršena primenom GLM procedure. Uticaj svih analiziranih faktora na obe osobine sperme bio je značajan do vrlo visoko značajan, a posmatrani faktori u većoj meri objašnjavaju fenotipsku varijabilnost VOL u odnosu na MO. Najveće vrednosti VOL utvrđene su kod nerasta rase DL (164.96±3.58 ml), a MO kod nerasta rase DLW (85.45±0.94%). Nerasti DD imali su VOL i MO ispod proseka svih analiziranih rasa. Između nerasta unutar ispitivanih genotipova utvrđene su razlike

($p>0.05$, $p<0.05$, $p<0.01$, $p<0.001$), a nerast rase DLW u odnosu na sve ispitivane neraste imao je najveći volumen ejakulata sa progresivnom pokretljivošću spermatozoida od 90.47%. Volumen, odnosno pokretljivost su varirali ($p<0.01$, odnosno $p<0.05$) pod uticajem sezone, a u periodu septembar-oktobar nerasti su imali ejakulate sa vrednostima VOL iznad proseka i najnižom MO. S obzirom na najveći VOL i MO iznad godišnjeg proseka, u junu mesecu je bilo moguće dobiti najveći broj doza po ejakulatu sa optimalnom fertilnom sposobnošću.

References

BANASZEWSKA D., KONDRACKI S. (2012): An assessment of the breeding maturity of insemination boars based on ejaculate quality changes. Folia Biologica (Kraków), 60, No 3-4, 151-162.

FRANGEŽ R., GIDER T., KOSEC M. (2005): Frequency of boar ejaculate collection and its influence on semen quality, pregnancy rate and litter size. Acta vet. Brno, 74, 265-273.

KANOKWAN K. (2011): Association and expression study of CD9, PLCz and COX-2 as candidate genes to improve boar sperm quality and fertility traits. Institut für Tierwissensechaften, Abt. Tierzucht und Tierhaltung der Rheinischen Friedrich-Wilhelms- Universität Bonn. Inaugural-Dissertation.

KONDRACKI S., WYSOKIŃSKA A., KOWALEWSKI D., MUCZYŃSKA E., ADAMIAK A. (2009): Season's influence on the properties of male domestic pig semen. Rozprawy naukowe Pope John Paul II State School of Higher Vocational Education in Biała Podlaska, Vol. III**,** 177-187.

KUNOWSKA-SLÓSARZ M., MAKOWSKA A. (2011): Effect of breed and season on the boar's semen characteristics. Annals of Warsaw University of life sciences- SGGW, Animal Science, 49, 77-86.

OKERE C., JOSEPH A., EZEKWE M. (2005): Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. Journal of animal and veterinary advances, 4, 10, 885-888.

SAS INST. INC. (2002-2003): The SAS System for Windows, Cary, NC.

SMITAL J. (2009): Effects influencing boar semen. Animal reproduction, 110, 3, 335-346. *(abstract)*

SMITAL J. (2010): Comparison of environmental variations in boar semen characteristics of six breeds and their crossbreds over an eight-year period. Research in pig breeding, 4, 1, 26-32.

STANČIĆ B., GAGRČIN M., RADOVIĆ I. (2003): Uticaj godišnje sezone, rase i starosti nerastova na kvalitet sperme (1. Nativna sperma). Biotechnology in animal husbandry, 19, 1-2, 17-23.

ŠERNIENÉ LORETA, RIŠKEVIČIENÉ VITA, BANYS ANTANAS, ŽILINKAS HENRIKAS (2002): Effects of age, and season on sperm qualitative parameters in Lithuanian white and Pietren boars. Veterinarija ir zootechnika, 17 (39).
WOLF J., SMITAL J. (2009a): Effects in genetic evaluation for semen traits in Czech large white and Czech landrace boars. Czech J. Anim. Sci., 54, 8, 349-358.
WOLF J., SMITAL J. (2009b): Quantification of factors affecting semen traits in artificial insemination boars from animal model analyses. Journal of animal science, 87, 1620-1627.

Received 17 June 2013; accepted for publication 28 June 2013

Biotechnology in Animal Husbandry 29 (2), p 311-319 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.087'652
DOI: 10.2298/BAH1302311V

EFFECT OF PHYTOGENIC ADDITIVES ON PERFORMANCE, MORPHOLOGY AND CAECAL MICROFLORA OF BROILER CHICKENS

M. Vukić-Vranješ[1], N. Tolimir[1], Đ. Vukmirović[2], R. Čolović[2], V. Stanaćev[3], P. Ikonić[2], S. Pavkov[4]

[1]Institute of Science Application in Agriculture, 68b Blvd. D. Stefana, [Belg]rade 11000, R.Serbia
[2]Institute of Food Technology, 1 Blvd. Cara Lazara, Novi Sad 21000, R.Serbia
[3] Perutnina Ptuj – Topiko a.d., Petefi brigade 2, Bačka Topola 24300, R. Serbia
[4]Institute Goša, Milana Rakića 35, Belgrade 11000, R. Serbia
Corresponding author: e-mail: marinavranjes@vinfeed.com
Original scientific paper

Abstract: The objective of the research is to investigate effects of phytogenic additives as broiler feed supplements on production and slaughtering performances, morphological parameters and caecal microflora of fattening chickens of Ross 308 provenance. The research was based on two trials. In both trials, two feeding treatments were studied: a control diet with no supplemental feed additives (K) and a diet with a phytogenic additive supplemented to the control diet (1000g/t of Biomin PEP 1000, during the whole period). In the first trial, a total of 304 chickens were studied, in two treatments with four repetitions per pen with 38 chickens. The second trial included 16800 chickens, and it was conducted as a macro trial, that is, a pen with 8400 chickens was the subject of the trial. The results have shown that the chickens fed with the supplemental phytogenic additive have achieved significantly more favourable feed conversion rate. Differences in body mass and mortality rate between the trial group and the control were not statistically significant. Phytogenic additives did not have significant effects on breast portion and abdominal fat. Adding the phytogenic additive did not have significant effects on morphological parameters, but there was a tendency to increasing villous height and crypt depth. In both trials, the chicken groups fed with the supplemental phytogenic additive had less of all investigated groups of bacteria, and the differences were statistically significant for *Enterobacteriaceae* and *Staphylococcus* in the first, and *Enterobacteriaceae* and *Enterococcus* in the second trial. It can be concluded that the diet with the supplemental phytogenic additive had a positive effect on broiler performances, jejunum morphology parameters and caecal microflora.

Key words: broilers, phytogenic additive, performance, jejunal morphology, caecal microflora

Introduction

Modern strategies in broiler production are there to improve profitability, addressing productivity, legislation and consumers' requirements. A ban on the use of antibiotics as growth promoters in 2006 (*Castanon, 2007*) has led to a need for finding efficient yet safe additives for improving production performances with no negative effects on animal health and welfare, quality of food of animal origin, human health and the environment (*European Commission, 2003*). As replacement for antibiotics, most frequently used alternative growth stimulators in broiler production are probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogenic additives (*Perić et al., 2009*). Phytogenic additives, as natural substances, have been recognized as a very promising alternative solution, as they meet the requirements of consumers in terms of food safety and solve the problem of bacteria resistance that occurs as a result of using antibiotics as growth promoters (*Silva Cardoso et al., 2012*).

Phytogenic additives comprise a wide range of plants and spices, as well as their derivatives that, as supplemental to basal broiler diets, positively affect production performances, animal health and quality of products (*Windisch et al., 2008*). Effects of phytogenic additives are mostly related to antimicrobial, antiviral, and antioxidative activities (*B lükbaşi and Erhan, 2007*).

Some research on broilers indicate that phytogenics have positive effects on production performances (*Hashemi and Davoodi, 2010*; *Steiner et al., 2008*). Positive effects on production performances can be related to effects that phytogenic additives have on caecal microflora (*Roofchaee et al., 2011*; *Giannenas et al., 2003*), digestive enzyme activity (*Basmacioğlu Malayoğlu et al., 2010*) and caecal morphohistology (*Perić et al., 2010*). Positive effects of phytogenic additives on slaughtering performances of broilers were indicated in the research of *Jafari et al. (2011)* and *Mansoub* (*2011).* However, available literature shows that the use of phytogenic additives does not necessarily result in positive effects on production (*Kirkpinar et al., 2011*) and slaughtering performances (*Scheuermann et al., 2009*), nor caecal microflora (*Cross et al., 2007*). The major reasons for different effects of phytogenic additives, according to *Yang et al. (2009),* are differences among plant species that are used as additives, time of their picking, technology of additive production and the interaction between additives and other dietary components. The mechanism of phytogenic additives has not been explained entirely, and *Cross et al. (2007)* point out that the efficiency of phytogenic additives depends on differences in their chemical composition.

The objective of this research is to investigate effects of phytogenic additives as a broiler feed supplement on production and slaughtering performances, morphological parameters and microbiological status of caecum.

Material and Methods

The investigation on the effects of phytogenic additives was conducted through two trials. The first trial covered the period from the day 1 to the day 35, and the second trial from the day 1 to the day 42. Two feeding treatments were studied: a control diet with no supplemental feed additives (K) and a diet with a phytogenic additive supplemented to the control diet (1000g/t of Biomin PEP 1000, during the whole period). Phytogenic additive Biomin PEP 1000 (Biomin® GmbH Austria) comprises essential oregano, anise and citrus oil, as well as fructooligosaccharides. The main composition of diets was the same in both trials and it was in accordance with hybrid requirements.

In the first trial, 304 chickens were studied, in two treatments with four repetitions per pen with 38 chickens. Control weighing of all chickens was done with a precision balance on the day one and in each stage of the trial. The second trial was conducted in two objects, on 16800 chickens, i.e. 8400 chickens per feeding treatment (object). Control weighing of chickens was done with a precision balance, on a 4% sample.

Chickens were fed *ad libitum*. Feed conversion rate was calculated for different stages and for the whole trial, based on data on feed consumption and chicken growth per different periods, whereas all dead chickens were weighed and taken into the calculation. The death of chickens was recorded on a daily basis.

In order to study slaughtering performances, twelve 35-day old chickens and twelve 42-day old chickens of each gender were randomly chosen for the first and second trial respectively, for each feeding treatment. The chickens that were studied for slaughtering performances were processed according to the *Rulebook on quality of poultry meat (1981)*. When calculating the portion of certain carcass parts, they were calculated in relation to “ready to grill” carcass mass.

At the end of the starter period (21 days) and the end of the trial (42 days), 8 broilers from each group were slaughtered and small intestines were removed. Samples of jejunum were fixed in Bouin Solution, and after histological procedure stained with haematoxylin and eosin. Villous height, crypt depth and villous height: crypt depth ratio was determined using the light microscope and software for image analysis (IM1000, Leica Microsystems GmbH, Germany). A minimum of 15 measurements were made for each parameter per chicken.

In the first trial, on the day 21 and 35, and in the second trial, on the day 35 and 42, target types of bacteria were determined with conventional microbiological techniques on 6 samples. 1 g of caecal content of each group was transferred under aseptic conditions into 9 ml of buffered peptone water. Subsequently, it was homogenized by using a Vortex for 60 sec. Following homogenization, tenfold

serial dilution for each sample was made in sterile peptone–salt water until they were diluted to 10^{-8}. The results were expressed as LOG_{10}/g caecal content.

For data processing, STATISTICA 7 computer program, ANOVA, MANOVA and LSD post-hoc test were used.

Results and Discussion

The Table 1 shows production performances by feeding treatments in the first and the second trial. In the first trial, the chickens fed with the supplemental phytogenic additive had significantly more favourable feed conversion rate, while the differences in body mass after the starter period and in the whole trial, as well as mortality rate were not statistically significant. In the second trial, the chickens fed with diets with additives had bigger final body mass, more favourable feed conversion rate and lower mortality rate compared to the control.

The positive effect of phytogenic additives on broiler body mass can be linked with the results of the research of *Mountzouris et al. (2011)* and *Perić et al. (2010),* in which phytogenic additives based on oregano, anise and citrus expressed positive effects in terms of increasing chicken body mass.

Table 1 Effect of phytogenic additives on productive performances of broilers

Treatment	Trial 1					Trial 2		
	Body mass, g		Feed conversion		Mortality, %	Body mass, g	Feed conversion	Mort-ality, %
	Day 21	Day 35	1-21 days	1-35 days	1-35 days	Day 42	1-42 days	1-42 days
Control	803.86	1761.20	1.65 [a]	1.74[a]	2.00	2410.00	1.770	5.02
Sd	*4.81*	*50.21*	*0.04*	*0.03*	*2.09*			
Phytogenic	777.46	1753.00	1.52 [b]	1.68[b]	1.88	2471.00	1.725	4.52
Sd	*34.69*	*76.46*	*0.05*	*0.03*	*1.25*			

a-b Values without the same superscript in a column are significantly different (P <0.05)

The determined improvement in feed conversion in both trials is in compliance with the results of *Mansoub (2011)* and *Mountzouris et al. (2011).* The positive effect of phytogenic additives on feed conversion rate could be linked with effects that additives in this research had on caecal microflora, in terms of reducing the number of bacteria (Table 4) and caecal morpho-histology, i.e. elongation of villous height and crypt depth (Table 3).

The Table 2 shows slaughtering performances of broilers, i.e. yield and the portion of breast, thigh and drumstick, and abdominal fat, by feeding treatments.

Table 2 Effect of phytogenic additives on slaughtering performances of broilers

Treatment	„Ready to grill“ carcass	Breast		Thigh and drumstick		Abdominal fat ,g	
		Yield, g	Portion, %	Yield, g	Portion, %	Yield, g	Portion, %
Trial 1							
Control	1216.30	431.23	35.48	350.11	28.79	8.12	0.66
Sd	*42.97*	*17.34*	*1.57*	*21.41*	*1.52*	*4.84*	*0.38*
Phytogenic	1174.05	411.98	35.09	334.12	28.44	10.22	0.87
Sd	*49.45*	*29.83*	*2.06*	*28.03*	*1.72*	*4.10*	*0.35*
Trial 2							
Control	1752	653.00	37.25	459.90	26.24	14.60	0.83
Sd	*61.80*	*43.00*	*1.60*	*22.50*	*0.9*	*10.60*	*0.60*
Phytogenic	1764	648.60	36.76	486.00	27.54	14.40	0.82
Sd	*51.80*	*26.90*	*1.30*	*21.60*	*0.7*	*6.7*	*0.40*

a-b Values without the same superscript in a column are significantly different (P <0.05)

The values obtained for breast portion and thigh and drumstick portion were very approximate, and the differences between them were significant. It can be said that no significant effects of phytogenic additives on the investigated properties were determined, and there were no particular tendencies as a result of feeding treatments. The obtained results are in compliance with the research of *Jia – Chi et al. (2012)*, in which no effects on abdominal fat were determined. However, unlike this research, covering the same issue, *Mansoub* (2011) and *Jafari et al.* (2011) determined the biggest breast portion and the lowest abdominal fat in chickens fed with a supplemental phytogenic additive.

The Table 3 shows effects of phytogenic additives on jejunum morphology parameters, by feeding treatments.

Table 3 Effect of phytogenic feed additives on jejunum morphology of broilers

Treatment	Villous height, µm	Crypt depth, µm	Villous/Crypt ratio	Villous height, µm	Crypt depth, µm	Villous/Crypt ratio
	21-day period			42-day period		
Control	923.83	256.95	3.64	1254.06	303.09	4.14
Phytogenic additive	1050.65	267.11	3.95	1175.13	286.12	4.09

a-b Values without the same superscript in a column are significantly different (P <0.05)

Adding supplemental phytogenic additives did not have significant effect on an increase in villous height, crypt depth and their interrelation. The observed

morphological parameters were higher in 21-day old chickens fed with the supplemental phytogenic additive, yet this trend was not determined in 42-day old chickens. The results are in compliance with the research of *Jia – Chi et al. (2012),* who also did not determine the effect of oregano, anise and citrus based supplemental phytogenic additives on villous height, crypt depth and their interrelation in the jejunum. Unlike this research, in the research of *Perić et al. (2010),* adding phytogenic additives on the day 42 resulted in a significant increase in villous height and crypt depth.

Number of bacteria in caecal content in the first and second trial is shown in the Table 4 by feeding treatments and trial periods.

Table 4 Number of bacteria in caecal content (LOG $_{10}$/g caecal content)

Bacteria	Trial 1				Trial 2			
	Control		Phytogenic		Control		Phytogenic	
	Day 21	Day 35	Day 21	Day 35	Day 35	Day 42	Day 35	Day 42
Total aerobic bacteria	9.14	8.48	8.95	8.33	7.77	7.87[a]	7.59	7.39[b]
Echerichiae coli	8.88	7.73	7.85	6.38	7.66	7.03	6.80	6.49
Enterobacteriaceae	9.04[a]	8.02[a]	8.66[b]	6.62[b]	7.60	7.41[a]	6.98	6.17[b]
Enterococcus	6.99	6.02	6.83	5.49	6.07	5.44[a]	5.42	4.52[b]
Spaphylococcus	5.73	4.77[a]	5.75	4.13[b]	5.61	4.36	5.34	4.17
Lactobacillus spp	-	-	-	-	9.00	9.04	8.67	8.69
Clostridiuum	-	-	-	-	7.05	7.01	6.89	6.90

a-b Values without the same superscript in a row are significantly different (P <0.05)

Having analysed data from the Table 4, one can conclude that in both trials and in all periods of time, chickens fed with the supplemental phytogenic additive had lower values of all bacterial target groups. In the first trial, those differences were statistically significant for *Enterobacteriaceae* and *Spaphylococcus*, and in the second trial for *Enterobacteriaceae* and *Enterococcus*. Adding phytogenic additives resulted in the reduction of *Lactobacillus* spp, which is in compliance with the research of *Jia – Chi et al. (2012).* However, *Mountzoris et al. (2011)* determined a growing tendency of *Lactobacillus* spp after adding phytogenic additives, what is not in compliance with this research, but they obtained similar results regarding the reduction of total bacteria and Clostridium. The reduction in number of *Echerichiae coli* is in compliance with the research of *Roofchaee et al. (2011).*

Conclusion

Adding phytogenic additives involved giving oregano, anise and citrus based phytogenic additives to chickens of Ross 308 provenance, for improving production performances, intestinal morphology and microbiological status.

Adding phytogenic additives resulted in the improvement of broiler production performances, above all feed conversion rate. A positive effect was achieved on jejunum morphology parameters and caecal microflora. These effects show that phytogenic additives can contribute to the improvement of production performance, and therefore production efficiency as well.

Acknowledgment

The research was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, the project TR- III 46012.

Uticaj fitogenih aditiva na performanse, morfologiju i cekalnu mikrofloru brojlerskih pilića

M. Vukić-Vranješ, N. Tolimir, Đ. Vukmirović, R. Čolović, V. Stanaćev, P. Ikonić, S. Pavkov

Rezime

Cilj istraživanja je da se ispita uticaj fitogenog aditiva kao dodatka u ishrani brojlera, na proizvodne i klanične performanse, morfološke parametre i cekalnu mikrofloru tovnih pilića provenijence Ross 308. Ispitivanje je zasnovano na dva eksperimentalna ogleda. U oba ogleda ispitivana su dva tretmana ishrane: kontrolna hrana bez aditiva (K) i hrana za dodatkom fitogenog aditiva u osnovnu smešu (1000g/t Biomin PEP 1000, tokom celog oglednog perioda). U prvom ogledu ispitano je ukupno 304 pilića, prema dizajnu 2 tretmana sa 4 ponavljanja po boksu sa 38 pilića. U drugom ogledu ispitivanje je obuhvatilo 16800 pilića, a izvedeno po principu makroogleda, odnosno objekat od 8400 pilića bio je jedinica posmatranja. Rezultati su pokazali da su pilići hranjeni sa dodatkom fitogenog aditiva ostvarili signifikantno povoljniju konverziju hrane. Razlike u telesnoj masi i mortalitetu ogledne i kontrolne grupe nisu bile na nivou statističke značajnosti. Fitogeni aditivi nisu imali signifikantan uticaj na udeo grudi i abdominalne masti. Dodatak fitogenih aditiva nije rezultirao signifikantnim uticajem na morfološke parametre, ali je postojala tendencija povećanja visine resica i dubine kripti. U oba ogleda, grupe pilića sa dodatkom fitogenog aditiva imale su manji broj svih ispitivanih ciljnih bakterijskih grupa, a razlike su bile i na nivou statističke značajnosti za Enterobacteriaceae i Spaphylococcus u prvom i za Enterobacteriaceae i Enterococcus u drugom ogledu. Na osnovu dobijenih rezultata može se zaključiti da je ishrana sa dodatkom fitogenog aditiva indukovala pozitivan uticaj na performanse brojlera, morfološke parametre jejunuma i mikrofloru cekuma.

References

BASMACIOĞLU MALAYOĞLU H., BAYSAL S., MISIRLIOGLU Z., POLAT M., YILMAZ H., TURAN N. (2010): Effects of Oregano Essential Oil with or without Feed Enzymes on Growth Performance, Digestive Enzyme, Nutrient Digestibility, Lipid Metabolism and Immune Response of Broilers fed on wheat-soybean meal diets. British Poultry Science, 51, 1, 67-80.

B LÜKBAŞI S.C., ERHAN M.K. (2007): Effect of Dietary Thyme (Thymus vulgaris) on Laying Hens Performance and Escherichia coli (E. coli) Concentration in Feces. International Journal of Natural and Engineering Sciences, 1, 2, 55-58.

CASTANON J.I. (2007): History of the Use of Antibiotics as Growth Promoters in European Poultry Feeds. Poultry Science, 86, 2466-2471.

CROSS D.E., McDEVITT R.M., HILLMAN K., ACAMOVIC T. (2007): The Effect of Herbs and Their Associated Essential Oils on Performance, Dietary Digestibility and Gut Microflora in Chickens from 7 to 28 Days of Age. British Poultry Science, 48, 4, 496-506.

European Commission (2003): Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal of European Union L 268, 29-43.

GIANNENAS I., FLOROU-PANERI P., PAPAZAHARIADOU M., CRISTAKI E., BOTSOGLOU N.A., SPAIS A.B. (2003): Effect of Dietary Supplementation with Oregano Essential Oil on Performance of Broilers after Experimental Infection with Eimeria Tenella. Arch. Anim. Nutr. 57, 2, 99-106.

HASHEMI S.R., DAVOODI H. (2010): Phytogenics as New Class of Feed Additive in Poultry Industry. Journal of Animal and Veterinary Advances, 9, 17, 2295-2304.

JAFARI B., REZAIE A., EBADI A., GHIAMIRAD M., AHMADIZADEH C. (2011): Evaluation of Medicinal Plant Oil (*Teucrium Polium*) in Diets of Broilers. Journal of Applied Biological Science, 1, 12, 583-586.

JIA-CHI H., STEINER T., AUFY A., TU-FA L. (2012): Effects of Supplemental Essential Oil in Growth Performance, Lipid Metabolites and Immunity, Intestinal Characteristics, Microbiota and Carcass Traits in Broilers. Livestock Science, 144, 3, 253-262.

KIRKPINAR F., ÜNLÜ H.B., ZDEMIR G. (2011): Effects of Oregano and Garlic Essential Oils on Performance, Carcase, Organ and Blood Characteristics and Intestinal Microflora of Broilers. Livestock Science, 137, 219-225.

MANSOUB N.H. (2011): Performance, Carcass Quality, Blood Parameters and Immune System of Broilers Fed Diets Supplemented with Oregano Oil (*Origanum* sp.). Annals of Biological Research, 2, 6, 652-656.

MOUNTZOURIS K.C., PARASKEVAS V., TSIRTSIKOS P., PALAMIDI I., STEINER T., SCHATZMAYR G., FEGEROS K. (2011): Assessment of a Phytogenic Feed Additive Effect on Broiler Growth Performance, Nutrient

Digestibility and Cecal Microflora Composition. Animal Feed Science and Technology, 168, 3, 223-231.
PERIĆ L., MILOŠEVIĆ N., ŽIKIĆ D., BJEDOV S., CVETKOVIĆ D., MARKOV S., MOHNL M., STEINER T. (2010): Effects of Probiotic and Phytogenic Products on Performance, Gut Morphology and Cecal Microflora of Broiler Chickens. Archiv Tierzucht, 53, 3, 350-359.
PERIĆ L., ŽIKIĆ D., LUKIĆ M. (2009): Application of Alternative Growth Promoters in Broiler Production. Biotechnology in Animal Husbandry 25, 5-6, 387-397.
ROOFCHAEE A., MEHRDAD I., EBRAHIMZADEH M.A., AKBARI M.R. (2011): Effect of Dietary Oregano (*Origanum vulgare* L.) Essential Oil on Growth Performance, Cecal Microflora and Serum Antioxidant Activity of Broiler Chickens. African Journal of Biotechnology, 10, 32, 6177-6183.
Rulebook on quality of poultry meat (1981). Pravilnik o kvalitetu mesa pernate živine (1981). Službeni list SFRJ br. 1, januar 1981. god.
SCHEUERMANN G.N., CUNHA JUNIOR A., CYPRIANO L., GABBI A.M. (2009): Phytogenic Additive as an Alternative to Growth Promoters in Broiler Chickens. Ciência Rural, 39, 2.
SILVA CARDOSO V., RIBEIRO de LIMA C.A., FREIRE de LIMA M.E., GOMES DORNELES L.E., GRACAS MIRANDA DANELLI M. (2012): Piperine as a Phytogenic Additive in Broiler Chickens. Pesquisa Agropecuária Brasileira, 47, 4.
STEINER T., PERIĆ L., MILOŠEVIĆ N., ĐUKIĆ STOJČIĆ M., BJEDOV S. (2008): Efficiency of a Phytogenic Feed Additive in a Performance Trial with Broilers. Proceedings from 7. BOKU Symposium TIERERNÄHRUNG: Tierernährung in Spannungsfeld zwischen Lebensmottelproduktion, Energieerzeugnung und Umweltschutz, 4 Dezember, Wien, pp. 95-100.
WINDISCH W.M, SCHEDLE K., PLITZNER C., KROISMAYR A. (2008): Use of Phytogenic Products as Feed Additives for Swine and Poultry. Journal of Animal Science, 86, 14 (E. Suppl.), E140-E148.
YANG Y., IJI P.A., CHOST M. (2009): Dietary Modulation of Gut Microflora in Broiler Chickens: a Review of the Role of Six Kinds of Alternatives to In-feed Antibiotics. World's Poultry Science Journal, 65, 97-114.

Received 2 April 2013; accepted for publication 20 June 2013

Biotechnology in Animal Husbandry 29 (2), p 321-329 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.087'652
DOI: 10.2298/BAH1302321S

DIFFERENT SOURCES AND LEVELS OF VEGETABLE OILS IN BROILER CHICKEN NUTRITION

V.Ž. Stanaćev[1], D. Milić[1], N. Milošević[2], V.S. Stanaćev[2], Z. Pavlovski[3], Z. Škrbić[3], N. Puvača[2]

[1]Perutnina Ptuj-Topiko ad., Petefi Brigade 2, 24300 Bačka Topola, Republic of Serbia.
[2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia.
[3]Institute for Animal Husbandry, Auto put 16, P. Box 23, 11080 Beograd, Republic of Serbia.
Corresponding author: vidica.stanacev@stocarstvo.edu.rs
Original scientific paper

Abstract: The aim of this study was to investigate the effects of soybean, flax and rapeseed oil on productive performance and carcass quality of broilers chickens. At the beginning of the experiment six groups of 40 one day-old chicks, hybrid line Cobb 500 was formed, with five replications. For nutrition two feed mixtures were used. During the first two weeks, in preparation period, chickens were fed with starter mixture, and then with grower mixture until the end of the experiment. Control group was fed with mixture of standard composition and quality based on corn and soybean meal with the addition of 4% and 8% soybean oil, while the experimental group included 4% and 8% flaxseed oil and 4% and 8% rapeseed oil. The experiment lasted 35 days. During the experimental period, chickens were fed and watered *ad libitum* and microclimatic conditions were constantly monitored. Control of body weight and feed consumption was carried out every seven days. At the end of the experiment 10 chickens from each group were sacrificed for the purpose of testing the carcass quality. Upon completion of the experiment, control group achieved weight of 2122g and 2053g, and the experimental group with flax oil 2164g and 2094g, and the group with rapeseed oil weighs of 2121g and 2081g. Chickens on treatment with 4% flax oil in the diet achieved significantly higher body mass ($P<0.05$) at the end of the experiment compared with the groups who were on treatment with 8% rapeseed and soybean oil. Soybean oil at a rate of 4% showed significantly ($P<0.05$) increased final body weight compared to body mass of chicks in a group with the addition of 8% soybean oil. Feed conversion ratio was lowest in the group with the addition of 4% flax oil and rapeseed oil, and the highest in the group with the addition of 8% flax oil. The differences found in the carcass quality between the control and experimental groups were not statistically significant ($P>0.05$). The largest amount of abdominal fat (18.9 g) was in the group with the addition of 4% rapeseed oil and

lowest in the group with the addition of 8% soybean oil (12.6 g). Based on these results it can be concluded that the addition of 4% oil showed better productive results, but had no effect on carcass quality of chickens.

Key words: flaxseed oil, rapeseed oil, carcass quality, nutrition, chickens.

Introduction

High genetic potential of hybrids, which are used for the production of chicken meat, meet the needs of the market at the age of 35 days, and chicken meat with altered structure of fat and polyunsaturated ω-3 fatty acids, substantially reduces cardiovascular diseases. In recent years, special attention has been focused on the effects of nutritional oils and fats, as health is concerned, which is primarily dependent on the presence of single fatty acids, as well as their relationship. The World Health Organization (*Whitworth, 2003*) recommended that in the human diet, fat should provide 15-30% of energy, of which saturated fatty acids were represented less than 10%, polyunsaturated fatty acids (PUFA) of 6-10%, n-6 PUFA of 5-8% n-3 PUFA of 1-2%, and less than 1% trans fatty acids. In poultry nutrition, fats and oils are a source of energy. Their energy value is greater than all the other nutrients present in the mixture, and more than twice compared to the carbohydrates. Mixtures usually contain fats or oils from different sources, which contribute to variations in the chemical composition and nutritive value (*Wiseman et al., 1996; Leeson and Summers, 2005*). In recent years, researchers have studied the impact of type and quantity of oil to increase the intensity, the efficiency of feed utilization, carcass quality and meat quality of chickens. In this way, tests are performed by *Nobakht et al. (2011)* and found that 4% of pure soybean, rapeseed and sunflower oil, and their mixtures in the diets for broiler chickens have significant impact on production performance, carcass quality and content of vitamin E in the breast meat. The same authors found that the best feed conversion ratio (1.83) is in the group with a mixture of 2% of the oil from rapeseed and soybean with the lowest relative share of the gizzard (2.52%), while the highest amount of vitamin E in white meat (22.05 mg/kg) was recorded in the group with a mixture of all three oils. *Lopez-Ferrer et al. (2011)* concluded that 2 and 4% flaxseed oil in diets for broilers, with the addition of fat to 8% affects the fatty acid composition of tissue, while the production parameters showed minor differences between treatments. The yield carcass weight and meat quality between the groups were not significant. However, the results of *Bartos et al. (2004)* showed a negative effect of addition of 6% of linseed oil to the broiler diet mixture when it comes to carcass quality. The introduction of 3% rapeseed oil in the diet of broiler chickens showed a significant increase in body weight compared to the control group, while the difference in mass of the liver, white and red meat were not significant

(*Shahyar et al., 2011*). Addition of 4% flax oil in the diet of broiler chickens has resulted in a higher concentration of fat in the liver, compared with chickens on treatment with 4% rapeseed oil. Rapeseed oil in mixtures for broiler chickens leads to a reduction in lipid content in the edible parts of the carcass, especially saturated fatty acids in white muscle and liver, as well as monounsaturated fatty acids of red and white meat, liver, and gizzard (*Zanini et al., 2006*). *DeWitt et al. (2009)* showed that the introduction of 6% sunflower and fish oil leads to improved feed conversion of broiler chickens, which is consistent with previous research of *El Yamany et al. (2008)* in terms of production performance of Japanese quail. The research of *Stanaćev et al. (2012)*, which aimed to investigate the effect of different vegetable oils in the diet of broiler chickens, concluded that the use of 4 and 8% flaxseed oil does not exhibit significant differences in production parameters and carcass quality. Previous research of *Stanaćev et al. (2011)* came to the conclusion that the inclusion of extruded rapeseed in quantities of 10, 15 and 20% in chicken diets, significantly affect the final body weight compared with the control group. Also same authors *(Stanaćev et al., 2011)*, noted significant changes when it comes to the fatty acid composition of lipids in chicken meat under the influence of feeding treatment, where was recorded the reduction of linoleic acid by 20% and increase in the content of linoleic acid by 50%. Given the above, the aim of this study was to examine the production parameters and carcass quality of broilers 35 days old, fed with different amounts of soybean, flaxseed and rapeseed oils.

Material and Methods

Trials were conducted under production conditions on the experimental estate "Pustara" in Temerin, on the floor system. At the beginning of the experiment six groups of 40 one day-old chicks hybrid line Cobb 500 were formed, with five replications. For nutrition two feed mixtures were used. During the first two weeks of chickens preparation period, starter mixture containing 21% protein were used, while then grower mixture with 20% protein was used until the end of the experiment. Control group was fed with mixture of standard composition and quality based on corn and soybean meal with the addition of 4% and 8% soybean oil, while the experimental group included 4% and 8% flaxseed oil and 4% and 8% rapeseed oil (Table 1). The experiment lasted 35 days. During the experimental period, chickens were fed and watered *ad libitum* and microclimatic conditions constantly monitored. Control of body weight and feed consumption was carried out every seven days. At the end of the experiment (on 35^{th} day), from each group 10 birds (5 males and 5 females), with mean body weight were sacrificed for the purpose of testing the carcass quality. Operation such as bleeding, scalding, plucking, evisceration and cooling were performed. Classically processed carcasses was measured and cut to the basic anatomical parts and measured (Regulations:

Fig. Gazette of SFRY, No.1/81 and 51/88). Evaluations were conducted on the basis of yield and weight of certain body parts. For proper interpretation of the results, appropriate statistical methods of ANOVA and Tacky post-hoc test of software package STATISTICA 12 were used.

Table 1. Experimental plan with chickens

Group and Treatment	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
Oil source	Soybean	Soybean	Flaxseed	Flaxseed	Rapeseed	Rapeseed
In grower	4%	8%	4%	8%	4%	8%

Results and Discussion

Based on the obtained results it can be concluded that this set of experiments demonstrated significant differences ($P<0.05$) between the different types and amounts of vegetable oils. During the preparation period chicks had a uniform body weight. Upon completion of the experiment at 35 day, small rise in depression in group on treatment with 4% rapeseed oil is noted, compared to the control group, while the III, IV and VI groups were superior in body weight. Highest body weight of 2164g achieved chickens of group III on treatment with 4% flax oil, which is 1.97% higher than in the control group with the same amount of soybean oil, and the lowest weight was chicken group V with 4% rapeseed oil with 2121g or 0.05% less than in the control group (Table 2). During the third week, significant differences ($P<0.05$) between groups II and IV are visible, while in the fourth week highly significant differences ($P<0.01$) is appeared between the control group with 8% soybean oil and experimental groups III, IV, V and VI, as well as between groups I and VI, until the fifth week when body mass of chickens had almost leveled for the same amount of oil. Statistically significant difference was maintained between the II and VI group at 8% soybean and rapeseed oil.

Table 2. Body weight of 35 day old chickens, g

Chicken age (weeks)	Group, treatment and amount of oil					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%- Soybean	8%-Soybean	4%-Flaxseed	8%-Flaxseed	4%-Rapeseed	8%-Rapeseed
IW	42	42	42	42	42	42
1	185	185	183	190	187	190
2	468±35,3	469±38,1	468±28,3	468±33,2	469±42,5	469±33,6
Index, %	*100*	*100*	*100*	*99,78*	*100,21*	*100*
3	986±57,2	967^{b}±58,3	989±52,8	997^{B}±54,7	995±64,5	977±55,4
4	1457^{bD}±155,3	1422^{ABCD}±134,1	1523^{A}±127,3	1532^{B}±125,6	1515^{C}±154,1	1575^{D}±90,8
5	2122^{E}±231,5	2053^{ae}±212,0	2164^{A}±260,2	2094±231,5	2121±255,1	2081^{a}±223,7
Index, %	*100*	*100*	*101,97*	*101,99*	*99,95*	*101,36*

Same big letters in the same row = highly significant (P<0.01); same big and small letters in the same row = significant (P<0.05); same small letters in the same row = not significant (P>0.05)

The use of different types and amount of oil was found to have different efficiency of feed utilization (Table 3). In the preparatory period steady feed consumption per kilogram of gain was recorded (1.30 to 1.36). However, in experimental period from thread to fifth weeks of age, it can be seen that most efficiently exploiting feed was in III and V group with the addition of 4% flax and rapeseed oil (1.60), while the highest conversion of 1.68 and 1.63 kg/kg gain is recorded in the IV and VI group who were on treatment with 8% of flax and rapeseed oil.

Table 3. Feed conversion, kg/kg

Period	Group, treatment and amount of oil					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-Soybean	8%-Soybean	4%-Flaxseed	8%-Flaxseed	4%-Rapeseed	8%-Rapeseed
1	1,13±0,06	1,16±0,06	1,14±0,06	1,12±0,11	1,14±0,04	1,08±0,03
2	1,35±0,04	1,34±0,05	1,30±0,03	1,35±0,09	1,36±0,06	1,33±0,02
Index, %	*100*	*100*	*96,29*	*100,74*	*100,74*	*99,25*
3	1,38±0,14	1,36±0,14	1,39±0,22	1,42±0,03	1,41±0,03	1,41±0,03
4	1,49±0,03	1,48±0,04	1,41±0,06	1,50±0,02	1,47±0,03	1,50±0,09
5	1,62±0,03	1,61±0,04	1,60±0,04	1,68±0,04	1,60±0,02	1,63±0,05
Index, %	*100*	*100*	*98,76*	*104,34*	*98,76*	*101,24*

Average values of carcass weight, dressing percentage and weight of certain body parts, as well as their relative share of the weight of dressed carcass, shown in Table 4, indicate that there is very little difference in all tested parameters and that the effect of feeding treatment on carcass yield was not statistically significant (P>0.05). The relative contributions of valuable body parts by weight of dressed carcass shows that breast as one of the most valuable parts, had the largest representation in the carcass, which ranged from 29.57 to 32.41%, followed by back 18.11 to 19.82 %, then the thigh from 12.69 to 13.65% and leg with 10.79 to 11.43%. The wings have the lowest representation with 9.15 to 9.58%. The amount of abdominal fat was relatively small, ranging between 12.6 and 18.9 g. Since abdominal fat is a good indicator of total fat content, it can be concluded that the carcasses were not greasy.

Table 4. Chicken carcass quality

Group and treatment	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
Source and amount of oil	4%-Soybean	8%-Soybean	4%-Flaxseed	8%-Flaxseed	4%-Rapeseed	8%-Rapeseed
Chicken mass, g						
Before slaughter, g	2157	2075	2170	2118	2097	2121
Carcass weight, g (CP)	1755	1706	1827	1756	1792	1781
Dressing, % (CP)	81,34	82,23	84,17	82,91	85,43	83,98
Carcass weight, g (RG)	1476,0	1426,1	1532,3	1474,4	1503,0	1488,6
Dressing, % (RG)	68,42	68,72	70,61	69,61	71,67	70,18
More valuable body part mass, g						
Wings	160,6±10,8	163,3±20,7	169,0±14,3	165,8±13,2	168,4±24,3	170,4±13,9
Thigh	189,6±17,9	189,1±17,3	201,5±22,8	195,5±22,4	194,9±25,5	204,1±23,2
Drumstick	239,2±17,3	222,2±23,9	232,9±51,9	231,3±24,0	231,5±36,1	234,4±25,9
Breast	566,1±73,7	528,8±74,3	591,6±46,9	562,7±57,5	578,2±77,1	527,0±45,7
Back	320,5±17,1	322,7±29,7	337,3±59,5	319,1±47,9	330,0±43,3	352,7±30,3
Total	1476,0	1426,1	1532,3	1474,4	1503,0	1488,6
Index, %	*100*	*100*	*103,81*	*103,38*	*101,82*	*104,38*
Less valuable body part mass, g						
Abdominal fat	18,6±6,1	12,6±4,0	17,4±4,7	15,4±5,3	18,9±5,7	17,5±5,3
Liver	36,3±4,3	36,1±4,1	35,8±4,9	37,1±4,6	37,0±5,5	37,6±8,0
Heart	10,0±1,7	10,2±2,0	10,4±1,7	10,5±1,2	10,1±1,1	9,9±1,1
Gizzard	29,3±5,2	27,2±4,8	30,1±4,2	27,9±5,5	27,4±3,8	29,2±6,8
Head	44,5±4,6	48,8±5,0	50,3±4,9	46,1±4,8	49,2±5,4	46,8±6,3
Neck	75,2±13,2	76,6±11,6	77,3±9,7	75,1±8,1	78,5±13,0	77,9±12,4
Legs	64,8±9,4	68,6±7,8	73,0±13,5	69,7±11,4	67,5±11,2	73,8±14,1
Total	278,7	280,2	294,4	281,9	288,7	292,8
Index, %	*100*	*100*	*105,60*	*100,60*	*103,55*	*104,50*
Relative share of more valuable body parts, %						
Wings	9,15	9,42	9,26	9,44	9,39	9,58
Thigh	10,79	11,08	11,03	11,11	10,86	11,43
Drumstick	13,65	12,99	12,69	13,16	12,88	13,13
Breast	32,25	30,98	32,41	32,09	32,29	29,57
Back	18,27	18,93	18,47	18,11	18,39	19,82

Conclusion

Based on these results it can be concluded that the chickens on treatment with 4% flax oil in the diet achieved highest body mass at the end of the experiment compared with the groups who were on treatment with 8% of vegetable oil. The differences were statistically significant ($P<0.05$). Highest body weight (2164g) was in chickens of group III with 4% flax oil, and the smallest in chickens of V group (2121g) with 4% rapeseed oil in diet. The largest amount of abdominal fat (18.9 g) was in the fifth group with the addition of 4% rapeseed oil and lowest in the control group (II) with the addition of 8% soybean oil (12.6 g). Based on these results it can be concluded that the addition of 4% oil showed better productive results, but had no effect on chicken carcass quality.

Acknowledgement

The paper is part of the projects TR 31033 and III 46012, funded by the Ministry of Science and Technology of the Republic of Serbia.

Različiti izvori i nivoi ulja u ishrani brojlera

V.Ž. Stanaćev, D. Milić, N. Milošević, V. Stanaćev, Z. Pavlovski, Z. Škrbić, N. Puvača

Rezime

Cilj ovog rada je bio da se ispita efekat ulja soje, lana i uljane repice na proizvodne parametre i kvalitet trupa brojlera. Na početku ogleda formirano je šest grupa sa po 40 jednodnevnih pilića linijskog hibrida Cobb 500, u pet ponavljanja. Za ishranu su korišćene dve smeše. Tokom prve dve nedelje pripremnog perioda, za ishranu pilića je korišćena starter, a potom grover smeša do kraja eksperimenta. Kontrolna grupa je hranjena smešom standardnog sastava i kvaliteta na bazi sojine sačme i kukuruza sa dodatkom 4% i 8% ulja soje, dok je u eksperimentalnim grupama bilo uključeno 4% i 8% lanenog ulja, odnosno 4% i 8% ulja repice. Ogled je trajao 35 dana. U toku eksperimentalnog perioda piliću su hranjeni i napajani po volji, a mikroklimatski uslovi redovno kontrolisani. Kontrola telesne mase i utroška hrane je vršena svakih sedam dana. Na kraju eksperimenta žrtvovano je po 10 pilića iz svake grupe za potrebe ispitivanja kvaliteta trupa. Po završetku eksperimentalnog perioda, kontrolna grupa je ostvarila masu od 2122g i 2053g, a ogledne grupe sa uljem lana 2164g i 2094g, dok su grupe sa repičinim uljem

ostvarile masu od 2121g i 2081g. Pilići na tretmanu sa 4% ulja lana u ishrani ostvarili su statistički značajno veću telesnu masu (P<0,05) na kraju eksperimenta u poređenju sa grupama koje su bile na tretmanu sa 8% ulja repice i soje. Ulje soje u količini od 4% je takođe statistički značajno (P<0,05) uticalo na povećanje završne telesne mase u poređenju sa telesnom masom pilića u grupi sa dodatkom 8% sojinog ulja. Konverzija hrane je bila najniža u grupi sa dodatkom 4% ulja lana i uljane repice, a najviša u grupi sa dodatkom 8% ulja lana. Utvrđene razlike u kvalitetu trupa između kontrolne i oglednih grupa nisu bile statistički značajne (P>0,05). Najveća količina abdominalne masti (18,9g) je u grupi sa dodatkom 4% ulja repice, a najmanja u grupi sa dodatkom 8% ulja soje (12,6g). Na osnovu dobijenih rezultata može se zaključiti da je dodatak 4% ulja ispoljio bolje prozvodne rezulate, ali nije uticao na kvalitet trupa pilića.

References

BARTOS A., PAL L., BANYAI A., HORVATH P., WAGNER L., DUBLECZ K. (2004): Effect of different oil (fat) supplemented diets on the performance, carcass quality and fatty acid composition of tissues of broiler chicks. Allattenyesztes es Takarmanyozas, 53(1), 63-78.

DEWITT J.C., COPELAND C.B., STRYNAR M.J., LUEBKE R.W. (2009): Perfluorooctanoic acid–induced immunomodulation in adult female chicks. Environ Health Perspect, 116, 645–650.

EL YAMANY A.T., HEWIDA M., EL ALLAWY H., LAILA D., ABD EL-SAMEE A., EL-GHAMRY A. (2008): Evaluation of using different levels and sources of oil in growing Japanese quail diets. American-Eurasian Journal of Agricultural & Environmental Science, 3(4), 577-582.

LEESON S., SUMMERS J.D. (2005): Ingredient evaluation and diet formulation: Flaxseed. Commercial Poultry Nutrition. 3rd ed. University Books, Guelph, Ontario, Canada, 44-46.

LOPEZ-FERRER S., BAUCELLS M.D., BARROETA A.C., GALOBRAT J., GRASHORN M.A. (2011): n-3 enrichment of chicken meat. 2. Use of precusors of long-chain polyunsaturated fatty acids: linseed oil. Poultry Science, 80, 753-761.

NOBAKHT A., SAFAMEHR A., SOZANY S., GALANDARI I., TAGHAVI E., GHABOLI I. (2011): Comparison of effects of using different levels of animal and vegetable fats and their blends on performance of laying hens. Brazilian Journal of Poultry Science Research, 1, 1433-1437.

SHAHRYAR H.A., NOBAR R.S., LAK A., LOTFI A. (2011): Effect of dietary supplemented canola oil and poultry fat on the performance and carcass characterizes of broiler chickens. Current Research Journal of Biological Sciences, 3(4), 388-392.

STANAĆEV V., KOVČIN S., STANAĆEV V., PUCAREVIĆ M., PUVAČA N. (2011): Extruded canola seed in improving chicken fattening and fatty acid composition. Kuwait Journal of science and Engineering, 38(1A), 71-80.
STANAĆEV V., MILOŠEVIĆ N., STANAĆEV V., PUVAČA N., MILIĆ D., BJEDOV S., PLAVŠA N., BEUKOVIĆ D., LJUBOJEVIĆ D. (2012): Sources and effects of different levels of oil on production performance and broiler carcass quality. Proceedings of 6th Central European Congress on Food – CEFood, Novi sad, 23-26 May, 1625-1629.
WHITWORTH J.A. (2003): 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. Journal of Hypertension, 21(11), 1983-1992.
WISEMAN S.A., MATHOT J.N.N.J., DE FOUW N.J., TIJBURG L.B.M. (1996): Dietary non-tocopherol antioxidants present in extra virgin olive oil increase the resistance of low-density lipoproteins to oxidation in rabbits. Atherosclerosis, 120, 15-23.
ZANINI S.F., COLNAGO G.L., PESSOTTI B.M.S., BASTOS M.R., CASAGRANDE F.P., LIMA V.R. (2006): Body fat of broiler chickens fed diets with two fat sources and conjugated linoleic acid. International Journal of Poultry Science, 5(3), 241-246.

Received 10 June 2013; accepted for publication 22 June 2013

Biotechnology in Animal Husbandry 29 (2), p 331-336 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.05'652
DOI: 10.2298/BAH1302331L

THE EFFECT OF TRANSPORTATION ON BROILER MEAT pH AND TENDERNESS

H. A.W. Lengkey[1], J. A. Siwi[1], P. Edianingsih[1], F. J. Nangoy[2]

[1] Universitas Padjadjaran, Bandung, Indonesia
[2] Universitas Sam Ratulangi, Manado, Indonesia
Corresponding author : lengkeyhendronoto@gmail.com
Original scientific paper

Abstract : 180 broilers, five weeks old broilers were used to asses the effect of transportation on broiler meat pH and tenderness, has been carried in Bandung, Indonesia. Using Completely Randomized Design (CRD), with five levels (P-0 slaughter at the farm, P-1 one hour transportation before slaughter, P-2 two hours transportation before slaughter, P-3 three hours transportation before slaughter, and P-4 four hours transportation before slaughter) and four times replications. Results indicated the meat pH are between 6.33 – 6.63. Even the result shows that the pH values increase when the transportation time more longer, but this results are between the normal meat pH (between 5.2 – 6.6). The meat tenderness were between 124.75 – 176.50 mm/g/10 sec. The meat tenderness was decreased when the transportation was prolonged.

Key words: broiler meat pH, tenderness, transportation.

Introduction

A substantial amount of research about the factors influencing the sensory quality of meat. Sometimes there are relationships between ultimate pH, and sensory traits of meat (tenderness, juiciness, and flavor), but increasing ultimate pH was shown to have detrimental effects on color. Therefore, pre-slaughter conditions which affect muscle glycogen level at slaughter and subsequent ultimate pH, could affect the sensory quality of meat. This study was designed to evaluate the possibility of controlling the sensory quality of meat by varying the transportation of broiler.

Handling and transportation, besides improving the animal productivity also will improve the meat quality. Losses during handling and transport will cause losses to the meat industry. Because of the rough handling and transportation will cause stress and the meat quality will affected, that induced some problems, such as dark cutters, carcass shrinkage, even the death losses and morbidity in the long

distance rough transportation. Meat quality defects will affect the marketing of the meat. Losses during handling and transport are due to bruises, dark cutters, death and pale soft exudatives (PSE).

Transport may involve individual and large groups animals. During transportation, animals interact with the physical facilities around them, and the biotic environment which consists of both conspecifics and stockpersons (*Gonyou, 1993*). Loading poultry onto transport vehicle, movement is accomplished by more attractive route, and also by providing more space, better lighting, or some feature that involve individual. The response of animals to handling and transport depends on their sensory capabilities (*Kilgour and Dalton, 1984*). Broilers are reared in large group in environmentally controlled, and then transported from growing sites to the slaughter plant.

Rearing conditions has significant effect on quality of carcass; also for size and conformation of carcass, problems with legs, skin damages, the parameters of carcass quality (*Škrbić, et. al. 2009*). Fresh meat must look good to the consumer, when selecting meat for purchase. Therefore color and textural characteristics are critical important, the most typical color of poultry meat, is gray-white to dull red. Some of the loss of protein hydration caused by pH decline and rigor mortis is recovered during subsequent storage of meat. Lowering of muscle pH due to the accumulation of lactic acid is one of the most significant postmortem changes. Tenderness, juiciness, color and flavor may be influenced by changes occurring during conversion of muscle to meat. Carcass that have rapid pH decline, are often results in the stress animal, that are fatigue, excitement, restraint and shock; and are slaughtered before have sufficient time to replenished the muscle glycogen stores, will results in a high ultimate pH (*Aberle, et al, 2001*). Several steps in the marketing process, loading onto trucks, transportation, have severity of effects on the meat quality. The negative attributes of high temperature-low pH combinations are associated with improvements in meat tenderness. Ante- and pre-slaughter stresses influence the quality of poultry meat. Transport for up to six hours, did not significantly influence the tenderness of chicken breast and thigh meat. However, birds in crates on waiting lorries may be subjected to temperatures 10 to 12^{o}C higher than ambient in warm climates area, showed the toughness of breast meat increased in heat-stressed birds (*Froning et al. 1978; Gschwindt and Ehinger, 1978; and Ehinger and Gschwindt, 1981 in Cross and Overby, 1988).*

Transportation of poultry is a complicated multifactorial stressful and traumatic event and in order to understand its impact on the bird, stress must be well defined and understood (Elrom, 2000). Transportation is considered a major stressor for farm animals and might have deleterious effects on health, well-being, performance, and ultimately, product quality. Livestock are transported by land, sea and air. During transport, animals are exposed to environmental stresses including heat, cold, humidity, noise, motion and social regrouping. Transportation involves a series of handling and confinement situations (*Tarrant and Grandin,*

2000). When consumers assess meat quality, most rank tenderness before juiciness and flavor. In carcass, muscle pH and color are indicators of tenderness. Water holding capacity and keeping quality are related to pH (*Wythes, et al., 1982*). When the physical stresses associated with transportation can have important effect on meat quality.

The objectives of this research are to find the effect of transportation on broiler meat pH and tenderness.

Materials and Methods

180 broilers were used for this study, using Completely Randomized Design (CRD), with five levels (P-0 slaughter at the farm, P-1 one hour transportation before slaughter, P-2 two hours transportation, P-3 three hours, and P-4 four hours transportation) and four times replications. The broiler were transported in the crate 100cm x 60cm x 30cm (l x w x h), and in each crate for 18 broilers. The broilers were transported in the car, and the speed average is 50-60 km/hr; transported to the slaughterhouse for 1 hours, 2 hours, 3 hours and 4 hours. This treatment was four time replicates. P-0, the broilers was slaughter at the farm, P-1 was slaughter after one hours transportation, P-2 after 2 hours transportation, P-3 after 3 hours transportation, and P-4 after 4 hours transportation. And then muscle pH and the meat tenderness was checked. Sensory analysis was carried out on samples cooked in the pan at a temperature of 70^0C. Tenderness were scored using Universal penetrometer 1/10 TH MM DV. The meat pH used Jenway pH meter type 3310.

Results and Discussion

Broiler Meat pH

Table 1. Broiler meat pH after transportation

	P-0	P-1	P-2	P-3	P-4
I	6.36	6.36	6.54	6.25	6.70
II	6.40	6.40	6.63	6.50	6.58
III	6.26	6.24	6.51	6.64	6.64
IV	6.30	6.56	6.20	6.73	6.60
Total	25.32	25.56	25.88	26.12	26.52
Average	6.33	6.39	6.47	6.53	6.63

From Table 1, the meat pH are between 6.33 – 6.63. The normal meat pH values are between 5.2 – 6.6; so these results are between the normal meat pH. Even the result shows that the pH values increase when the transportation time is

longer. The environmental stress factor can result in changes in the metabolites of muscle. Transportation of poultry marketing can be one of the most severe segments of the process. Muscle tissue shrinkage and reduction of dressed carcass weight can result from uncomfortable or prolonged transportation. These changes in turn, are responsible for differences in the ultimate properties of meat. Many related changes in meat quality and product performance result from alterations in tissue pH. Resting animals before slaughter allows them to recover from the journey, adapt to the surroundings and replenish muscle glycogen concentrations. Rest usually reduces pH values in sheep and beef carcasses (*Shorthose, 1977 in Wythes et al, 1982*). High pH values can occur, if animals do not rest adequately because of noise, abattoir activities and other disturbances. With careful attention to handling and transport, producers should be able to sell the maximum weight of bruise-free, tender meat. The changes of meat color, depends on the meat pH. In carcasses, muscle pH and color are indicators of tenderness. Water holding capacity and keeping quality are related to pH. An increase or decrease of the pH, will increases the intensity of the color changing when the meat was cooked. (*Wythes, et al., 1982*).

Broiler Meat Tenderness

Table 2. The effect of treatment on tenderness (mm/g/10sec)

	P-0	P-1	P-2	P-3	P-4
I	180	163	128	142	121
II	171	169	150	129	134
III	189	152	142	123	120
IV	166	147	151	129	124
Total	706	631	571	523	499
Average	176.50	157.75	142.75	130.75	124.75

From Table 2, the meat tenderness was between 124.75 – 176.50 mm/g/10 sec. The meat tenderness was decreased when the transportation was prolonged. When the broiler was slaughter at the farm, the tenderness are higher (176.50 mm/g/10 sec), and then gradually decrease as the transportation was prolonged. The broiler that transported for 4 hours, the meat tenderness was 124.75 mm/g/10sec. The skill of driver and the quality of the road appear to be more important in determining transport stress and losses value than the distance traveled (*Tarrant and Grandin, 2000, in Smith, et al. 2004*).

Tenderness of meat is distinctly important to the consumer, and many factors influence tenderness. It is known and widely recognized that meats may vary greatly in tenderness. Tenderness of broilers is rarely a problem because they are young when slaughtered. Rough transportation is detrimental to meat quality. Of the various attribute (tenderness, juiciness and chewiness), which contribute to

the texture of poultry meat, tenderness has attracted the most attention to the consumers. Processing factors might thus be expected to play an important role in influencing meat tenderness. When arrived at the slaughterhouse in an exhausted state which could be related to fatigue, after long transportation. After 4 hours journey, the broiler were more fatigue than the broiler were not transported. That is why, the broilers transported for 1 hours will decrease the tenderness compared to the broilers that was slaughter at the farm. As the transportation was prolonged, then the tenderness was decreased. Tenderness score in calves was lower in calves transported for 11h than for 1 h (*Fernandez, et. al., 1996*). According to *Nicol and Saville-Weeks (1993*), the improving of handling and transportation will increase the tenderness of the poultry meat, because the poultry were less stressful. Improvements in welfare during handling and transport procedures will improve and reduce losses associated with poor carcass quality (*Eldridge et al., 1982*).

Conclusion

Careful transportation, with good maintenance of handling and transport facilities in all parts of the harvesting chain will significantly reduce the level of bruising and improve meat quality. Improving the transport system, e.g. need better vehicles and careful transportation system, will improve the meat quality since birds have to travel for longer distance.

Uticaj transporta na pH i mekoću mesa brojlera

H. A.W. Lengkey, J. A. Siwi, P. Edianingsih, F. J. Nangoy

Rezime

180 brojlera, uzrasta pet nedelja, su korišćeni u oceni uticaj transporta na pH i mekoću mesa brojlera, urađena je u Bandungu, Indonezija. Koristeći potpuno slučajni dizajn ogleda (Completely Randomized Design - CRD), sa pet nivoa (P-0 klanje na farmi, P-1 sat transporta pre klanja, P-2 dva sata transporta pre klanja, P-3 tri sata transporta pre klanja, i P-4 četiri sata transporta pre klanja), i četiri ponavljanja. Rezultati pokazuju pH mesa između 6.33 – 6.63. Rezultati takođe pokazuju da se pH vrednosti povećavaju kada prevoz traje duže vreme, ali to su rezultati između normalne vrednosti pH mesa (pH između 5.2 – 6.6).Vrednosti za mekoću mesa su između 124.75 - 176.50 mm/g/10 sec. Vrednosti za mekoću mesa se smanjuju sa povećanjem dužine transporta.

References

ABERLE, E. D., J. C. FOREST, D. E. GERRARD and E. W. MILLS. (2001). Principles of Meat Science. 4th ed. p. 109 – 116.

CROSS, H. R. and A. J. OVERBY. (1988). Meat Science, Milk Science and Technology. Elsevier. Amsterdam. p. 145.

ELDRIDGE, G. A., WYTHES J.R., ARTHUR R.J., MEISCHKE H.R.C., VOWLES W.J., HUTSON G.D., BARNETT J.L., HEMSWORTH P.H., DAVIES G.T. (1982). Handling and Transport of Meat Animals in Relation to Efficiency, Meat Quality and Welfare. Proceedings of the Australian Society of Animal Production 14: 127-129.

ELROM, K. (2000). Handling and Transportation of Broilers Welfare, Stress, Fear and Meat Quality. Israel Journal of Veterinary Medicine. Vol. 55 (2).

FERNANDEZ, X., G. MONIN, J. CULIOLI, ISABELLE LEGRAND, and Y. QUILICHINI. (1996). Effect of Duration of Feed Withdrawal and Transportation Time on Muscle Characteristics and Quality in Friesian-Holstein Calves. J. Anim. Sci. 74:1576-1583.

GONYOU, H. W. (1993). Behavioural Principles of Animal Handling and Transport. In Grandin, T. (ed.). 1993. Livestock Handling and Transport. CAB International. Wallingford, UK. p. 11 – 20.

GRANDIN, T. (ed.). (1993). Livestock Handling and Transport. CAB International. Wallingford, UK. Pp. 320.

KILGOUR, R. and DALTON, D. C. (1984). Livestock behavior: A Practical Guide. Collins Technical Books, Glasgow. UK.

NICOL, C. and C. SAVILLE-WEEKS, (1993). Poultry Handling and Transport. In GRANDIN (ed). (1993). Livestock Handling and Transport. CAB International. Wallingford. UK. p. 273 – 288.

SKRBIC, Z., Z. PAVLOVSKI, and M. LUKIC.(2009). Stocking Density – Factor of Production Performance, Quality and Broiler Welfare. Biotechnology in Animal Husbandry 25 (5-6). Belgrade, Zemun. p. 359 – 372.

SMITH, G.C., T. GRANDIN, T.H.FRIEND, D. LAY, Jr., AND J. C. SWANSON.(2004). Effect of Transport on Meat Quality and Animal Welfare of Cattle, Pigs, Sheep, Horses, Deer and Poultry. www.grandin.com/behaviour/effect.of.transport.html/ p 1-16.

TARRANT, V. AND T.GRANDIN. 1993. Cattle Transport. In: Grandin, T. (ed) 1993. Livestock Handling and Transport. CAB International. Wallingford, UK. p. 109 – 126.

WYTHES, J.R., R.J.ARTHUR, AND H.R.C. MEISCHKE. (1982). The Handling and Transport of Livestock in Relation to Meat Quality. Proceedings of the Australian Society of Animal Production 14: 116-119.

Received 21 May 2013; accepted for publication 26 June 2013

Biotechnology in Animal Husbandry 29 (2), p 337-344 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.087'652
DOI: 10.2298/BAH1302337S

COMMERCIAL ENZYME INFLUENCE ON BROILERS FED DIFFERENT LEVELS OF WHEAT

S. Seifi

Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
P. Box: 46168-49767
Corresponding author: saeedseifi@umz.ac.ir
Original scientific paper

Abstract: An experiment was conducted to evaluate the efficacy of multi-enzyme supplement (Natuzyme®) in diets containing wheat on the broilers performance. A total of one hundred twenty day old male broiler chicks (Arbor Acres) were randomly assigned to 6 treatments with a 2×3 factorial arrangement (0, 150 and 300 gram per kilogram wheat and 0 and 0.025 gram per kilogram enzyme). Chickens were fed with starter, grower and finisher diets during 1-21, 22-35, 36-49 days of the age, respectively. Body weight gain, feed intake, and feed conversion ratio were determined weekly and productivity efficiency index was determined at the end of experiment. Litter moisture was evaluated at the age 21 days. The results indicated that although different levels of multi-enzyme and wheat had no significant effect on body weight gain, feed intake, feed conversion ratio, productivity efficiency index and litter moisture but caused partial improvement in these parameters.

Key words: broilers, performance, multi-enzyme, wheat

Introduction

Wheat grains contain relatively high concentrations of non-starch polysaccharides (NSPs), which are indigestible by broilers. NSPs problems are due to their viscous nature, to the physiological and morphological effects on the digestive tract and to their interaction with the intestinal microflora (*Gao et al., 2007*). There are two mechanisms proposed for the anti-nutritional effect of soluble NSPs. One mechanism refers to the fact that starch and protein in wheat endosperm are encapsulated by the cell wall NSPs. The second mechanism is related to higher digesta viscosity as a direct effect of soluble NSPs because the diffusion of the digestive enzymes is reduced (*Amerah et al., 2008*). Enzyme supplementation of diets based on wheat can reduce the mentioned adverse effects (*Bedford, 2000*). Enzymes decrease the viscosity of gut contents, resulting in improvements in nutrient digestibility and performance when added to poultry diets (*Marquardt et*

al., 1994). The positive effects of supplementing NSP-hydrolyzing enzymes on soluble NSPs are related to lower jejunal viscosity values and a faster passage rate of the diet through the digestive tract, which may also limit the potential development of pathogenic microbes (*Bedford and Morgan, 1996*). The present study was conducted to investigate the effects of a commercial multi-enzyme on performance and litter moisture of broilers that fed by different levels of wheat.

Materials and Methods

Birds, housing and management: A total of one hundred twenty 1-d-old Arbor Acres broiler chicks (average 41 ± 0.3g body weight) were randomly assigned to 6 treatments of 4 floor pen replicates. The birds were housed in deep litter pens and reared from day one to seven weeks. The temperature of the experimental room was maintained at 32±2°C during the first week of trial and then reduced by 2°C each week till it reached 24°C which was maintained for the rest of the period. No vaccine was used in this experiment.

Experimental diets: 0 (Control group), 15 and 30% wheat and 0 and 0.025% multi-enzyme were added to diet, respectively. Commercial multi-enzyme (Natuzyme®) which used in this experiment was obtained from *Trichoderma longibrachiatum*. The complex was contained xylanase, cellulose, protease, α-amilase, pectinase, phytase, β-glucanase, amyloglucosidase, pentosanase and hemicellulase. Compositions of the basal diet and calculated nutrient levels for the experiment are presented in Table 1.

Data collection: Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) were determined weekly and mortality was recorded when occurred. Productivity efficiency index (PEI) was determined at the end of experimental period. Litter moisture (LM) was evaluated at the age of 21 days. The samples of litter were taken off, dried at 70° C and weighted after 24 hours, and then samples moisture were evaluated.

Statistical Analysis: The experiment had a completely random design with a 2×3 factorial arrangement (analysis of variance , ANOVA, SPSS Inc., Chicago , IL): $Y_{ijk} = \mu + a_i + b_j + a_i b_j + e_{ijk}$, where Y_{ijk} is the observation of a chick fed the levels of wheat i and multi-enzyme inclusion j , a_i is the levels of wheat (0, 150, 300 gram/kg wheat) and b_j is the multi-enzyme inclusion (0, 0.025 gram/kg Natuzyme®), $a_i b_j$ is the interaction between levels of wheat and multi-enzyme inclusion and e_{ijk} is the error term. Duncan multiple range test was used to determine confidence intervals for all pair wise differences between means. The general linear model was used to determine the main effects of factors and any

possible interactions between factors. Significance was accepted at the $p<0.05$ level.

Table 1. Formulation of the diet and estimated chemical composition of experimental rations

Experiment Period	Starter (1-21 Days)			Grower (22-35 Days)			Finisher (36-49 Days)		
Ingredients	No wheat	15% wt. wheat	30% wt. wheat	No wheat	15% wt. wheat	30% wt. wheat	No wheat	15% wt. wheat	30% wt. wheat
Corn	45.55	32.52	20.53	49.96	37.93	35.93	54.34	42.31	30.29
Soybean meal	38.34	37.12	35.89	33.06	31.83	30.65	27.98	26.76	25.54
Wheat bran	7.59	5.82	4.06	7.64	5.88	4.00	7.58	5.82	4.05
Wheat	0	15.00	30.00	0	15.00	30.00	0	15.00	30.00
Oil	5.18	5.18	5.18	5.30	5.30	5.60	6.11	6.11	6.11
Di calcium phosphate	2.17	2.17	2.18	1.91	1.92	1.99	1.78	1.78	1.79
Calcium carbonate	0.98	0.97	0.96	0.91	0.90	0.85	1.02	1.01	1.00
L-methionine	0.19			0.20			0.20		
Lysine	0.14	0.17	0.20	0.15	0.19	0.25	0.07	0.10	0.14
Salt	0.37	0.35	0.33	0.37	0.35	0.33	0.43	0.43	0.43
Premix[1]	0.5			0.5			0.5		
Calculated Analysis									
M.E. (Kcal.Kg)	2850			2950			3050		
C.P.	22.5			20.0			18.0		
Lysine	1.38			1.25			1.05		
Methionine	0.539			0.526			0.493		
Methionine + Cystine	0.920			0.880			0.820		
Calcium	1.00			0.90			0.90		
P (available)	0.50			0.45			0.42		
Sodium	0.16			0.16			0.18		

[1]supplied per kilogram diet: vitamin A= 9000 IU , vitamin D3= 2000 IU, vitamin B1= 18 IU, vitamin B3= 9.8 IU, vitamin B6= 2.9 IU, vitamin B12= 0.15 IU, vitamin E= 18 IU, vitamin K3= 2mg, vitamin B2=6.6 mg, vitamin B5 = 29.7 mg, vitamin B9= 1 mg, vitamin H2= 0.1 mg, Cholin chloride= 500 mg, Mn= 99.2 mg, Zn= 84.7 mg, Fe = 5mg, Co= 1mg, Se=0.2 mg, I= 0.992 mg

Results and Discussion

BWG, FI, FCR, PEI and LM did not significantly affected ($P>0.05$) by different levels of wheat (Table 2). Diet supplemented with Natuzyme® had no significant effect on BWG, FI, FCR, PEI and LM ($P>0.05$). Supplementation diets, including different levels of wheat, with Natuzyme® increased BWG partially in comparison with control diets. FCR and PEI also improved by multi-enzyme. Throughout the entire experiment the highest BWG was reported in diet with 0%

wheat plus Natuzyme®, whereas birds received diet included 30% wheat without Natuzyme® had the lowest BWG. The highest FI and FCR were recorded in 0% wheat without Natuzyme® fed group, whereas birds received 15% wheat without Natuzyme® had the lowest FI and FCR. The chicks were fed with 30% wheat plus Natuzyme® and control (0% wheat + 0% Natuzyme®) diets had the highest and lowest PEI, respectively. Enzyme consumption in all of the treatments except the birds fed with 15% wheat with plus enzyme, decreased LM in comparison with diets with different level of wheat, without Natuzyme®.

Table 2. Effect of different levels of wheat and Natuzyme in broiler diet on performance and litter moisture.

Treatments		BWG (gr)		FI (gr)		FCR (gr/gr)		PEI	Litter moisture (%)
Wheat (%)	Enzyme (%)	1-21 days	1-49 days	1-21 days	1-49 days	1-21 days	1-49 days	49 days	21 day
0	0	644.5	2728.5	964.0	5963.1	1.496	2.232	238.9	28
15	0	634.7	2673.4	958.3	5542.6	1.510	2.097	244.5	22
30	0	621.2	2662.8	954.0	5797.2	1.535	2.198	239.0	24
0	0.025	614.5	2765.0	986.8	5832.7	1.606	2.124	258.5	22
15	0.025	636.9	2697.8	965.1	5903.5	1.501	2.189	243.0	26
30	0.025	616.1	2710.8	936.7	5683.5	1.517	2.101	258.6	23
Pooled SEM		14.4	44.2	12.3	80.8	0.03	0.04	8.4	1
Probability									
Wheat		NS	NS	NS	NS	NS	NS	NS	NS
Enzyme		NS	NS	NS	NS	NS	NS	NS	NS
Wheat x Enzyme		NS	NS	NS	NS	NS	NS	NS	NS

NS: Not Statistically Significant; BWG: Body Weight Gain; FI: Feed Intake; FCR: Food Conversation Ratio; PEI: Productivity Efficiency Index = {[Body Weight (kg) × Livability (%)]/ [Duration of Experiment (d) × Feed Conversation Ratio]} ×100; SEM : Standard Error of the Means

The results of this study showed that using of wheat in diet had no significant effect on BWG (P>0.05) although 15 and 30% wheat in diets decreased BWG by an average of 2.01 and 2.40 percent, respectively. This finding is in

accordance with other studies (*Silva et al., 2002; L'azaro et al., 2003; J'ozefiak et al., 2007*). Decreasing of BWG can be attributed to the presence of soluble arabinoxylans in wheat that they are generally believed to be responsible for the majority of the anti-nutritive activity of NSPs in poultry by virtue of their capacity to increase intestinal viscosity (*Choct and Annison, 1992*). Therefore, it is possible that the wheat used in this trail was situated in category of low NSPs wheat.

The present study showed that addition of Natuzyme® to diets with 15 and 30% wheat had no significant effect on BWG, but improved it slightly. At the end of experiment, BWG in broilers fed diets supplemented with enzyme were higher (1.77%) than broilers fed diets without enzyme that is in accordance with previous researches (*Engberg et al., 2004; Wang et al., 2005*). The positive influence of Natuzyme® on broiler performance has been explained by an improved digestibility (*Silva et al., 2002; L'azaro et al., 2003; J'ozefiak et al., 2007*). When xylanase adds to wheat based diet, a greater proportion of NSPs may be hydrolyzed. In addition xylanase can effectively reduce digest viscosity, presumably by cleaving the large molecules into smaller fragments. Therefore only high molecular weight soluble arebinoxylans are responsible for increased digest viscosity (*Bedford and Classen, 1992*). In this survey, Natuzyme® had no enough efficiency. This result might be caused by the presence of enzyme inhibitors or using different variety of wheat, and it is possible that level of wheat in the diet was low.

Using of 15 and 30% wheat in diets decreased FI, up to level of 7.1 and 2.8%, respectively. This is disagreement with the result of previous researches (*Silva et al., 2002; L'azaro et al., 2003; J'ozefiak et al., 2007*). In other hand, addition of Natuzyme® to diets including wheat increased FI partially (approximately 0.7%). This is in agreement with results of *Marron et al. (2001)* and *Gao et al. (2008)*, but this is disagreement with *Nejib et al.* (*2002), L'azaro et al. (2003)* and *Yuben and Ravindran (2004)*. These differences among results may be caused by using different levels of wheat and enzyme. Insignificant effects of diets contain wheat on FCR and PEI may be due to use wheat with low NSPs that had a good quality and has no negative effect on broilers performance. In comparison with diets without enzyme, using enzyme in diets improved FCR (1.70%) and PEI (5.21%), nonetheless using enzyme supplementation had no effect. Whereas several researchers, *Yuben and Ravindran (2004*); *Wang et al. (2005); Gao et al. (2008)* reported that addition of xylanase to diets containing wheat improved FCR significantly.

In respect to the content of LM at 21 d, the enzyme did not significantly ($P>0.05$) affect this parameter. This result is in agreement with *leeson et al. (2000)*. Although, enzyme did not significantly affect LM, it had a decreasing trend. The reason of insignificant effect of enzyme on LM is not clear, but NSPs content may not be enough to affect this parameter significantly.

Conclusion

In conclusion, although NSPs-hydrolyzing enzyme preparations (Natuzyme®) in diets with different levels of wheat had no significant effect on broilers performance; it can improve it. This improvement is achieved through the enzyme's influence on digestion of broiler chick.

Uticaj upotrebe komercijalnih enzima na brojlere hranjene različitim nivoima pšenice

S Seifi

Rezime

Eksperiment je izveden da se proceni efikasnost više enzimskih dodataka (Natuzime ®) u ishrani koja sadrži pšenicu na performanse brojlera.Ukupno sto dvadeset jednodenvnih muških brojlera Arbor Acres () je podeljeno na 6 tretmana sa 2 3 faktora (0, 150 i 300 grama po kilogramu pšenice; 0 i 0.025 grama po kilogramu enzima). Pilići su hranjeni sa starter, grover i finišer obrocima tokom 1-21, 22-35, 36-49 dana starosti, respektivno. Prirast telsne težine, unos hrane, i konverzija hrane su utvrđivani nedeljno a indeks efikasnost i produktivnost određen je na kraju eksperimenta. Vlažnost prostirke je ocenjena u uzrastu 21 dana. Rezultati su pokazali da različiti nivoi multi- enzima i pšenice nisu imali značajan uticaj na prirast telesne mase, unos hrane, konverziju hrane, indeks produktivnosti i efikasnosti, vlažnost prostirke, ali je registrovano delimično poboljšanja u ovim parametrima.

References

AMERAH A.M., RAVINDRAN V., LENTLE R.G., THOMAS D.G. (2008): Influence of particle size and xylanase supplementation on the performance, energy utilization, digestive tract parameters and digesta viscosity of broiler starters. British Poultry Science, 49, 455-462.

BEDFORD M.R., CLASSEN H.L. (1992): Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is affected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. Journal of Nutrition, 122, 560-569.

BEDFORD M.R., MORGAN A.J. (1996): The use of enzymes in poultry diets. World's Poultry Science Journal, 52, 61-68.
BEDFORD M.R. (2000): Exogenous enzymes in monogastric nutrition, their current value and future benefits. Animal Feed Science and Technology, 86, 1-13.
CHOCT M., ANNISON G. (1992): Antinutritive effect of wheat Pentosanas in broiler chicken: Role of viscosity and microflora. British Poultry Science, 33, 821-834.
ENGBERG R.M., HEDEMANN M.S., STEENFELDT S., JENSEN B.B. (2004): Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poultry Science, 83, 925-938.
GAO F., JIANG Y., ZHOU G.H., HAN Z.K. (2007): The effect of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat based diets. British Poultry Science, 48, 480-488.
GAO F., JIANG Y., ZHOU G.H., HAN Z.K. (2008): The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. Animal Feed Science and Technology, 142, 173–184.
J'OZEFIAK D., RUTKOWSKI A., JENSEN B.B. ENGBERG R.M. (2007): Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract. Animal Feed Science and Technology, 132, 79-93.
L'AZARO R, GARCIA M., MEDEL P., MATEOS G.G. (2003): Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poultry Science, 82, 132-140.
LEESON S., CASTON L., KIAEI M.M., JONES R. (2000): Commercial enzymes and their influence on broilers fed wheat and barley. Journal of Applied Research, 9, 242-251.
MARRON L., BEDFORD M.R., MCCRACKEN K.J. (2001): The effects of adding xylanase vitamin C and copper sulfate to wheat-based diets on broiler performance. British Poultry Science, 42, 439-500.
MARQUARDT R.R, BOROS D., GUENTER W., CROW G. (1994): The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reevei* enzyme preparation. Animal Feed Science and Technology, 45, 363-378.
NEJIB M., SERGE M., LUC S., BERNARD Q., MICHEL L. (2002): Effects of xylanase and B-glocanase addition on performance, nutrient digestibility and physicochemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. Animal Research, 51, 395-406.
SILVA S.S.P., SMITHARD R.R. (2002): Effect of enzyme supplementation of a rye-based diet on xylanase activity in the small intestine of broilers, on intestinal

crypt proliferation and nutrient digestibility and growth performance of the birds. British Poultry Science, 43, 274-282.
WANG Z.R., QIAO S.Y., LU W.Q., LI D.F. (2005): Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat based diets. Poultry Science, 84, 875-881.
YUBEN B.WU., RAVINDRAN V. (2004): Influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements And carcass characteristics of broiler chickens. Animal Feed Science and Technology, 116, 129-139.

Received 12 March 2013; accepted for publication 24 May 2013

Biotechnology in Animal Husbandry 29 (2), p 345-352 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.087
DOI: 10.2298/BAH1302345P

THE INFLUENCE OF SELENIUM SUPPLEMENTATION OF ANIMAL FEED ON HUMAN SELENIUM INTAKE IN SERBIA

Z. Pavlović[1], I. Miletić [2], Ž. Jokić [3], J. Stevanović [4],S.Šobajić[2], Z.Bulat[5]

[1]Institute for Public Health Požarevac, Jovana Šerbanovića 14, 12000 Požarevac, Republic of Serbia Corresponding author: zpavlovicpo@yahoo.com
[2]Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Republic of Serbia
[3]Institute for Animal Science, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Beograd - Zemun, Republic of Serbia
[4]Association of Agriculture, Food-processing and Tobacco and Water Industry
Serbian Chamber of Commerce, Resavska 13-15, 11000 Belgrade, Republic of Serbia
[5]Department of Toxicology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Republic of Serbia
Original scientific paper

Abstract: The use of selenium as animal feed supplement in Serbia was approved in 1989 for some categories of pigs, sheep and poultry. From 2000 selenium in animal feed became a requirement for all categories of farm animals. The aim of this study was to determine the consumption of selenium by Serbian livestock and in poultry production between 1990-1991 and 2000-2008 and to analyze the selenium content of meat, milk and eggs sold on Serbian markets to gain insight into human selenium intake. Data indicate a low level of selenium inclusion in animal feed during 1990-1991 compared to during 2000-2008. These results coincide with Serbian regulations. From 2001 an organic form of selenium (selenized yeast) was introduced in small quantities (less than 8% of the total consumed selenium). Analysis of meat, milk and eggs from Serbian markets shows increased selenium levels compared to 1991. However, the estimated total human daily intake in Serbia is 40.9 μg, which is under the recommended quantity. Increasing the quantity of selenium included in animal feed and the substitution of inorganic selenium with selenized yeast could be beneficial for animal health and farm productivity. As a consequence a further improvement in the human selenium status in Serbia should be possible.

Key words: selenium, feed, mineral-vitamin premixes, foods animal origin

Introduction

Modern livestock farming and poultry production have three main challenges: To increase productivity, to have better quality products and to lower production costs. All targets can be met by improving animal genetics through breeding and selection, providing adequate nutritionally-balanced diets and improving management practice. Selenium is one of a number of dietary components that can have direct and/or indirect implications on productivity and health of farmed animals. The selenium content of foodstuffs reflects its level in soil. Many regions in the world, including Serbia, have very low selenium concentrations in the soil (*Combs, 2001*). Fodder from such soils cannot provide sufficient selenium to ensure optimal animal health and productivity (*Trenkovski, 1989*). Such a situation requires selenium supplementation of animal feed through mineral-vitamin premixes. Feed supplementation with selenium apart from helping to achieve optimal conditions for livestock farming and poultry production has another important benefit for humans: By increasing the level of selenium in meat, eggs and milk prevention of selenium deficiency in the Serbian population can be achieved.

Prior to this study there was no available data regarding selenium consumption as a function of feed supplementation of livestock and poultry in Serbia from 1990 to the present. Furthermore, there are insufficient data concerning selenium in food of animal origin and its influence on the human intake of selenium.

Our study had two main objectives. Firstly, to determine the consumption of selenium (as feed supplement) by Serbian livestock and in poultry production between 1990-1991 and 2000-2008 and secondly, to determine the selenium content of meat, milk and eggs from Serbian markets (selling foodstuffs produced in Serbia) and their contribution to human selenium intake.

Material and Methods

The consumption of selenium supplements for animal farming was calculated on the basis of Serbia's import of selenium as feed additive. Data regarding import of selenium supplements, production and import and export of mineral-vitamin premixes were obtained from the Serbian Chamber of Commerce. Human food consumption assessed by household budget surveys in 2007 (*Statistical Office of the Republic of Serbia, 2009*) was used to calculate the average daily intake of selenium.

Eggs (battery farmed hens, class A), milk (containing 2.8% fat), pork (leg), beef (leg) and poultry meat (breast) were collected from Serbian market. Selenium was determined using hydride generation atomic absorption spectrometry with a Varian SpectrAA-10 containing a VGA-76 hydride unit according to a method

described previously (Pavlovic et al., 2009). Method verification involved the use of certified reference material: NIST RM 8415 (whole egg powder), IAEA 155 (whey powder) and IAEA 407 (fish tissue). Our obtained results agreed with official certified values.

Results and Discussion

Mineral-vitamin premix production and its consumption in Serbia during 1990-1991 and 2000-2008 are shown in Table 1. During 2000-2008 the average year's premix production was only about one half of the year's production during 1990-1991. From 2000 increased import of mineral-vitamin premix combined with decreased premix production changed the structure of premix consumption in Serbia. During 1990-1991 imported mineral-vitamin premix accounted for about 16% of the total consumed premix, while from 2000 the value was 34%. Export of premix was small, less than 5% of the total quantity produced and that imported. The quantity of imported inorganic selenium during 1990-1991 was small (Table 1). From 2000 Serbia's import of inorganic selenium grew significantly but suffered from year-to-year fluctuations. During 2000-2008 the average import of inorganic selenium compounds amounted to 147 kg per year (as selenium). Average yearly premix production was 11066 tones. Therefore, average selenium content was 14.3 mg/kg premix. In 2001 importation of an organic form of selenium (selenized yeast) started in small quantities (about 12 kg per year, as selenium). Data regarding the selenium content of imported premixes are not available.

Table 1. Mineral-vitamin premix production in Serbia

year	Premix production, tons	Import of premix, tons	Export of premix, tons	Premix consumed in Serbia, tons	Import of Selenium,* kg
1990	22799	4883	899	26783	11,5
1991	22460	3475	252	25424	22,5
2000	10189	2435	0	12624	99
2001	8662	2788	29	11421	22,25
2002	15467	7121	233	22355	67,5
2003	14318	7862	251	21929	124
2004	8601	6557	530	14628	465
2005	8837	4119	215	12741	33,25
2006	9231	5503	517	14217	357,5
2007	9900	7282	303	16879	152
2008	14385	8694	416	22663	212

*selenium as feed additive

Table 2. Estimated Serbian selenium consumption from animal products

Foodstuff	Se concentration Average ± SD[1]	Range	Daily consumption	Daily Se contribution to diet, µg
Milk (n=15)	12,1 ± 3,61 µg/l	<5-16	0,490 l[2]	5,9
Pork (n=20)	112,7 ± 27,64 µg/kg*	79-189	83,1 g[3]	9,4
Beef (n=19)	95,7 ± 30,39 µg/kg*	44-163	17,8 g[3]	1,7
Poultry (n=20)	121,7 ± 17,98 µg/kg*	89-145	65,7 g[3]	8,0
Eggs (n=20)	186,1 ± 28,77 µg/kg*	138-247	36 g	6,7

[1]standard deviation, [2]calculated as milk and dairy, [3]fresh, frozen and processed item
* per kg wet weight

Animal-based food for human consumption is a good dietary source of selenium in Serbia (Table 2). The highest selenium concentration was found in eggs with an average of 186 µg/kg. Selenium in meat sold at Serbian markets was in the range 44–163 µg/kg for beef, 89–145 for chicken and 79–189 µg/kg for pork. Milk produced in Serbia is not rich in selenium as the average content was only 12.1 µg/l. Taking into consideration selenium food content and daily food consumption, pork is the richest source of human selenium intake in Serbia. Pork provides more than one sixth of the Recommended Dietary Allowance (RDA) for selenium (Food and Nutrition Board-USA Institute of Medicine, 2000). Poultry, eggs and milk and dairy produce also importantly contribute to selenium intake (14%, 12 % and 11% of RDA, respectively). Beef has a minor influence on selenium intake due to its low consumption in Serbia.

The first large-scale geochemical investigation of selenium in Serbia showed low selenium content in river sediments (*Maksimovic et al., 1985*). Four years later the low selenium content Se content of Serbian agricultural soil and selenium deficiency in animal feed were reported (*Trenkovski 1989*). Selenium supplementation of animal feed in Serbia started in 1989, by inorganic selenium compounds, for some categories of pigs, sheep and poultry but not for fattening pigs, hens, dairy cows and cattle (*Rulebook 15/89, 1989*). As a consequence of the limited use of selenium supplements insignificant quantities of selenium were imported during 1990-1991. Our results, together with the fact that at this time selenium supplements were not permitted for animals that provided the greatest contribution to human selenium intake in Serbia, are in agreement with results of *Djujic et al., (1995)* who reported poor human dietary selenium intake in Serbia in 1991 and low selenium in food animal origin for human consumption. Our findings coincided with those of *Maksimovic et al., (1992)* who reported that the mean serum selenium level in a healthy Serbian population (n=602) was below 45 µg/L.

The new period of selenium supplementation in the Serbian animal feed industry began in 2000 with the obligatory inclusion of selenium in animal feed for all farm animals. From 2000 onwards the yearly minimal requirement of selenium content in animal feed was 0.1 mg/kg for all categories of pigs and cows and 0.15 mg/kg for poultry feed, and using an organic form of selenium was approved (*Rulebook 20/00, 2000*). Our results show that selenium import during 2000-2008 was adequate for mineral-vitamin premix production and that the calculated average content of selenium in premixes satisfied the demands of Serbian regulations but not the needs of modern livestock and poultry production.

The analysis of selenium levels in food origin from animals in our study showed some disagreement between average selenium content in premixes and selenium content of animal food origin for human consumption. Given that the selenium content in mineral-vitamin premix is about 14 mg/kg, the analysis of food of animal origin for human consumption showed that the selenium content in some groceries was not adequate therefore putting into doubt the average level of selenium in premixes. However, selenium levels in pork and eggs were elevated compared to 1991 (as a consequence of higher selenium content in premixes, as required by Serbian regulations). In contrast, the selenium content of milk and beef are similar to findings by *Djujic et al., (1995).* We propose that suboptimal use of selenium supplements for dairy cows and other cattle was the cause. Selenium has been a supplement in broiler feed since 1989. Our results regarding the selenium content in poultry meat correspond to those of *Djujic et al., (1995*) and confirmed the use of selenium according to modern poultry production methods. A possible explanation for the discrepancies mentioned above is that imported selenium is included in premixes for some categories of farmed animals in quantities according to the demands of modern livestock and poultry production, while on the other side feed for dairy cows and other cattle is not currently supplemented with selenium.

Research in Serbia from 2000 onwards reported improvement of selenium status in healthy Serbian population. *Mihajlovic et al., (2002*) found 71.8 µg Se/l in plasma of healthy persons, while *Pesut et al. (2005)* reported slightly lower plasma selenium content in healthy adults of both sexes in the central city area of Belgrade (66.9 µg Se/l). Except changes in feed industry, during last two decade in Serbia there weren't other activities that could be to influence dietary selenium intake of general population. We suppose that increment of selenium inclusion in animal feed is the mainly reason for improvement of Se status in Serbian population. Cited researches (*Mihajlovic et al., 2002; Pesut et al., 2005*), also indicate effectiveness of the selenium supplementation in animal feed programme.

In Serbia inorganic selenium compounds, primarily sodium selenite, are most often used for inclusion in animal feed. An organic form selenium (selenized yeast) has many advantages compared with inorganic selenium: greater deposits in meat, eggs and milk, better productivity by animals and a better effect in the prevention of some diseases (*Pavlovic et al., 2009, Pavlovic et al. 2010, Krstic et*

al., 2012). However, selenized yeast accounts for less than 8% of selenium included in animal feed in Serbia. Currently, the important reason for a low degree of supplementation with selenized yeast is economically-higher price (selenized yeast compared with sodium selenite).

Food of animal origin plays a significant role in human nutrition in Serbia. The average Serbian citizen consumes 61 kg meat, 180 l milk (as milk and dairy products) and 240 eggs per year (Statistical Office of the Republic of Serbia 2009). Therefore, the selenium content in such foodstuffs is important for human selenium intake. In 1991 the estimated average daily Se intake in the Serbian population was 29.7 μg (*Djujic et al., 1995*). The greatest part was provided by food of animal origin (20.5 μg), while cereals were ensured 7.3 μg and vegetables, fruits and alcoholic beverages less than totally 2 μg Se/day (*Djujic et al., 1995*). The composition of food consumed in Serbia in 2007 (*Statistical Office of the Republic of Serbia 2009*) revealed increased ingestion of pork, poultry and eggs, which together with elevated levels of selenium in pork and eggs, resulted in an increased daily intake of selenium by food originating from farmed animals (31.7 μg) compared with 1991 (*Djujic et al., 1995*). However, the currently estimated total human daily intake in Serbia is 40.9 μg, which is still under the recommended quantity and points to the need increased.

Conclusion

Clearly selenium supplementation of animal feed affects the selenium level in food. However, there are still many points for improvement. An increased quantity of selenium in animal feed and the substitution of inorganic selenium compounds with selenized yeast could offer benefits for animal health and farming productivity, and also for human selenium status. Improving the selenium status in Serbia's citizens via consumption of better quality food is an ongoing goal for agricultural and food technology companies.

Uticaj suplementacije stočne hrane selenom na humani status selena

Z. Pavlović, I. Miletić, Ž. Jokić, J. Stevanović, S. Šobajić, Z Bulat

Rezime

Upotreba selena kao suplementa za stočnu hranu odobrena je u Srbiji 1989. godine za neke kategorija svinja, ovaca i živine. Od 2000. propisan je obavezan

sadržaj selena u hrani za sve domaće životinje. Cilj rada bio je istraživanje upotrebe selena za suplementaciju stočne hrane selenom u periodima 1990-1991 i 2000-2008 i analiza sadržaja selena u namirnicama životinjskog porekla. Rezultati ukazuju na povećanu upotrebu selena kao aditiva za stočnu hranu od 2000. i povećan sadržaj selena u svinjskom mesu i jajima u poređenju sa podacima iz 1991. Procenjeni dnevni unos selena stanovnika Srbije je 40.9 μg što je značajno manje od preporučenog dnevnog unosa selena. Povećana upotreba selena u ishrani domaćih životinja, i zamena neorganskih jedinjenja selena seleniziranim kvascem, pored povoljnih efekata na zdravlje i produktivnost životinja, dovela bi do daljeg poboljšanja statusa selena stanovnika Srbije.

References

COMBS G.F.Jr. (2001) Selenium in global food systems. Br. J. Nutr. 85, 517-547.

DJUJIC I., DJUJIC B., TRAJKOVIC Lj. (1995) Dietary intake of selenium in Serbia: results for 1991. Serbian Academy of science and arts (Department of natural and mathematical sciences). Scientific meetings 6, 81–87.

FOOD AND NUTRITION BOARD – USA Institute of Medicine (2000). Dietary References Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. Washington: National Academy Press, pp 284-324.

KRSTIC B., JOKIC Z., PAVLOVIC Z., ZIVKOVIC D. (2012) Options for the production of selenized chicken meat. Biol. Trace Elem. Res. 146:68-72.

MAKSIMOVIC Z., DUJIC I., JOVIC V., RSUMOVIC M. (1992) Selenium deficiency in Serbia and possible effects on health. Bull. Acad. Serbe. Sci. Arts, 33, 65-83.

MAKSIMOVIC Z., RSUMOVIC M., RADOSEVIC P. (1985) Selenium in certain river sediments in Serbia (Yugoslavia). Bull. Acad. Serbe. Sci. Arts, 26, 101-109.

MIHAJLOVIC M.B., VASILJEVIC Z., SOBAJIC S., JOVANOVIC I.B., PESUT O., MATIC G. (2002) Antioxidant status of patients with acute myocardial infarction. Trace Elem. Electrolytes, 19(4), 171-173.

PAVLOVIC Z., MILETIC I., JOKIC Ž., PAVLOVSKI Z., ŠKRBIC Z., SOBAJIC S. (2010) The Effect of Level and Source of Dietary Selenium Supplementation on Eggshell Quality, Biol Trace Elem Res, 133(2):197-202

PAVLOVIC Z., MILETIC I., JOKIC Z., SOBAJIC S. (2009) The Effect of Dietary Selenium Source and Level on Hen Production and Egg Selenium Concentration. Biol. Trace Elem. Res. 131(3), 263-270.

PESUT O., BACKOVIC D., SOBAJIC S. (2005) Dietary selenium supplementation of pigs and broilers as a way of producing selenium enriched meat. Acta Vet (Beograd) 55, 483-492.

PRAVILNIK O KVALITETU I DRUGIM ZAHTEVIMA ZA HRANU ZA ŽIVOTINJE. (2000) Službeni list SRJ 20/00, 3-31.

PRAVILNIK O KVALITETU STOČNE HRANE (1989) Službeni list SFRJ 15/89, 381-402.
STATISTICAL OFFICE OF THE REPUBLIC OF SERBIA (2009) Household Budget Survey 2007. Bulletin 493, pp 71.
TRENKOVSKI V. (1989) Selenium content of soils and feeds from different regions of Serbia, “in Serbian, with English abstract”. Stočarstvo 43, 331-336.

Received 7 March 2013; accepted for publication 12 June 2013

Biotechnology in Animal Husbandry 29 (2), p 353-362 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.087'639.211
DOI: 10.2298/BAH1302353O

IMPACT OF MANNANOLIGOSACCHARIDES ON PERFORMANCE TRAITS OF RAINBOW TROUT

S. Obradović[1]*, B. Živković[2], V. Đekić[3], M. Šekler[4], M. Živkov-Baloš[5], M. Marković[6]

[1]Faculty of economics and engineering management, Novi Sad;
[2]Institute for Animal Husbandry, Belgrade-Zemun;
[3]Centre for Small Grains, Kragujevac;
[4]Veterinary Specialist Institute, Kraljevo;
[5]Scientific Institute of Veterinary Medicine, Novi Sad; [6]Faculty of Veterinary Medicine, Belgrade
Corresponding author: obradovi@sbb.rs
Original scientific paper

Abstract: The effect of mannanoligo saccharides (MOS) as a food additive, applied at a concentration of 0.2% (O-I group of fish) and 0.3% (O-II group of fish) on morphometric characteristics and primary production of the Californian trout was investigated. The experiment was conducted on 450 fish divided into three groups with 150 individuals in each group, and lasted 40 days. The analysis of obtained results established the beneficial effect of the applied additives on morphometric parameters of the growth rate of fish, their final mass and final linear dimensions, and their growth, but no statistically significant differences (p> 0,05) were established. The highest body weight and body length of studied groups was achieved by trout of O-II group (118.49 g, 19.59 cm), followed by trout of O-I group (118.04 g, 19.58 cm) and the lowest K-group fish (115.94 g, 19.56 cm). Adding MOS in the feed mixture for trout had a beneficial effect (p> 0.05) in the exercise of lower feed conversion (FC) and better values of main production indicators: condition factor (FC), protein efficiency ratio (PER), the specific rate growth rate (SGR) and production index (PI) compared to the K group of fish that were fed diets without added mannanoligo saccharides. The best results were achieved by O- II fish group, and expressed in relative terms in comparison to the K group of fish: better feed conversion by 5.61%, a better utilization of protein feed value by 5.94%, a higher value of condition factor by 1.73 %, the specific growth rate by 2.26% and a better value of production index by 8.27%.

Keywords: mannanoligosaccharides, rainbow trout, production indicators

Introduction

Ban of antibiotics as growth promoters and increase in demand for environmentally safe products, modern aquaculture management strategies

in fish nutrition, involve the use of biologically active substances in order to achieve maximum production output. Nowadays, a range of biofunctional preparations are used as feed additives, which exert a positive effect on production traits and health of farmed animals and fish, but do not have direct nutritional effects (*Adams, 2004*).

Prebiotics mannan oligosaccharides (MOS) and supplements based on it are complex carbohydrates derived from the cell wall of yeast *Saccharomyces cerevisiae* and are non-digestible food ingredients. The form present in the cell wall (α-1, 3 and α-1, 6 branched mannans) is particularly effective in binding to pathogens in the digestive tract of animals and fish (*Spring et al., 2000*). Their use contributes to the increased vitality of animals and fish, reduces mortality, stimulates production of immunoglobulins that enhance the immune system, and also improves the conversion and absorption of food, which leads to good production results and positive economic effect (*Ferketi et al., 2002*). mannanoligosaccharides are now the most used in monogastric animal nutrition.

Considering the above facts relating to the applicability of biologically active substances as food additives, the aim of this paper is to show the potential of mannanoligosaccharides as a food additive on the production characteristics of yearling rainbow trout (1 + years).

Material and methods

The experiment was conducted on 450 of sorted and approximately uniform specimens of rainbow trout aged 1 + years of which were formed into three experimental groups of 150 fish each. Studied groups of fish were distributed into 3 individual pools of 20 m^3 volume, with the same water inlet of 24 l/sec, and the flow of water comprising of 69changes within 24 hours. The experiment lasted 40 days.

Fish in all three experimental groups were fed with standard mixture for feeding fattening trout with 40% of the total protein. The control group of fish was fed feed without additives, and other experimental group of fish was fed diets supplemented with either mannanoligo saccharides (MOS) as follows: O-I group was fed mixture containing 0.2% MOS and O-II group mixture containing 0.3% MOS.

The amount of food and number of daily meals were determined on a daily basis according to pre-determined food tables, adjusted to the water temperature and body weight of fish during the test (*Phillips, 1970*). Consumed food was chemically analyzed at the beginning of the experiment using standard testing methods (AOAC, 1990), except that the energy content was obtained by calculation. Ingredients and chemical composition of the mixture used for feeding fish in the experiment is shown in Table 1

Table 1. Ingredients and chemical composition of the used mixture

Component ,%	**K**	**O-I**	**O-II**
Corn	11,30	11,10	11,00
Fish meal	48	48	48
Soybean meal	19	19	19
Sunflower meal 33%	3	3	3
Lime	1,9	1,9	1,9
Mono-Ca-phosphate	0,5	0,5	0,5
Iodized salt	0,3	0,3	0,3
Premix	1	1	1
Soybean oil	15	15	15
Mannanoligo saccharides (BioMos)	-	0,2	0,3
Average chemical composition of used mixtures (%) VSM			
Water	9,33	9,31	9,30
Ash	11,00	11,10	11,14
Proteins	40,39	40,41	40,35
Fibre	2,66	2,64	2,70
Dry matter (DM)	90,67	90,69	90,70
Metabolic energy ME MJ/kg (calculation)	15,06	15,10	15,08

Composition of used premix/kg mixture: Vitamin A 20000 IJ/kg; Vitamin D_3 3000;Vitamin E 80 mg/kg; Vitamin K_3 5 mg/kg; Vitamin B_1 15 mg/kg; Vitamin B_2 25 mg/kg; Vitamin B_6 15 mg/kg; Vitamin B_{12} 0,04 mg/kg; Vitamin C 500 mg/kg; Niacin 100 mg/kg; Ca-pantotenat 50 mg/kg; Biotin 1 mg/kg; folic acid 4 mg/kg; Cholin chloride 100 mg/kg; Fe 40 mg/kg; Cu 10 mg/kg; Mn 40 mg/kg; Zn 40 mg/kg; J 10 mg/kg; Se 0,05 mg/kg; Co 1 mg/kg; Mg 50 mg/kg; Antioxidant BHT 100 mg/kg.

Based on the shown chemical composition of the mixture it can be concluded that the quality of the mixture was such that it meets the optimal nutritional requirements of rainbow trout and meets the requirements that are placed in the design of the experiment (*NRC, 1993, Official Gazette of RS No.4/2010*).

Control measurements of body mass and body length of fish were performed at the beginning, middle and end of the experiment. In order to determine the production characteristics of fish from each group two hours after feeding 20 individuals were cought, according to the method of random sampling. Individual body weight of fish was determined by measuring on a decimal technical scale (accuracy to 10-2 g) and body length using the ihtiometer (accuracy to 0.1 cm).

Based on the determined measurement results in order to determine the effect of mannanoligo saccharides as additive to the mixtures, the following product parameters were analyzed: total body weight gain, average individual weight gain and total body length, feed conversion ratio (FC), condition factor (FC), specific growth rate (SGR), protein efficiency ratio (PER) and production index (PI). In order to determine the hygienic conditions in the pools, indices of fish stocking density (Ig) and flow (Ip) were determined

These parameters were calculated using the following forms:

$HK = H / TM_2 – TM_1$

$FK = TM_2 (g) / L_2^3 (cm)$

$SGR = [(TM_2 - TM_1) / (T_2 - T_1)] \times 100$

PER = Ptm / Up

Where: H-consumed food; TM_2 - Final body weight (g); TM_1 - initial weight (g); L_2 - Final total body length (cm); T_2-T_1 - the number of days the experiment; Ptm - gain of body weight (g); Up - consumption of protein (g).

According to *Piper et al. (1982)* the following was calculated:

Ig = [TM / L] x Q and Ip = [TM / L] x P

Where: TM-weight (g); L - total body length (cm); Q - volume of the fish pool (m^3), P - the water flow (l/sec).

Statistical analysis was performed using analysis of variance with assessment of statistical significance using the t-test.

Results and Discussion

The results on the impact of adding different concentrations of mannanoligo saccharides in the feed mixture of rainbow trout on the performance are presented in Tables 2 and 3

Table 2 Comparative overview of the values of some production traits

Indicator	Groups		
	K	O-I	O-II
Initial body weight (beginning of trial), g	82,30	82,20	82,30
Average total body length (beginning of trial), cm	19,16	19,16	19,16
Production indicators at the end of the trial			
Average body weight (TM), g	115,94	118,04	118,49
Average total body lemngth (L), cm	19,56	19,58	19,59
Total fish weight, kg	17,39	17,71	17,77
Total gain of body weight, kg	5,05	5,38	5,43
Average individual gain of TM, g	33,64	35,84	36,19
Difference (%)		6,54	7,58
Average individual increase of L, cm	0,400	0,420	0,430
Difference (%)		5,00	7,50
Total feed consumption, kg	9,06	9,15	9,20
Average daily feed consumption per fish, g	1,510	1,525	1,533
Difference (%)		0,99	1,55
Feed conversion ratio (FC)	1,795	1,702	1,695
Difference (%)		-5,21	-5,61
Protein efficiency coefficient (PER)	1,392	1,469	1,475
Difference (%)		5,49	5,94
Condition factor (FK)	0,0155	0,0157	0,0158
Difference (%)		1,50	1,73
Specific growth rate (SGR)	2,10	2,24	2,26
Difference (%)		6,54	7,58
Production index (PI)	2,421	2,601	2,622
Difference (%)		7,40	8,27

At the beginning of the experiment all fish groups were roughly equal in terms of body weight and body length, which is the result of the previous sorting to obtain the more uniformh groups. Upon completion of the experiment, i.e. 40 days, the highest body weight values were recorded in O-II fish group (118.49 g), followed by trout of O-I group (118.04 g), and the lowest in K-group (115.94 g). Average values of body length were highest in fish of O-II group (19.59 cm) and lowest in group K (19.56 cm). The differences of the final body weight and total body length of fish between the groups (Table 3) were not statistically significant ($p > 0.05$). Realized values of morphometric parameters analyzed had directly influenced the results in the gain. Expressed in relative terms, O-II group fish achieved a higher average weight gain compared to fish in K group and 7.58% of the total body length of 7.50%, while the fish of O-I group for the same parameters had better results by 6.54% and 5.00%, respectively. For these two parameters between the groups statistical analysis did not establish a significant difference ($p > 0,05$).

Table 3 Average values of body weight (g) and total body length (cm), 40 days of the experiment

Group	n	$\bar{X}$	min	max	$S_{\bar{X}}$	Sd	t- test
Average values of body weight							
K- group	20	115,94	108,20	124,30	1,040	4,649	p>0,05
O-1 group	20	118,04	113,50	127,60	0,950	4,248	
O-2 group	20	118,49	110,10	128,30	1,032	4,634	
Average values of total body length							
K- group	20	19,56	19,18	19,91	0,061	0,273	p>0,05
O-1 group	20	19,58	19,16	19,93	0,063	0,283	
O-2 group	20	19,59	19,15	19,95	0,066	0,287	

Obtained results are in agreement with the data by researchers who established that the addition of MOS to mixtures for various species of fish, has stimulating effect on the increase in the value of their growth and weight gain, but without statistical significance (*Binh et al., 2008; Dimitroglou et al. , 2011; Sara et al. 2011*). According to other researchers, who have examined the younger fish categories, the positive effects of adding MOS to fish food caused significantly better growth of examined fish (*Staykov et al., 2005; Čuljak et al. 2006; Ognean et al., 2009*).

Based on the obtained production results (Table 2) it can be concluded that the presence of MOS in the mixtures for fattening trout, O-I and O-II group of fish, caused realization of greater feed intake and better feed conversion value and the ratio of protein efficiency. The lowest total food consumption had fish in group K (9.06 kg), and the highest trout in O-II group (9.20 kg) and average daily feed

intake of fish in the O-II group was by 1.55%, and O-I group by 0.99% higher than in the K group.

In the analysis of feed conversion (FC) as the interaction of growth and food consumption, it is evident that the best value for this production parameter was achieved by O-II fish group (1.695), followed by O-I group (1.702), and the worst results of the value of this parameter were established for the fish group K (1.795). As for the relative values of utilization of food protein/protein efficiency (PER), it was found that the O-II group fish performed better for this parameter compared to the group K by 5.94%, while the O-I group of trout by 5.49% in comparison to the K group. Significance test for differences in determined average values for feed intake, conversion and utilization of proteins in food pointed to the absence of a statistically significant difference ($p > 0,05$).

The results in terms of feed conversion are in accordance with the results of *Hossu et al. (2005)* who found that the addition of MOS had positive impact on feed conversion in the experiment with gilthead (*Sparus aurata*), but without significant differences. Similar results have been presented by *Peterson et al. (2010)* in whose research the addition of MOS (0.2% and 0.4%) had no significant effect on feed conversion. However, the results obtained in present study are somewhat lower than the data of *Staykov et al. (2005),* who report that the addition of prebiotics to trout food induced increase of the efficiency of utilization of food by 9.01% and 10.16% ($p < 0,05$). Also, *Čuljak et al. (2006)* indicate that the addition of MOS of 0.6% to carp food contributed to a better feed conversion ratio values by 22.81% ($p < 0.05$) and PER by 22.49% ($p < 0 , 05$).

Average values of condition factor (FC) as an indicator of relations of body weight and total body length of fish, indicate that the groups of fish where the MOS was applied as a dietsupplement, achieved better value of the condition factor. Compared with the K group of fish, O-II group had better values of this parameter by 1.73%, and trout of O-I group by 1.50%, which justifies the above said statement that the application of 0.3% MOS in food achieved the best weight and length gain of the tested fish. The results are lower than the values presented by *Sara et al. (2011).* In their research, adding prebiotic group (0.2% + BioMos SelPlex NuPro 0.03% + 2%) led to improved FC by 5.23%.

According to the data presented in Table 2, it can be concluded that the fish of O-II group compared to K group achieved better values of fish specific growth rate (SGR) by 7.58% and O-I group of fish by 6.54%. Statistically significant differences between the groups were not observed ($p > 0,05$). The obtained results were in line with that of other researchers *Binh et al. (2008), Sarah et al. (2011),* while slightly lower in terms of significance were results reached by *Čuljak et al. (2006), Ognean et al. (2009).*

The value of production index (PI) ranged from 2.421 (K group) to 2,622 (O-II group). The highest value of this ratio was observed in fish of O-II group, and as a result more vitality and better feed conversion in this group.

During the experiment there were no disorders in the health status of fish, and the mortality in treated trout was relatively low (K group, 2 fish; O-I group, 1 fish, O-II group, 2 fish). Of the total number of fish with which the experiment was started, by the end of the trial 1.11%died, indicating a good condition of farmed fish and favorable environment conditions. Stocking index values ranged from 0.0044 (K group) to 0.0045 (O-I and O-II group), while flow index values ranged from 0.0556 (K group) to 0.0567 (O-II group). The recorded values of Ip and Ig are in accordance with standards of sanitation for raising trout (*Piper et al. 1982, Klontz, 1991*). From the results of fish mortality in this study, it can be concluded that the use of MOS in the mixtures had no effect on mortality, and that the applied stocking density and carried hygienic measures caused low percentage of deaths and good health of the fish.

Conclusion

Based on the conducted research, it can be concluded that the use of mannanoligo saccharides (MOS) added to mixtures for rainbow trout had a positive effect on the tested production results: morphometric indicators of fish growth rate, feed conversion, condition factor, protein efficiency ratio, specific growth rate and production index.

This especially relates to best production results achieved in the O-II group fish, which used MOS in concentration of 0.3%. Slightly lower values of recorded production parameters of fish in O-I group, which were fed mixture containing 0.2% MOS, while the lowest studied parameters were established in the K group of trout. Despite the favorable effect, the differences in average values of production parameters between studied groups were not statically significant ($p > 0.05$).

For this reason, there is a need for further research in order to find the optimal dose of mannanoligo saccharides as food additive, which would allow a wider application in intensive fish farming.

Uticaj mananoligosaharida na proizvodne osobine kalifornijske pastrmke

S. Obradović, B. Živković, Vera Đekić, M. Šekler, M. Živkov-Baloš, M. Marković

Rezime

Ispitivan je uticaj mananoligosaharida (MOS) kao aditiva hrane, primenjenog u koncentraciji od 0,2% (O-I grupa riba) i 0,3% (O-II grupa riba) na

morfometrijske karakteristike i osnovne proizvodne pokazatelje gajenja kalifornijske pastrmke. Ogled je sproveden na 450 riba podeljenih u tri grupe sa po 150 jedinki u svakoj grupi i trajao je 40 dana. Analizom dobijenih rezultata, ustanovljen je povoljan efekat primenjenog aditiva na morfometrijske pokazatelje tempa rasta riba, kako njihove završne komandne mase i završnih dužinskih mera, tako i njihovog prirasta, ali bez utvrđenih statistički značajnih razlika ($p>0,05$). Najveću prosečnu telesnu masu i dužinu tela kod ispitavanih grupa, ostvarile su pastrmke O-II grupe (118,49 g i 19,59 cm), zatim pastrmke O-I grupe (118,04 g i 19,58 cm), a najmanju K-grupa riba (115,94 g i 19,56 cm).

Dodavanje MOS-a u smeše za ishranu pastrmki je imalo povoljan uticaj ($p>0,05$) na ostvarivanje niže konverzije hrane (HK) i boljih vrednosti osnovnih proizvodnih pokazatelja: faktora kondicije (FK), koeficijenta proteinske efikasnosti (PER), specifične stope rasta (SGR) i proizvodnog indeksa (PI) u odnosu na ribe K grupe, koje su hranjene smešama bez dodatka mananoligosaharida. Najbolje rezultate su ostvarile ribe O-II grupe, a iskazano u relativnim pokazateljima u odnosu na K grupu riba: bolju konverziju hrane za 5,61%; bolju vrednost iskorišćenja proteina hrane za 5,94%; veću vrednost faktora kondicije za 1,73%; specifične stopa rasta za 2,26% i bolju vrednost proizvodnog indeksa za 8,27%.

References

ADAMS C.A. (2004): Nutricines in poultry production: focus on bioactive feed ingredients. Nutrition abstracts and reviews: Series B 74, Nutritional Services department, Kemin Europa, Belgium. 1-12.

AOAC - Association of Official Analytical Chemists (1990): Official Methods of Analysis. Washington, DC: AOAC. p. 246.

BINH V., TRUC N., HUNG L. (2008): Effects of BioMos (mannan oligosaccharide) on growth performances and fish health improvement of tra catfish (*Ictalurus punctatus*). Faculty of Fisheries. Nong Lam University Ho Chi Minh city. Vietnam. Presentation Alltech.

ČULJAK V., BOGUT I., HAS-SCHON E., MILAKOVIĆ Z., CANECKI K. (2006): Effect of Bio-Mos on performance and health of juvenile carp. Proceedings of Alltech's 22 Annual Symposium. Abstracts of posters presented Lexington. April 23-26, 2006. Lexington, KY, USA.

DIMITROGLOU A., REYNOLDS P., RAVNOY B., JOHNSEN F., SWEETMAN J.W. JOHANSEN J., DAVIES S. (2011): The Effect of Mannan Oligosaccharide Supplementation on Atlantic Salmon Smolts *(Salmo salar L.*) Fed Diets with High Levels of Plant Proteins. Journal of Aquaculture & Research Development. Special section S1-011., 1-6.

FERKET P.R., PARKS C.W., GRIMES J.L. (2002): Mannan oligosaccharides *versus* antibiotics for turkeys. Nutritional Biotechnology in the Feed and Food

Industries. Proc. of Alltech's 18th International Symposium. T. P. Lyons and K. A. Jacques, eds. Nottingham University Press, Nottingham, UK., 179-184.
HOSSU B., SALNUR S., GULTEPE N. (2005a): The effects of yeasts derivatives (Bio-Mos®) on digestibility of Gilthead sea bream (*Sparus aurata*). In: Nutritional Biotechnology in the Feed and Food Industries: Proceedings of Alltech's 21th Annual Symposium (Suppl., Abstracts of Posters presented). May 23-25, Lexington, KY, USA, pp 5.
HOSSU B., SALNUR S., GULTEPE N. (2005b): The effects of yeasts derivatives (Bio-Mos®) on growth of Gilthead sea bream (*Sparus aurata*). In: Nutritional Biotechnology in the Feed and Food Industries: Proceedings of Alltech's 21th Annual Symposium (Suppl., Abstracts of Posters presented). May 23-25, Lexington, KY, USA, pp 5.
KLONTZ G.W. (1991): Fish for the Future: Concepts and Methods of Intensive Aquaculture. Text Number 5, Idaho Forest, Wildlife and Range Experiment Station, University of Idaho., 28-39.
NRC (1993): Nutritien requirements of fish. National Academy Press. Washington DC.
OGNEAN L., BARBU A. (2009): The estimation of biostimulator potential of some fodder additives based on the main hematological and biometrical indices of brook trout (Salvelinus fontinalis M.). Annals of Romanian Society for cell biology. Vol. XIV, Issue 2., 292-296.
PETERSON C.b., BRAMBLE S.T., MANNING B.B. (2010): Effects of Bio-Moşon Growth and Survival of Channel Catfish Challenged with *Edwardsiella ictaluri*. Journal of the world aquaculture society. Vol. 41, No. 1, 149-155.
PHILIPS, A.M. (1970): Trout feeds and feeding. Manual of fish culture.U.S.Department of the Interior,Washington. DC., 1- 49.
PIPER, R.G., MCELWAIN I.B., ORME L.E., MCCRAREN J.P., FLOWER L.G., LEONARD J.R. (1982): Fish hatchery management. U.S. Fish and Wildlife Service, Washington, D.C. p.517.
PRAVILNIK O KVALITETU HRANE ZA ŽIVOTINJE (Službeni glasnik RS br.4/2010)
SARA A., BARBU A., GABOR E., BENTEA M. (2011): Researches regarding the combined fodder additives (Bio-Mos+Nupro+Selplex) effects on the productive performances and health of rainbow trout (Oncorhynchus Mykiss W.). Animal Science and Biotechnologies. 44 (2)., 61-66.
SPRING P., WENK C., DAWSON K.A., NEWMAN K.E. (2000): The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poult. Sci. 79., 205-211.
STAYKOV Y., DENEV S., SPRING P. (2005): Influence of dietary Bio-Mos on growth, survival and immune status of rainbow trout (Salmo gairdneri irideus G.) and common carp (*Cyprinus carpio L.*). Nutritional Biotechnology in the Feed and

Food Industries. Proceedings of Alltech's 21st Annual Symposium, Lexington, Kentucky, USA, 22-25 May 2005. Edition: First Edition, Publisher: Nottingham University Press, United Kingdom, 333-343.

Received 26 April 2013; accepted for publication 20 June 2013

Biotechnology in Animal Husbandry 29 (2), p 363-371 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.2.03
DOI: 10.2298/BAH1302363P

EVALUATION OF QUALITY OF SEMI-NATURAL GRASSLANDS OF CENTRAL SERBIA UPON PHYTOSOCIOLOGICAL AND NUMERICAL ANALYSIS

M. Petrović[1], S. Aćić[2], V. Zornić[1], B. Anđelković[1], Z. Dajić-Stevanović[2], S. Babić[1]

[1] Institute for forage crops, Globoder, 37251 Kruševac, Republic of Serbia
[2] Faculty of agriculture, Belgrade University, Nemanjina 6, 11080 Zemun - Beograd, Republic of Serbia
Corresponding author: mirjana.petrovic@ikbks.com
Original scientific paper

Abstract: The aim of this paper is to present a numeric-statistical model by which it is possible to evaluate the quality of the observed grassland upon floristic and vegetation assessment. Thanks to this new methodological approach, the impact of each individual plant species on overall quality of the grassland could be estimated. The main goal was to determine species which significantly determine the pastoral value of the grassland. The quality (pastoral value) of the grassland was calculated using the numerical values of quality index of each individual species of each relevé of the community. For testing this numerical method, the total of 11 relevés of 4 grassland communities of hilly-mountainous area of Mt. Kopaonik was used. Analyzed vegetation includes previously unpublished phytocenological relevés as result of our own field research. All analyzed relevés were obtained using the method of the Swiss-French phytocenological school. The results showed that the best and the worst quality were determined for the ass. *Festuco-Brometum erectii*, and the ass. *Nardetum strictae*, respectively. Species that highly contributed to good and bad quality of grassland were *Arrhenatherum elatius, Festuca rubra, Dactylis glomerata, Trifolium repens*, etc., and *Carduus alpestris, Hieracium hoppeanum, Ornithogalum umbelatum*, respectively

Key words: semi-natural grasslands, numerical index of grassland quality, numerical assessment of grassland quality, pastoral value, cluster analysis, multivariate analysis.

Introduction

Grasslands are one of the most important ecosystems. During the last century in developed countries their utilization shifted from weakly exploited areas into productive resources equal to any crop production (*Kemp and Michalk, 2007*). Considering their high distribution in Serbia, at about 1.4 million ha, grasslands have great importance both for grazing and biodiversity conservation, as indicated

that hilly-mountainous (semi) natural grasslands are the floristically richest vegetation units in the country (*Dajic Stevanovic et al., 2010*). Moreover, the most presented grassland communities of Serbia were also evaluated for high diversity and considerable stocks of medicinal and aromatic plants (*Dajić et al., 2000*).

Distribution, quality and botanical composition of pastures and meadows largely depend on the management, whereas excessive utilization, such as over-grazing and fertilization at one point, and abandonment from the other, cause dramatic effects on floristic and vegetation diversity (*Dajić Stevanovic et al., 2010*). Mt. Kopaonik, the largest in the central Serbia, is known for long tradition in use of semi-natural meadows and pastures, encompassing the height gradient between 450 and 2017 m a.s.l., above the upper forest zone, where form the final stage of vegetation formation. According to information gathered from local population, each of 11 studied grasslands was or is still used either for grazing or mowing. In order to evaluate the quality of meadows and pastures of the Mt. Kopaonik for forage production, researchers from the Institute for forage crops from Krusevac, Serbia, have been conducting numerous experiments starting from 1960's (*Đorđević and Radojević, 1968, Stošić 1973*), until today (*Lazarević et al., 2009*). The quality of grasslands was evaluated through chemical parameters of dry matter and botanical composition. Indirect evaluation of a grassland quality using pastoral value of each individual species was applied by different authors (*De Vries et al., 1942, with a scale of quality indices from 0 to10; Klapp et al. 1953, scale from -1 to 8; Jurko, 1990, scale from -3 to 5; Dajic Stevanovic et al., 2008, scale from -2 to 10, etc*). Upon application of numerical values as indicators of a quality of the grassland within its phytocoenological relevés, it is possible to obtain an indirect evaluation of the grassland quality and the impact of individual species on total pastoral value, which could facilitate decision-making in proper grassland's use, including application of various agricultural practices.

The aim of this study was the testing of numeric-statistical model applied on set of phytocoenological relevés of semi-natural grasslands of the Mt. Kopaonik to estimate the quality of each grassland community, as well as to evaluate the significance of particular species, mainly indicators of particular functional groups, within the total pastoral value of a community.

Materials and methods

The sampling of the phytocenological records was conducted in area of Mt. Kopaonik in central Serbia and was performed on 11 plant communities distributed at higher mountain zone, between 1400m and 1700m a.s.l. Phytocenological approach of Braun-Blanquet from 1964 was used, whereas species nomenclature followed "Flora of Serbia" (*Josifović, 1970-1977*). The collected relevés belong to the following grassland communities: *Agrostietum vulgaris* (relevés no. 3,4,5,6 and 9 in Table 1.), *Festucetum rubrae* (relevés no. 8 and 10 in Table 1.), *Festuco-Brometum erecti* (relevés no. 7 in Table 1.) and *Nardetum strictae sensu lato*. (relevés no. 1,2 and 11 in Table 1.)

Table 1: 11 phytosociological relevés, and 24 of 37 species which number of occurrences are greater than in four relevés . Quality of each grassland (releve), the quality of each single species, influence of each species on grassland quality, membership of species to clusters are shown.

Species	Phytocenologicel relevés											The quality of species	Species impact on grassland quality											Average species impact	Cluster
	1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11		
Agrostis vulgaris	0	0	3	2	3	3	0	0	2	1	1	4	0	0	22	7.4	13	16	0	0	11	2.4	3.8	10.9	I
Arrhenatherum elatius	1	1	0	0	1	+	1	0	1	0	0	8	7.8	7.7	0	0	5.9	2.7	6	0	9.7	0	0	6.6	II
Festuca rubra	1	1	2	2	0	1	0	2	+	1	1	4	2.3	2.2	8.8	7.4	0	1.7	0	6.8	0	2.4	3.8	4.4	II
Dactylis glomerata	0	0	+	+	+	1	0	+	0	1	+	8	0	0	3.1	2.9	2.3	6.8	0	2.9	0	8.4	4.4	4.4	II
Trifolium repens	+	+	+	1	0	0	0	0	0	+	0	8	3.1	3	3.1	7.4	0	0	0	0	0	3.3	0	4	II
Anthoxanthum odoratum	1	1	+	+	0	+	0	0	2	1	+	4	2.3	2.2	0.9	0.8	0	0.7	0	0	11	2.4	1.5	2.7	II
Lathyrus pratensis	+	+	0	0	0	0	0	+	+	+	+	6	2	1.9	0	0	0	0	0	1.8	2.5	2.1	2.9	2.2	II
Trifolium alpestre	0	0	0	0	+	+	1	+	0	+	0	6	0	0	0	0	1.4	1.7	3.6	1.8	0	2.1	0	2.1	II
Lotus corniculatus	+	+	0	0	0	+	+	0	0	0	+	6	2	1.9	0	0	0	1.7	1.4	0	0	0	2.9	2	II
Briza media	0	0	0	0	+	0	0	1	1	1	+	4	0	0	0	0	0.6	0	0	1.8	2.9	2.4	1.5	1.9	II
Leontodon hispidus	1	1	0	0	1	0	+	0	0	0	+	4	2.3	2.2	0	0	1.5	0	0.5	0	0	0	1.5	1.6	II
Vicia villosa	0	0	0	0	1	1	1	0	+	1	0	4	0	0	0	0	1.5	1.7	1.3	0	1.1	2.4	0	1.6	II
Veratrum album	0	0	0	+	+	1	0	0	1	1	1	-2	0	0	0	-2.4	-2	-6	0	0	-7.2	-6.5	-7.4	-5.3	III
Hypericum maculatum	1	0	+	1	0	0	0	0	1	0	+	-2	-6.1	0	-2.3	-6.1	0	0	0	0	-7.2	0	-2.9	-4.9	III
Genista sagittalis	2	2	1	+	0	+	1	1	0	+	1	0	-12	-12	-3	-1.3	0	-1.4	-3.4	-3.8	0	-1.4	-3.7	-4.7	III
Hypericum perforatum	0	1	0	0	0	+	+	+	0	+	0	-2	0	-5.9	0	0	0	-2.4	-2.2	-2.6	0	-2.5	0	-3.1	III
Nardus stricta	2	2	0	0	0	0	0	0	0	0	2	2	0.1	-1.9	0	0	0	0	0	0	0	0	-1.9	-1.8	IV
Bromus erectus	0	0	+	+	0	+	1	0	0	+	0	4	0	0	1.1	0.7	0	0.7	1.5	0	0	0	0	1	IV
Carduus alpestris	0	0	R	R	1	+	0	1	1	0	0	0	0	0	-0.6	-0.7	-3	-1.4	0	-3.8	-3.8	0	0	-1.4	IV
Hieracium hoppeanum	1	1	+	+	0	0	1	0	0	0	+	1	-1.9	-1.9	-0.7	-0.8	0	0	-2.2	0	0	0	-0.7	-2.2	IV
Ornithogalum umbella.	0	0	0	0	+	+	0	+	+	0	+	0	0	0	0	0	-1.2	-1.4	0	-1.5	-1.5	0	-1.4	-1.4	IV
Silene sendtneri	+	+	R	R	+	0	+	+	+	+	+	0	-1.3	-1.3	-0.6	-0.7	-1.2	0	-1.3	-1.5	-1.5	-1.4	-1.4	-1.4	IV
Campanula abietina	+	+	1	1	+	0	0	0	0	+	0	1	-0.8	-0.7	-1.7	-2	-0.7	0	0	0	0	-0.8	0	-1.2	IV
Viola tricolor v.mac.	0	0	+	1	0	+	0	0	+	0	+	1	0	0	-0.7	-2	0	-0.8	0	0	-0.8	0	-0.7	-1.1	IV
Thymus pulegioides	+	+	0	1	0	0	0	0	+	+	+	1	-0.8	-0.7	0	-2	0	0	0	0	-0.8	-0.8	-0.7	-1	IV
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1	IV
Grassland quality	**2.0**	**2.4**	**2.3**	**2.7**	**2.9**	**2.7**	**2.7**	**2.5**	**2.2**	**2.4**	**2.4**														

Values of species abundance (R, +, 1, 2, 3, 4 and 5) were transferred into its percentage equivalents (1, 2, 5, 17, 37, 62 and 87, respectively).

The quality of the grassland's species was assessed upon *Šoštarić-Pisarčić and Kovačević (1968)* by translating of their descriptive values into numerical system, and upon *Peeters and Dajić (2006)*. Such approach includes the scale from -2 to 10, with the lowest value for a poisonous plant and the highest for the best forage quality plant, respectively. Thus, each species of phytocoenological relevé was marked with number within this scale.

Based on the assessment of quality of individual species and abundance values, the overall quality of grassland (Q) was calculated by the following procedure: the number of species in observed grassland was indicated by n, species were marked with s_i ($i = 1, ..., n$). Value for land cover of the species s_i were indicated by c_i ($i = 1, ..., n$). The value for the quality of species s_i was indicated by q_i ($i = 1, ..., n$). Finally, the grassland quality was calculated by the following formula:

$$Q = \frac{\sum_{i=1}^{n} c_i \cdot q_i}{\max\left(\sum_{i=1}^{n} c_i, 100\right)}$$

where *max* denotes the larger of numbers $\sum_{i=1}^{n} c_i$ and 100. This way the division is done with the larger of those two numbers. For Q is $-2 \le Q \le 10$.

In addition to the overall quality of the grassland, the impact of individual species on its quality was calculated as well. The impact of species was calculated in two steps after determining the average total pastoral value of the grassland as described. The first step was to calculate the value of grassland quality without presence of the observed species. In the second step it was calculated the percentage of a change of grassland quality by removing the observed species.

If the grassland achieves a better quality by removing of the observed species, than that species has a negative impact. Otherwise, the observed species will exhibit a positive impact.

Such approach was modeled as follows:

Q_i marked the quality of the grassland generated by removing species s_i from phytosociological table. The impact of the species s_i was marked as I_i and calculated by formula:

$$I_i = \frac{Q - Q_i}{Q} \cdot 100$$

Based on the impact, the species were classified using k-means clustering method. By this method, a set of data (species) was divided into predetermined

number of clusters. In our analysis a set of species was divided into four clusters. In order to verify the validity of the results of cluster analysis, the principal component analysis (PCA) was also performed. PCA analysis was performed on species occurring at least in half of the total phyto-sociological records. These analyses were performed using the statistical package Statistica8 (*StatSoft, 2007*).

Results and Discussion

Phytocenological relevés were classified into the following associations: *Agrostietum vulgaris, Festucetum rubrae, Festuco-Brometum erecti and Nardetum strictae* (Tab. 1). In addition to coverage values of each individual species of the relevé, the quality value for each species is presented, as well as results for the quality of grasslands (Q), the impact on the quality of individual species on grassland (%), the average effect of the species calculated for all relevés, and species affiliation to particular cluster. Grassland quality is calculated using all species that occur in it. Of that number 37 species occur in four or more relevés, but 24 species are shown in Table 1. In Table 1, species *Nardus stricta* and *Bromus erectus* are also present as community edificator, although degree of their occurrence is less than four.

The obtained average values of the quality of individual grasslands ranged from 2 to 2.9. The lowest value was determined for the community *Nardetum strictae* **(**relevé no. 1 in Table 1), while the highest for the community *Agrostietum vulgaris* **(**relevé no. 5 in Table 1). Similar estimations for quality of these grasslands, but obtained upon measurements of yield of dry matter have been already reported (*Đorđević and Radojević, 1968*; *Peeters, 2004*).

Cluster analysis enabled grouping of species into four clusters (Tab. 1). The first cluster (Cluster 1) is made of only *Agrostis vulgaris* (the quality grade 4) as a species that has the greatest positive impact. Such high positive impact of a single species is here a consequence of its high coverage, since the quality index of the species is moderate. The second cluster (Cluster 2) consists of species *Festuca rubra* (4), *Anthoxanthum odoratum* (4), *Dactylis glomerata* (8), *Arrhenatherum elatius* (8), *Lathyrus pratensis* (6), *Trifolium repens* (8), *Briza media* (4), *Trifolium alpestre* (6) and others. These species with generally high marks for quality and significant abundance in the investigated associations have thus expressed the positive impact on the grassland quality.

The third cluster (Cluster 3) contains harmful and useless species which were abundant at surveyed sites, including *Genista sagittalis* (0), *Veratrum album* (-2), *Hypericum perforatum* (-2) and *Hypericum maculatum* (-2). The fourth cluster (Cluster 4) is made of species whose impact is more or less negligible, either due to their low abundance in the grasslands or due to their low quality value.

In case of the first grassland (relevé no. 1) whose quality was assessed as 2, it could be seen that obtained low quality value was depended mainly on high abundance of the *Hypericum maculatum* (lowering the average grassland quality for 6.1%) and *Veratrum album* (-12%), obviously having a negative impact on grassland quality (Tab. 1). However, in case of the fifth relevé, the contribution to general pastoral value was mostly depended on high proportion of *Agrostis vulgaris* (contributing for 13%) and *Arrhenatherum elatius* (5.9%), providing the highest determined grassland quality value (2.9).

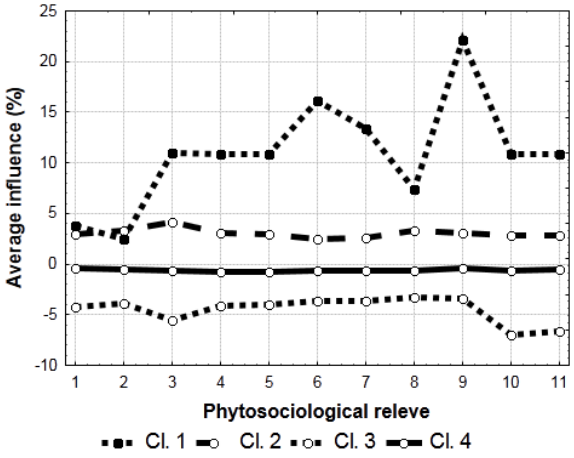


Figure 1: The average impact of the cluster members on each of the sites

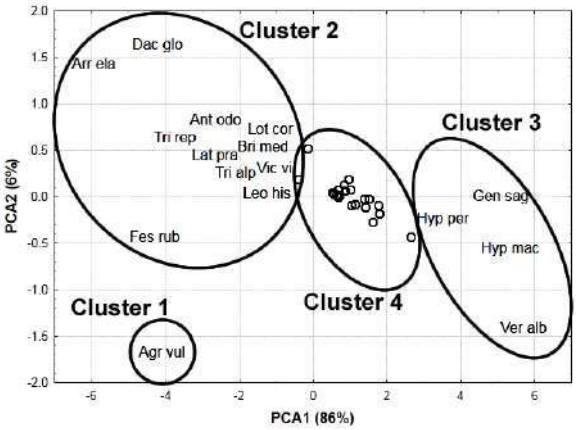


Figure 2: The principal components method. The names of species are presented by abbreviations, the first three letters of the name of the genus, the next three characters are the name of the species: Dac glo-*Dactylis glomerata*.Other names shown in Table 1.

Although communities with *Festuca rubra* i *Bromus erectus* are generally considered better, communities wih *Agrostis vulgaris* got here higher quality grade. It has appeared primarily due to high cover values of *Agrostis vulgaris*.

The average contribution of members of each cluster to the overall quality of the single grassland is presented (Fig. 1). Members of the first and the second cluster had, on average, a positive impact on each of the studied grasslands, while the impact of members of the third cluster was negative. The average impact of species of the fourth cluster was neither positive nor negative.

The results obtained using k-means clustering were confirmed using principal components analysis (PCA). It was shown that the first two axes cover 92% of the total variability of the data (Fig. 2). Species of a positive impact on the grassland quality are located on the left side, while species with a negative impact are grouped on the right side. Species that don't have a significant impact on the quality are located in the middle. The *Agrostis vulgaris*, which makes the cluster itself, is separated (lower left corner).

Conclusion

This paper presents a model for grassland quality estimation using numerical analysis approach in phytocenological data set. By this method, the evaluated quality of the studied grasslands of the Mt. Kopaonik ranged from 2 to 2.9. It was shown how each individual species contribute to the overall grassland quality upon its abundance and individual quality index. The classification method was assessed to identify the species that either increase or decrease the quality of the grassland in particular geographic region. Such modeling upon floristic composition and numerical analysis upon species abundance and individual quality index, it was possible to assess the value of grassland indirectly, which might be an useful approach for fast screening for sustainable livestock production.

Acknowledgment

Research was financed by the Ministry of Education and Science, Republic of Serbia, project TR 31057.

Procena kvaliteta polu-prirodnih travnjaka centralne Srbije korišćenjem fitocenoloških i numeričkih analiza

M. Petrović, S. Aćić, V. Zornić, B. Anđelković, Z. Dajić-Stevanović

Rezime

Travnjaci predstavljaju jedan od najznačajnijih prirodnih resursa; na teritoriji Srbije obuhvataju oko 1.400.000 ha. Koriste se kao izvor stočne hrane ali i kao resurs materija koje se primenjuju u farmaceutskoj industriji. Uzimajući u obzir njihov veliki značaj istraživanja na travnjacima u svrhu ispitivanja njihovog kvaliteta vrše se u Srbiji od 60-ih godina dvadesetog veka na ovamo. Cilj ovog rada je prikazivanje metode kojom se numerički ocenjuje kvalitet travnjaka, a pored toge se prikazuje i doprinos svake, pojedinačne vrste ukupnom kvalitetu. Doprinos vrste, koji je konkretan broj - pozitivan ili negativan, zavisi od njene pokrovnosti i numeričkog indeksa kvaliteta. Zahvaljujući dobijenim vrednostima doprinosa svake vrste kvalitetu izvršena je podela na klastere. To je najpre izvršeno metodom k-means clustering, zadata su četiri klastera, u prvom je samo jedna vrsta *Agrostis vulgaris*, u drugom su vrste koje pozitivno utiču na kvalitet, u trećem vrste koje negativno utiču na kvalitet a u četvrtom klasteru su neutralne vrste. Ova podela je proverena metodom glavnih komponenti, i na grafikonu je uočljivo razdvajanje vrsta na pomenute klastere.

Zahvaljujući ovoj metodi moguće je za kratko vreme dobiti podatke o kvalitetu nekog travnjaka i podatke o vrstama koje tu žive. Ti podaci o konkretnom travnjaku su dovoljni da se proceni njegova upotrebna vrednost kada je u pitanju stočarska proizvodnja a da se pritom izbegnu skupe hemijske analize.

References

DAJIC, Z., FABRI, S., MAKSIMOVIC, S. AND VRBNICANIN, S. (2000): Analysis of medicinal plants on meadows and pastures in Serbia. Proceedings of the First Conference on Medicinal and Aromatic Plants of Southeast European

DAJIĆ STEVANOVIĆ, Z., PEETERS, A., VRBNIČANIN, S., ŠOŠTARIĆ, I., AĆIĆ, S. (2008): Long term grassland vegetation changes: Case study Nature Park Stara Planina (Serbia). Community Ecology 9 (Supplement 1). 23-31

DAJIĆ-STEVANOVIĆ, Z., LAZAREVIĆ, D., PETROVIĆ, M., AĆIĆ, S. (2010): Biodiversity of natural grasslands of Serbia: state and prospects of utilization. XII International Symposium on Forage Crops of Republic of Serbia, Biotechnology in Animal Husbandry. 26, 1, 235-247.

De VRIES, D.M., t'HART, M.L., KRUJINE, A.A., (1942): Een waardeeing van grasland op grond van de plantkundige samenstelling. Landbouwkundig, Tijschrift, 54, 245-265.
ĐORĐEVIĆ, V., RADOJEVIĆ, D. (1968): Uticaj količina kompletnog đubrenja mineralnim đubrivima (NPK) u istom odnosu hraniva na produktivnost i kvalitet pašnjaka tipa Nardetum strictae. Zbornik naučnih radova, Vol II i III, 53-65.
JOSIFOVIĆ, M. (ed.) (1970-1977): Flora SR Srbije I-X. Srpska akademija nauka i umetnosti, Beograd.
JURKO, A. (1990): Ecological and socio-economic evaluation of vegetation (in Slovak). Priroda. Bratislava. ISBN 80-07-00391-6.
KEMP, D. R., AND MICHALK, D. L. (2007): Towards sustainable grassland and livestock management. Journal of Agricultural Science, 145, 543–564.
KLAPP, E., BOCKER, P., KONIG, F., STACHLIN, A. (1953): Wertzahlen der Grunlandpflanzen, Das Grunsland, 2, 5, 38-42.
LAZAREVIĆ D., STOŠIĆ M., DAJIĆ Z., TERZIĆ D., CVETKOVIĆ M. (2009): Productivity and quality of plant mass of meadow ass. Danthonietum calycinae depending on the fertilization and utilization time.. Biotechnology in animal husbandry, 25, Book 1, 133-142.
PEETERS, A. (2004): Wild and sown grasses. Published by Food and agriculture organization of the united natios and Blackwell publishing. Rome
PEETERS, A. AND DAJIĆ, Z. (2006): Grassland management study for the Stara Planina, Mt. Nature Park. Proposals of biodiversity restoration measures. Project report. Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia.
STATSOFT, INC. (2007). STATISTICA (data analysis software system), version 8.0. www.statsoft.com.
STOŠIĆ, M. (1973): Uticaj mineralnih đubriva na botanički sastav brdske livade tipa Danthonietum calycinae. Magistarski rad. Beograd.
ŠOŠTARIĆ-PISAČIĆ, K. I KOVAĆEVIĆ, J. (1968): Travnjačka flora i njena poljoprivredna vrednost. Znanje, Zagreb.

Received 26 March 2013; accepted for publication 22 June 2013

Biotechnology in Animal Husbandry 29 (2), p 373-383 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.312'62
DOI: 10.2298/BAH1302373T

SPATIAL DISTRIBUTIONS OF AIRBORNE DUST IN A COWS BARN EXPOSED TO INFLUENCE OF DIFFERENT VENTILATION RATES

G. Topisirović, D.V. Petrović, R. Maletić

University of Belgrade –Faculty of Agriculture, Nemanjina 6, 11080 Belgrade - Zemun, Republic of Serbia
Corresponding author: gogi@agrif.bg.ac.rs
Original scientific paper

Abstract: Information on the concentration of dust particles is an important microclimate parameter that characterizes the local environmental quality of each livestock building. Increased concentration of dust particles primarily affects the indoor air quality and, consequently, the animal and workers health. Among many others, ventilation rate is a vital parameter that controls the spatial distribution of airborne dust particles in livestock buildings. This was the main motive for authors of this paper to research the influence of rotation rate of under-roof axial fans (i.e. the air flow rate) on airborne dust particles distribution crossover the barn specified for tied cows breeding. During a series of performed experiments, six different air flow rates have been maintained in the range between 0 $m^3 \cdot h^{-1}$ and 48000 $m^3 \cdot h^{-1}$. Flow rate has been controlled by special electronic control unit, which provided six different rotation rates of two under-roof fans, including the neutral regime (natural ventilation only). Measurements have been performed at four typical height levels (0,5 m; 1,0 m; 1,5 m and 2,0 m), cross-over the three lateral and four longitudinal characteristic building sections. Consequently, 48 measuring points were appropriately selected, in order to cover the indoor space in adequate way. Comparative analysis of air flow velocities and dust concentrations showed that this fan setup may give satisfactory results under adequate operational regime. Certain working regimes were recommended for use, and the third rotation rate step, generating the airflow of 37300 $m^3 \cdot h^{-1}$ or indoor air exchange level of approximately 25 h^{-1}, has been found as the most suitable.

Key words: fan flow rate, airflow velocity, airborne dust, livestock buildings.

Introduction

The term “airborne dust” commonly represents solid particles having a diameter up to 100 μm, which are suspended in the air. Within the total (inhalable) dust content, fraction of respirable dust should be distinguished and analysed separately, because it contains very small particles characterized by diameters less than 5 μm (*Jacobson, 2007*). Dust particles of this kind are of special engineers interest because of their capability of deep penetration in the lungs. In opposite,

larger particles are less dangerous because most of them are retained in the upper parts of the respiratory tract. The origin of dust in livestock buildings is primarily organic. It is most commonly produced from the animal body surface (dried skin particles), bedding, dried feces and concentrated feeds (*Wang et al., 2000*).

It has been recognized worldwide that an increased level of airborne dust concentrations may cause a variety of different health problems of breeding animals and working people. Consequently, the animal's and employee's productivity could be seriously decreased. However, recommendations for allowable concentrations of dust are given with respect to the human sensitivity. Nowadays, the most commonly accepted value of allowed concentration is 10 $mg \cdot m^{-3}$ for total and 5 $mg \cdot m^{-3}$ for respirable dust. In order to prevent the occurrence of long-term health problems, much more stringent limit has been recommended in the past: only 2.5 $mg \cdot m^{-3}$ for total and 0.2 $mg \cdot m^{-3}$ for respirable dust (*Jacobson et al., 2008*).

So far, a wide variety of research attempts related to enriching and verification of the available data bases and/or modeling the governing parameters of various production processes in animal husbandry have been made (see *Pearson and Sharples 1995*, *Petrović and Topisirović 1997*, *Petrović et al. 1998*, *Takai et al. 1998, Petrović et al. 1999, Jacobson et al. 2008, Topisirović and Djurić, 2008, Bartzanas et al. 2010,* among many others). Following this practice, this study analyzes the influence of multi regime roof fans flow rate on the concentration of particular airborne dust fractions. On the base of performed experiments, recommendations on fan usage and settings are given.

Material and Methods

In this study are presented experimental results of researching the effect of De Laval ventilation system Multifan with control unit STD (Manual 8 A thermostatic controller T15 - WD and DF 1300 fans) on the spatial distribution of airborne dust particles concentration.

This system can operate at six different rotation rate regimes. Three axial fans of indoor type, are located below the roof, but above the feeding alley. Maximum fan capacity is 48000 $m^3 \cdot h^{-1}$, at negligible pressure difference and the maximum rotation speed of 400 rpm. Measurements in the experimental cows barn are carried out at 48 measurement points (Fig. 1). These points were arranged in three lateral sections, with 4 vertical rows in each section, and the four height levels (0,5 m; 1,0 m; 1,5 m and 2,0 m). Lateral measuring sections were placed in 3 distinctive parts, as it is presented in Fig.1. The first section was set at 3.30 m from the front door to the feeding alley on the north side, while the other two lateral sections were placed in a way to provide the distance of 14.90 m between any of two subsequent sections. This way, the fans influence zones are covered. Vertical arrays are placed symmetrically above the feeding places and manure channels (*Curtis et al., 1996*). Height levels are at 50, 100, 150 and 200 cm, with the same goal as in the previous case. Concentrations of dust fraction ≤ 3 μm and ≤ 5 μm have been measured under carefully controlled indoor conditions. The

measurements have been performed for five different fan operating regimes and neutral regime (natural ventilation).

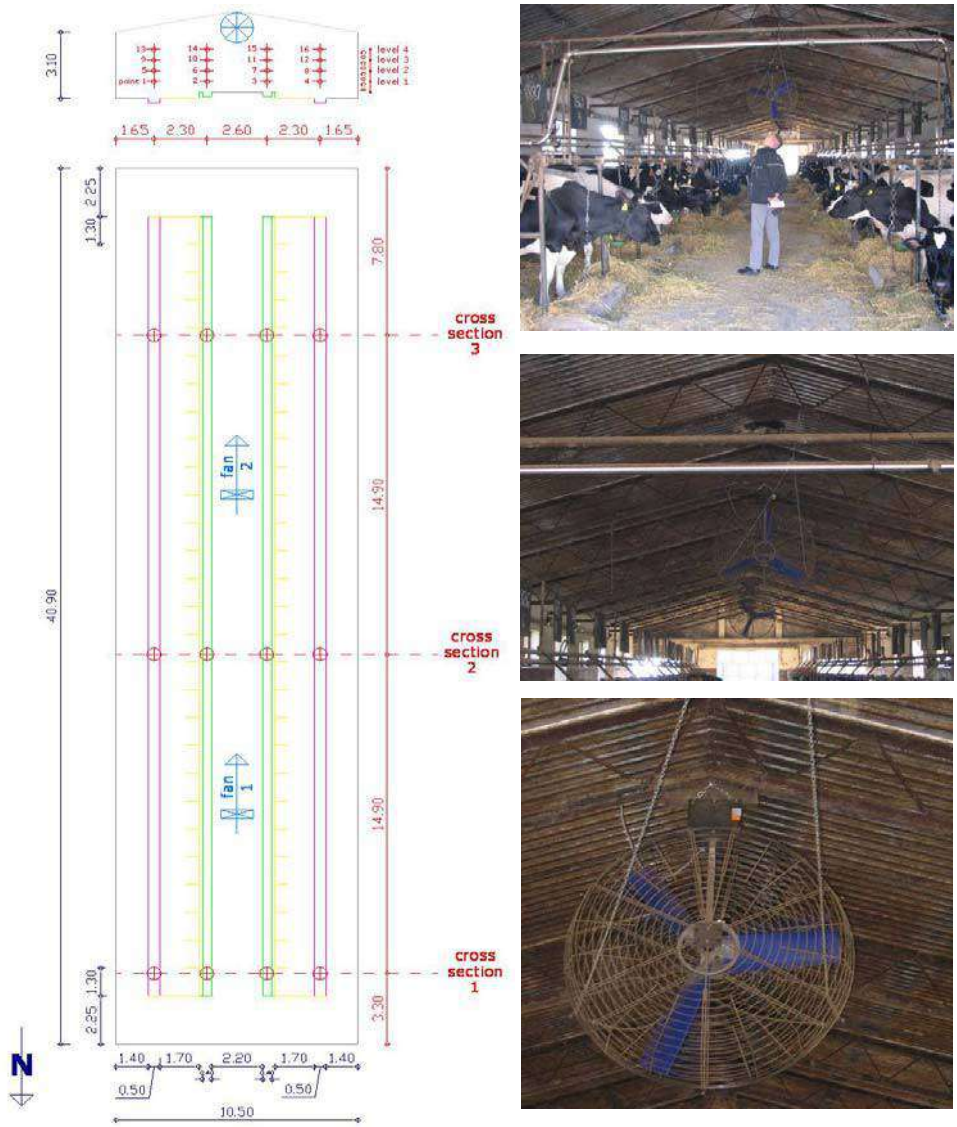


Figure 1. Building layout and measuring points setup (left) and photos of indoor ambient (right)

Results and discussion

During the first measurement series of dust concentration fans were switched off. In those conditions, concentrations of dust particles in different parts

of the experimental cow barn depend on natural ventilation, i.e. on the natural air flow and air humidity. Consequently, concentrations of dust fraction of up to 5 μm were higher compared to corresponding concentrations when roof fans were in any operating regime (Figures 2a to 5a, respectively).

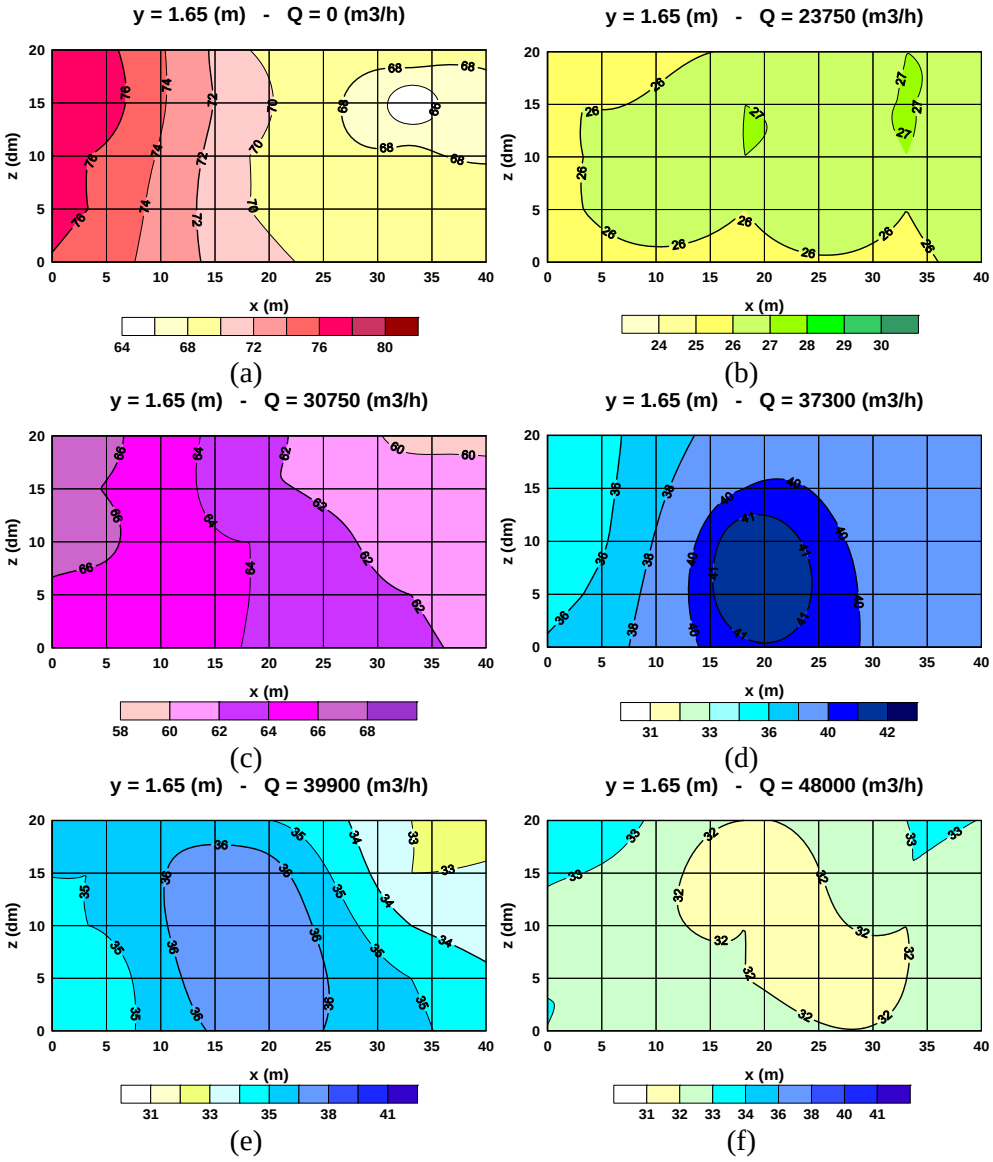


Figure 2. Distributions of 5 μm dust fraction concentrations along the first longitudinal cross section at 3,95 m from the eastern wall of the barn, under different operating regimes of roof fans

Concentrations were fairly uniform over the object volume, which confirms stationary state of air velocity fields during the absence of forced ventilation. Some deviations of the dust concentration field from this rule, that were rarely recorded, are caused by cows activity, different bedding composition, distribution of concentrated feeds etc.

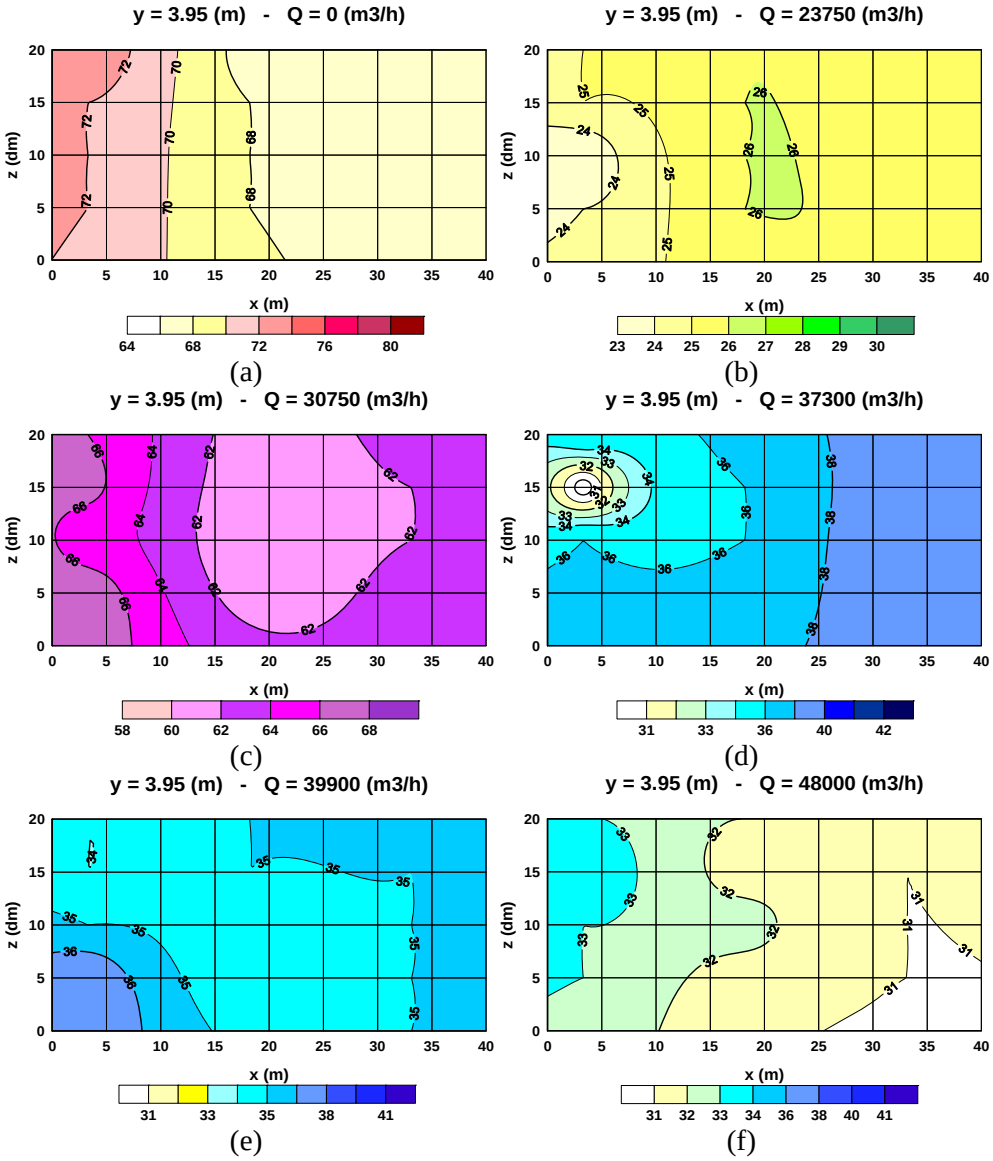


Figure 3. Distribution of 5 µm dust fraction concentrations along the second longitudinal cross section, at 3,95 m from the eastern wall of the barn, under different operating regimes of roof fans

The second series of measurements was performed under the controlled fans nominal air flow of 23750 $m^3 \cdot h^{-1}$. This operation regime resulted in the lowest airborne dust concentration, with respect to all other operating regimes (Figures 2b to 5b, respectively). In this mode, fans provide adequate air exchange, and acceptable air flow velocity, that does not re-suspend dust sediment from the floor and other surfaces.

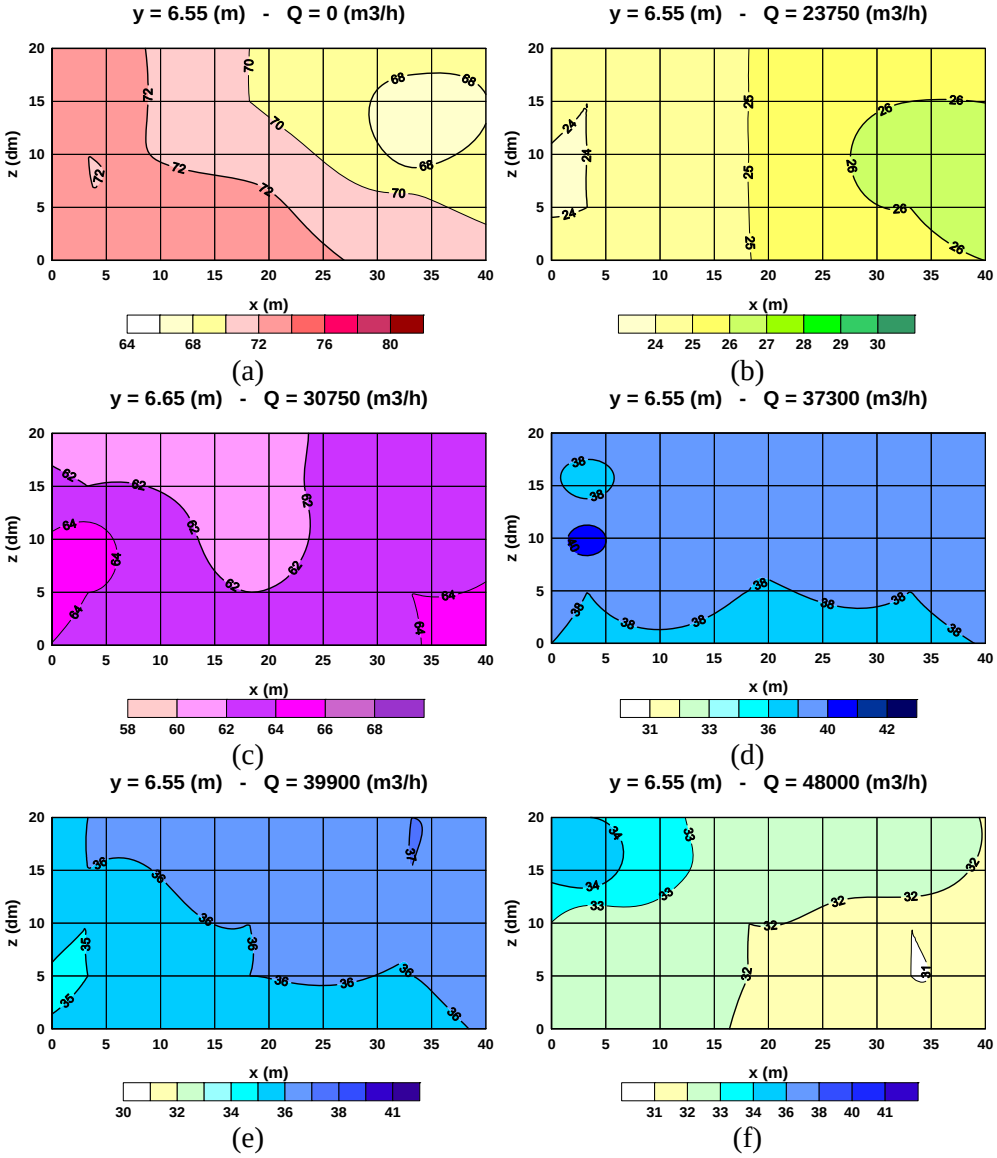


Figure 4. Distribution of 5 µm dust fraction concentrations along the third longitudinal cross section, at 6,55 m from the eastern wall of the barn, under different operating regimes of roof fans

All those issues resulted in reduced airborne dust concentrations in the stable, compared to other fans operating regimes - the average concentration of dust fraction with ≤ 5 µm in diameter decreased from 72 particles·ml^{-1} (natural ventilation, Q = 0 m^3·h^{-1} - Fig. 3a) to only 26 particles·ml^{-1} (forced ventilation, Q = 23750 m^3·h^{-1} - Fig. 3b), i.e. by 64%.

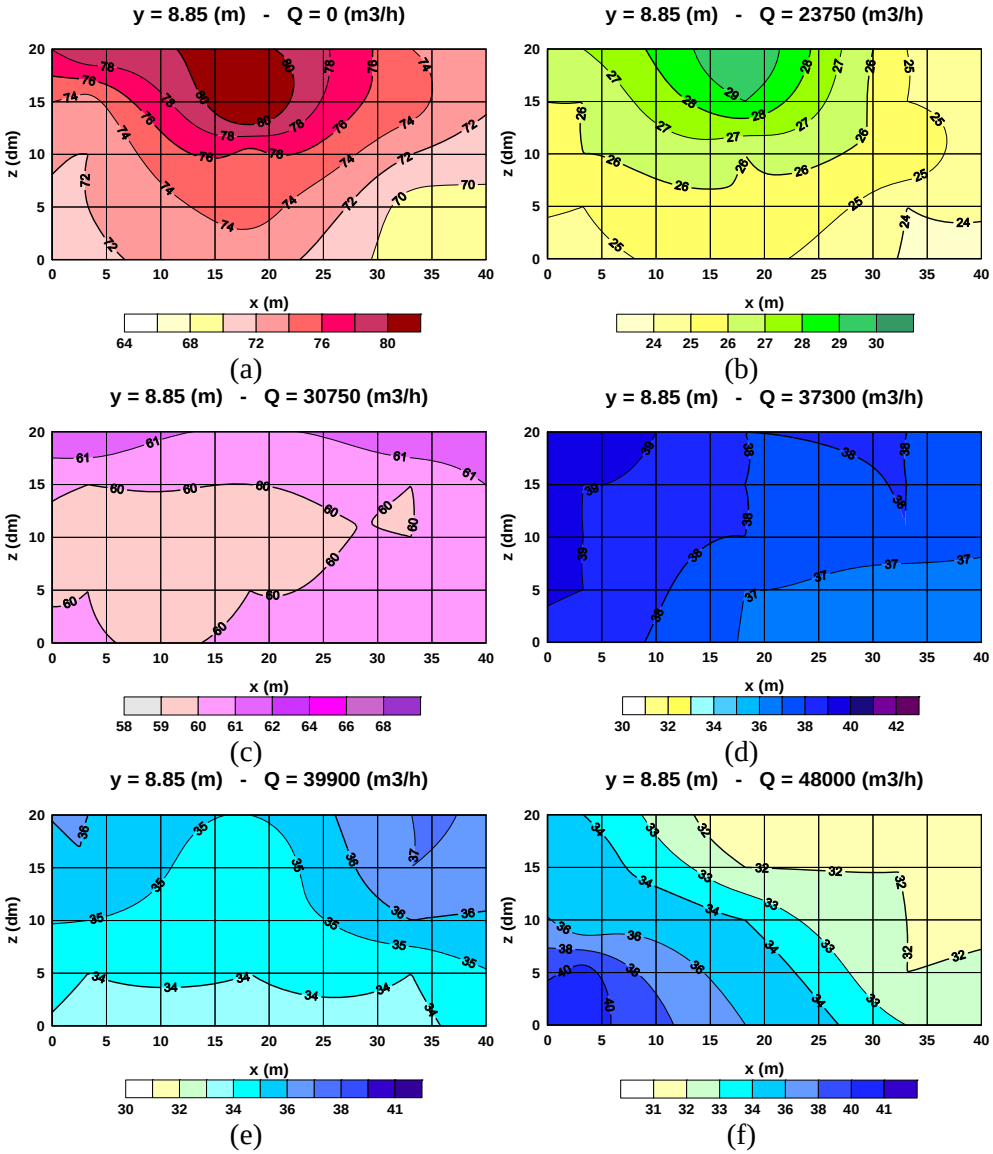


Figure 5. Distribution of 5 µm dust fraction concentrations along the fourth longitudinal cross section, at 8,85 m from the eastern wall of the barn, under different operating regimes of roof fans

The third series of measurements comprehended 2nd fan operating regime with nominal air flow rate of 30750 $m^3 \cdot h^{-1}$. The highest concentrations of dust particles in the experimental facility were recorded (Figures 2c to 5c, respectively) in this regime, compared to all the other regimes (excluding switched of fans). It can be expected that dust concentration would additionally decrease after a longer fan operation.

During the fourth (Figures 2d to 5d, respectively) *and fifth* (Figures 2e to 7e, respectively) *series of measurements*, that included 3rd and 4th fan operating regime (respectively) with nominal flow rates of 37300 $m^3 \cdot h^{-1}$ and 39900 $m^3 \cdot h^{-1}$ (respectively), reduction of concentrations of both dust fractions were observed. Fairly uniform dust concentration distributions were established in the barn. In these operating regimes, convective transport (exhaust) of dust particles exceeds its introduction into air flow as a result of settled particles raising from the floor and other surfaces within the facility.

The last, sixth series of measurements included 5th fan operating regime with maximum air flow of 48000 $m^3 \cdot h^{-1}$. The concentrations of airborne dust particles began to rise again (Figures 2f to 5f, respectively), as a result of intensive dust re-suspending from bedding, due to the increased airflow velocity in the house.

After comparison of airflow velocities and dust concentrations under different fan operating regimes, it can be concluded that, for air exchange in summer conditions, the optimal operating regimes are primarily 1st (23750 $m^3 \cdot h^{-1}$), and then 3rd (37300 $m^3 \cdot h^{-1}$) and 4th (39900 $m^3 \cdot h^{-1}$). In these three cases, along with optimal airflow velocities, the best effects of subjective feeling were achieved by intensive body heat drawing, while achieving the lowest concentration of both dust fractions.

Conclusion

Careful and detailed analysis of measurement results of dust concentration in the cow barn, with respect to the optimal fan airflow capacity requested in such buildings, it may be concluded that the best effects in dust concentration reduction were achieved by the lower fan rotation speeds. The first operational regime (23750 $m^3 \cdot h^{-1}$) of the ventilation systems is particularly favorable, but also acceptable are the third (37300 $m^3 \cdot h^{-1}$) and the fourth (39900 $m^3 \cdot h^{-1}$) regime. In those conditions very stable and effective dust concentration reduction was achieved, with airflow velocities that are within the optimal intervals for summer conditions (0.2 - 0.9 $m \cdot s^{-1}$).

Acknowledgements

The study was supported by the Ministry of Science and Technological Development of Serbia, under the projects:

- Optimization of technological procedures and zoo-technical resources on farms in purpose of milk production sustainability improvement (TR31086),
- Improvement of biotechnological procedures as a function of rational utilization of energy, agricultural products productivity and quality increase (TR 31051),
- Improvement and development new technological procedures in production of animal products, to achieve high quality and safe competitive products in market (III46009),
- Dynamical stability and instability of mechanical systems exposed to stochastic disturbances (OI 174011).

Raspodela koncentracija prašine u vazduhu objekta za krave pri različitim režimima ventilacije

G. Topisirović, D.V. Petrović, R. Maletić

Rezime

Koncentracija čestica prašine je je važan mikroklimatski parametar koji karakteriše kvalitet ambijenta svakog stočarskog objekta. Povećana koncentracija prašine primarno utiče na kvalitet stajskog vazduha, a time i na zdravstveno stanje životinja i ljudi. Pored ostalih, intenzitet ventilacije je značajan parametar u kontroli prostorne raspodele koncentracija prašine u stočarskim objektima. Ovo je bio motiv autora da sprovedu istraživanje uticaja režima rada krovnih aksijalnih ventilatora (tj. intenziteta strujanja vazduha) na raspodelu koncentracija prašine u staji za vezao držanje krava. Tokom izvođenja ogleda ispitivan je uticaj šest različitih intenziteta strujanja vazduha u opsegu od 0 $m^3 \cdot h^{-1}$ do 48000 $m^3 \cdot h^{-1}$. Režimom rada ventilatora je upravljao elektronski kontrolni uređaj, kojim je određivano šest različitih brojeva obrtaja za dva krovna ventilatora, uključujući i neutralni režim (samo prirodna ventilacija). Koncentracija je merena na četiri visine (0,5 m; 1,0 m; 1,5 m i 2,0 m iznad poda), po tri poprečna i četiri podužna preseka objekta. Tako je dobijeno 48 pravilno raspoređenih mernih tačaka, koje su ravnomerno pokrivale unutrašnji prostor staje. Komparativnom analizom brzina strujanja vazduha i koncentracija prašine zaključeno je da ispitivani položaj ventilatora može da da zadovoljavajuće rezultate pri odgovarajućem režimu rada. Preporučeni su najpovoljniji radni režimi za upotrebu. Kao najpovoljniji izdvojen

je treći ispitivani režim sa protokom vazduha od 37300 $m^3 \cdot h^{-1}$, odnosno 25 h^{-1} ostvarenih izmena vazduha.

References

BARTZANAS T., BAXEVANOU C., FIDAROS D., PAPANASTASIOU D., C.KITTAS C. (2010): Airborne particles and microclimate distribution in a livestock building. International Conference on Agricultural Engineering AgEng - Toward Environmental Technologies, Clermont-Ferrand (France), September 6-8, 2010, p. 1-9, itema.cereteth.gr/files/2010/07/B01-2010.pdf .

CURTIS L, RAYMOND S., CLARKE A. (1996): Dust and ammonia in horse stalls with different ventilation rates and bedding. Aerobiologia, 12, 4, 239-247.

JACOBSON L.D. (2007): Animal Structures: Air Quality (in: Encyclopedia of Agricultural, Food, and Biological Engineering). Taylor & Francis, Oxford, UK.

JACOBSON L.D., HETCHLER B.P., JANNI K.A., LINN J., HEBER A., CORTUS E. (2008): Animal and environmental performance of a retrofitted mechanical cross-ventilation system to a naturally ventilated free stall dairy barn in the mid-west U.S. Livestock Environment VIII, 31 August - 4 September 2008, Iguassu Falls, Brazil 701, 408.

PEARSON C.C., SHARPLES T.J. (1995): Airborne Dust Concentrations in Livestock Buildings and the Effect of Feed. Journal of Agricultural Engineering Research, 60, 3, 1995, 145–154.

PETROVIĆ D., TOPISIROVIĆ G. (1997): Modeling the airborne dust particle size distribution in a swine farrow room. Agricultural Engineering, YU ISSN 0354-8457, UDC 631(05), 3, 3-4, 57-63.

PETROVIĆ D., TOŠIĆ M., TOPISIROVIĆ G. (1999): On the statistical prediction of water requirements in the milk cows barns. Beitrage zur “4. Internationalen Tagung “Bau, Technik und Umwelt in der Landwirtschaftlichen Nutztierhaltung”, München, 9.-10. März 1999, 467-470.

PETROVIĆ D., TOPISIROVIĆ G., SCHÖN H., TOŠIĆ M. (1998): Turbulent velocity field measurement in the air of swine housing buildings. Zbornik apstrakta naučno-stručnog skupa “Dan poljoprivredne tehnike – Informacione tehnologije i razvoj poljoprivredne tehnike", Beograd-Zemun. 101-102.

TAKAI H., PEDERSEN S., JOHNSEN J.O., METZ J.H.M., GROOT KOERKAMP P.W.G., UENK G.H., PHILLIPS V.R., HOLDEN M.R., SNEATH R.W., SHORT J.L., WHITE R.P., HARTUNG J., SEEDORF J., SCHROEDER M., LINKERT K.H., WATHES C.M. (1998): Concentrations and Emissions of Airborne Dust in Livestock Buildings in Northern Europe. J. agric. Engng Res. 70, 59-77.

TOPISIROVIĆ G., ĐURIĆ E. (2008): Numeričko predviđanje strujnog polja pri prirodnoj ventilaciji stočarskih objekata. Poljoprivredna tehnika, god. XXXIII, br. 3. 41 - 47. Poljoprivredni fakultet. Beograd.
WANG X., ZHANG Y., ZHAO L.Y., RISKOWSKI G.L. (2000): Effect of ventilation rate on dust spatial distribution in a mechanically ventilated airspace. Transactions of the ASAE., 43(6), 1877-1884.

Received 7 February 2013; accepted for publication 13 March 2013

Biotechnology in Animal Husbandry 29 (2), p 385-397 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.052'62
DOI: 10.2298/BAH1302385A

EFFECTS OF CROSSING OF DOMESTIC BREED WITH BEEF BREEDS ON THE QUALITY OF MEAT IN PR CHINA AND REPUBLIC OF SERBIA

S. Aleksić[1], Sun Fang[2], Liu Di[3] M.M. Petrović[1], V. Pantelić[1], N. Stanišić[1], D. Ostojić-Andrić[1], M. Petričević[1], D. Nikšić[1], N. Delić[1]

[1] Institute for Animal Husbandry, 11080 Belgrade, Republic of Serbia
[2] Institute of Animal Husbandry, Heilongjiang Academy of Agricultural Sciences, Harbin, PR China
[3] Heilongjiang Academy of Agriculture Science, Harbin, China
Corresponding author: saleksic@mail.com
Communication

Abstract: This paper presents the results of crossing Domestic Spotted breed with beef cattle breeds in the People's Republic of China and the Republic of Serbia. China is a big country of beef production and consumption. In 2012, beef production in China was 5,540,000 tons, which accounted for 9.7% of the global beef production, ranking the third in the world. The main sources of China's beef are from crossbreeding cattle (native breed crossbred with foreign beef cattle). Simmental cattle are the most-widely used beef cattle in China's improved beef cattle. China has cultivated its own Simmental after over 40 years' crossbreeding and improvement. China's consumers mainly have three demands for beef quality as follows: expensive beef produced from Wagyu crossbreed with better marbling; lean beef from Simmental, Charolais and Limousin crossbreeds, top parts supplied to hotels while common parts to supermarkets; veal from cow calves. Chinese researchers are carrying out researches which are centered on marbling beef, lean beef and veal on complete techniques of good breed, feeding management, slaughter and cutting and carcass classification. At present, researchers have lively interest in functional genomics of meat quality traits of cattle, they expect to use these methods to study meat quality traits and then improve the meat quality. Improved cattle breeds universally utilized in China are mainly Simmental followed by Charolais, Limousin, Wagyu and Angus. Other three cattle breeds including Belgian blue cattle, Piedmontese and Gelbvien have ever been applied, however, rarely used in present beef cattle production. Republic of Serbia has in the future to quickly and efficiently provide adequate quantities of top quality meat. One of the ways to increase the yield and quality of the meat is crossing of Domestic spotted cattle of lower production traits with French beef cattle breeds. Beef production in the EU is adapted to the consumer taste. Meat must have a light red colour, equally suffused with fat and with pronounced sensory characteristics

such as tenderness, juiciness, flavour and aroma. The results on the quality of meat of F1 generation crosses (Domestic Spotted breed with French beef cattle breeds Charolais and Limousine) indicate that by industrial crossing beef, meat-packing and organoleptic characteristics of meat can be improved.

Key words: cattle breeding, crossing, meat quality, the People's Republic of China, Republic of Serbia

Introduction

China is a big country of beef production and consumption. In 2012, beef production in China was 5,540,000 tons, which accounted for 9.7% of the global beef production, ranking the third in the world; China, ranking as the fourth largest beef consumer, consumed 5,524,000 tons of beef in 2012, which was 9.95% of the global beef consumption. In spite of this, China's Per-capita consumption of beef is 4.2 kg, ranking 26th all over the world (USDA, World Markets and Trade, In selected countries). Compared to 2011, China turned net export into net import of 49,204.13 tons of beef in 2012, and imported 61,404.41 tons of beef with the average price of $4,146.70/ ton. Due to the BSE in Brazil, China greatly increased beef import from Australia, and it will become the fourth largest beef exporter of Australia on June 30, 2013, with the import skipping from 6,000 tons one year ago to 50,000 tons according to the statistics given by a governmental research organization called the Australian Bureau of Resource Economics and Science. In 2012, China exported 12,200.29 tons of beef with the price of $6,582/ton (From: Integrated Information Network of China Customs - www.haiguan.info). The beef cattle slaughtered in China in 2012 was 24,000,000; year-end beef cattle stock was 65,000,000; the average carcass weight of crossbreeding cattle was 217 kg/head and that of yellow cattle (native breed) was 190 kg/head; yaks slaughtered was 2,070,000; year-end yak stock was 19,800,000; the average carcass weight of yaks was 120 kg/head (Cao Bing-hai, the chief scientist of China's cattle and yak industrial technology system).

Keeping in mind the needs of the developed countries to import high-quality beef, Republic of Serbia has in the future to quickly and efficiently provide adequate quantities of top quality meat. One of the ways to increase the yield and quality of the meat is crossing of Domestic spotted cattle of lower production traits with French beef cattle breeds (*Čobić et al., 1990; Aleksić et al., 1995, 1998,1999* and *Cepin, 2001*). Given that the population of Domestic Spotted cattle of lower production performance is a significant part of the population the total cattle population of Serbia, by breeding/improving this population, significant results in the production of quality beef would be achieved.

Beef production in the EU is adapted to the consumer taste. Meat must have a light red colour, equally suffused with fat and with pronounced sensory characteristics such as tenderness, juiciness, flavour and aroma (*Aleksić et al., 2001,2005,2006,2009*). EU member states exert a unified classification by so-called SEUROP system according the development of adipose tissue (1-5) (*Ostojić-Andrić et al., 2012*). Factors affecting the quality of meat can be divided into pre mortal *Dikcman et al.* (2005) (genetic basis, housing, preparation for slaughtering, etc.) and post mortal (slaughtering procedure and cooling of meat) *Thompson* (2002). Research indicates small differences in sensory traits of meat from different genotypes, and authors explanations is that they are mainly caused by the physiological age, since different breeds of the same age don't have the same slaughter maturity (*Harrington, 1985, Bucher, 1985*).

PR CHINA- Meat quality (crossing domestic breed x beef breeds)

China beef production and trade in 2012

The application of world's beef cattle breeds introduced to China

The main sources of China's beef are from crossbreeding cattle (native breed crossbred with foreign beef cattle), yaks, water buffalo in the south, and southern yellow cattle and Holstein bulls are becoming new sources supplying China beef. Improved cattle breeds universally utilized in China are mainly Simmental followed by Charolais, Limousin, Wagyu and Angus. Other three cattle breeds including Belgain blue cattle, Piedmontese and Gelbvien have ever been applied, however, rarely used in present beef cattle production.

Research on beef quality in China

Research process in functional genomics of meat quality traits of cattle

With the rapid development of China's economy, Chinese consumers have a fast growing beef consumption and higher demand for beef quality. The important indicators which affect the beef quality are marbling and tenderness, and those with good marbling and tenderness are favored by consumers. Studies have proved that beef marbling is highly related to beef tenderness and flavor, it can increase the tenderness, juiciness and flavor. At present, researchers have lively interest in functional genomics of meat quality traits of cattle, they expect to use these methods to study meat quality traits and then improve the meat quality.

Zhou Guoli et al.(2005) showed that HaeIII-RFLP of a mutated enzyme cutting site on intron 2 was found in H-FAPB gene of Luxi cattle (native beef cattle in Shandong province) by PCR-RFLP method. By applying least square principle

linear model to conduct a significance test for effect of molecular marker genotypes on meat quality including marbling grade and tenderness trait, the results showed that BB homozygous genotype in H-FABP gene of Luxi cattle had a greater impact on shearing force of beef tenderness ($P < 0.05$); while, differences among three genotypes AA, AB and BB were not significant for the grading of marbling.

A study by *Wen Li-zheng et al. (2008*) proved that Heart Fatty acid-binding proteins (H-FABP) could be used as candidate gene of improving the tenderness of red steppe cattle or QTL of meat traits. Another study by *Wen Li-zheng et al. (2008)* showed that H-FABP gene had a significant influence on the tenderness of red steppe cattle, and it could be a major gene affecting its tenderness.

Determination on Beef quality index

Research on beef quality of different breeds

In June, 2006, *Zhou Lei et al. (2007)* conducted an experiment to study the longissimus dorsi between 11-13 ribs of left half carcass of six cattle, including Holstein cattle, Holstein crossbreed (Holstein×Xinjiang brown cattle), yellow cattle, Xinjiang brown crossbreed (Xinjiang brown cattle×yellow cattle), Xinjiang brown cattle and Simmental crossbreed (Simmental×Xinjiang brown cattle). The determination was including myoglobin, water binding capacity, cooking loss, shearing force, collagen, moisture content, crude protein, intramuscular fat content. Results reflected that the flesh color, tenderness, protein, intramuscular fat content and other beef quality indexes of Xinjiang brown cattle were better than the other five breeds. Xinjiang brown cattle's crossbreeding with Holstein cattle and yellow cattle respectively made the shearing force value and collagen content respectively decrease by 1.26%, 6.31% and 5.34%, 8.57%; protein and intramuscular fat content respectively increased by 2.65%, 2.32% and 7.87%, 27.83%. Myoglobin content respectively increased by 5.36% and decreased by 8.20%. Xinjiang brown cattle had better improved beef quality of Holstein and yellow cattle. Detailed figures are shown in table 1.

Simmental cattle are the most-widely used beef cattle in China's improved beef cattle. China has cultivated its own Simmental after over 40 years' crossbreeding and improvement.

Niu Lei et al. (2011) conduced an experiment to assess the beef qualities of different parts on Chinese Simmental. They chose twelve 18-month Chinese Simmental cattle and slaughtered, took four parts including supraspinatus, *longissimus dorsi*, *psoas major muscle* and s*emitendinosus* on the left half-carcass of each cattle and chilled at 4 ℃ for 42-hour, and then conducted assessment for their physical indexes (cooking loss, shearing force, pH and flesh color), nutritional qualities (fat, protein and water content) and sensory evaluations (tenderness,

juiciness and flavor). SPSS17.0 statistical analysis software was utilized with methods of LSD and Duncan in order to do variance analysis.

Table 1 Results of physical and chemical beef quality traits from six beef breed

	Holstein	Xinjiang Brown × Holstein	Yellow Cattl e	Xinjiang Brown ×Yellow Cattle	Xinjiang Brown	Xinjiang Brown × Simmental
Myoglobin(umol/g)	0.112±0.002^{abc}	0.118±0.007^{abc}	0.122±0.007^{a}	0.112±0.004^{abc}	0.104±0.002^{bc}	0.100±0.008^{bc}
Rate dehydrate(%)	17.96±1.15^{b}	21.84±1.02^{a}	21.42±0.82^{a}	20.06±1.04^{ab}	22.11±0.98^{a}	21.62±1.22^{a}
Water holding capacity(%)	76.35±1.50^{a}	71.34±1.27^{b}	72.19±1.00^{b}	73.73±1.30^{b}	71.35±1.29^{b}	71.62±1.62^{b}
Cooking loss(%)	31.87±0.48	34.21±0.82	33.01±1.97	33.94±0.61	34.50±0.88	33.78±0.73
Shear force(kg)	7.92±0.34	7.82±0.32	8.08±0.39	7.57±0.21	6.85±0.65	7.55±0.53
Total collegen(mg/g)	6.56±0.32	6.21±0.39^{a}	6.77±0.51^{a}	6.19±0.34^{a}	5.06±0.35^{b}	6.14±0.21^{b}

Beef quality assessment on different parts of Chinese Simmental

Niu Lei et al. (2011) conduced an experiment to assess the beef qualities of different parts on Chinese Simmental. They chose twelve 18-month Chinese Simmental cattle and slaughtered, took four parts including supraspinatus, longissimus dorsi, psoas major muscle and semitendinosus on the left half-carcass of each cattle and chilled at 4 ℃ for 42-hour, and then conducted assessment for their physical indexes (cooking loss, shearing force, pH and flesh color), nutritional qualities (fat, protein and water content) and sensory evaluations (tenderness, juiciness and flavor). SPSS17.0 statistical analysis software was utilized with methods of LSD and Duncan in order to do variance analysis. When synthesizing physical indexes and sensory evaluations of nutrition, results displayed that the beef quality of psoas major muscle was better while that of semitendinosus was worse. There were no significant differences (P >0. 05) among all the tested parts in water content and flesh color while significant differences (P<0. 05) existed in other indexes. Detailed figures are shown in table 2.

Table 2 Results of physical beef quality traits for different parts of beef from Chinese Simmental

	Cooking loss (%)	Shear force (kg)	pH	Colour		
				L^*	a^*	b*
M.supraspinatus	38. 92±3. 06^{b}	5.93±1.35^{a}	5.98±0.19^{c}	40.50±2.62^{a}	22.98±2.14	10.92±2.14^{a}
M. longissimus dorsi	34.21±2.89^{a}	7.14±1.29^{b}	5.64±0.18^{ab}	41.74±3.56^{ab}	23.74±3.40	12.89±2.28^{a}
M. psoas major	32.71±3.42^{a}	5.43±0.70^{a}	5.74±0.14^{b}	43.98±3.23^{b}	24.07±3.44	11.17±3.21^{a}
M.semitendinosus	38.65±2.44^{b}	7.67±1.06^{b}	5.55±0.14^{a}	49.40±3.87^{c}	24.74±2.74	15.64±2.36^{b}

Note: Column data marked with different superscripts means significant difference (P<0. 05).Column data marked with same or no superscript

Table 3 Nutritional results for different parts of beef from Chinese Simmental (%)

	Fat (%)	Crude protein (%)	Humidity ratio (%)
M.supraspinatus	4.13±2.49^{ab}	21.05±1.06^{a}	75.13±3.87
M.longissimus dorsi	5.00±3.56^{b}	22.10±1.17^{b}	73.79±3.74
M.psoas major	4.66±2.27^{b}	21.88±1.42^{ab}	73.90±3.16
M.semitendinosus	2.28±1.04^{a}	22.31±0.97^{b}	75.75±1.96

Table 4 Sensory evaluations score for different parts of beef from Chinese Simmental

	tenderness	juiciness	flavor
M.supraspinatus	2.81±0.48^{a}	2.86±0.27^{a}	2.78±0.36^{b}
M. longissimus dorsi	2.74±0.43^{a}	2.89±0.30^{ab}	2.89±0.28^{b}
M.psoas major	4.07±0.24^{b}	3.13±0.31^{b}	3.28±0.22^{c}
M.semitendinosus	2.26±0.31^{a}	2.68±0.34^{a}	2.49±0.31^{a}

Features research of muscle fibers on the 6 beef cattle breeds in China *Xie Xiang-xue (2011)* used frozen sections of muscle fiber and succinic dehydrogenase (SDH) staining method to study muscle fiber features including type, diameter, and density of *longissimus dorsi*, *psoas major muscle*, and *biceps femoris* muscle from 6 beef cattle breeds (Limousin, Piedmont, China Simmental, Luxi cattle, Qinchuan cattle and Jinnan cattle) in 2011. The results showed that the proportions of red muscle fiber (type R) in *longissimus dorsi* of Luxi cattle and Limousin were 32.7% and 29.5% respectively, obviously higher than that of the other four breeds ($P < 0.05$); the highest proportions of type R fiber in psoas major muscle of Jinnan cattle and *biceps femoris* muscle of Luxi cattle were 42.7% and 48.7% respectively. Diameters of three types of muscle fibers in *longissimus dorsi* of Qinchuan cattle were 60.009, 61.109 and 67.800μm, which were obviously bigger

than that of the other five breeds ($P < 0.05$); diameters of red muscle fiber (type R) and intermediate fiber (type I) in psoas major muscle and *biceps femoris* muscle of Qinchuan cattle and Jinnan cattle were significantly larger than that of the other four breeds ($P < 0.05$). Densities of red muscle fiber (type R) in psoas major muscle of Piedmont and Limousin were 216.667 and 189.583 each fiber/mm^2, and densities of white muscle fiber (type R) were 200.926 and 261.11 each fiber/mm^2, both of them significantly higher than other breeds ($P < 0.05$); densities of muscle fiber (type R) in *longissimus dorsi* and *biceps femoris* muscle of Luxi cattle larger than the other breeds. The proportions of different muscle fiber types in muscular tissues of different parts in the 6 cattle breeds were not regular, while for diameters of muscle fibers, except that the fiber diameter of *biceps femoris* muscle of Jinnan cattle was the largest, the diameters of muscle fibers (3 types) in *longissimus dorsi* of the other five cattle breeds were obviously bigger than that of psoas major muscle ($P < 0.05$). Apart from Jinnan cattle, densities of muscle fibers in psoas major muscle of the other five cattle breeds were apparently bigger than that of *longissimus dorsi* and *biceps femoris* muscle ($P < 0.05$). For various cattle breeds, there were differences among proportions of types, diameters and densities of muscle fibers in the same part. Apart from Jinnan cattle, the other five cattle breeds had the features that the density of muscle fiber in psoas major muscle was the highest, and the diameter of *longissimus dorsi* was larger than that of psoas major muscle, while Jinnan cattle had their own features.

There are abundant beef cattle breeds of in China; however, crossbred cattle are the major breed at present. Since 2007, China's beef cattle stocks have been declining; meanwhile the consumption for the beef has been growing year by year. China's consumers mainly have three demands for beef quality as follows: expensive beef produced from Wagyu crossbreed with better marbling; lean beef from Simmental, Charolais and Limousin crossbreeds, top parts supplied to hotels while common parts to supermarkets; veal from cow calves. Chinese researchers are carrying out researches which are centered on marbling beef, lean beef and veal on complete techniques of good breed, feeding management, slaughter and cutting and carcass classification.

REPUBLIC OF SERBIA - Meat quality (crossing domestic breed x beef breeds)

One of the ways to improve the production of quality beef is a method of industrial crossing of Domestic Spotted breed with French fattening breeds. The examination included beef carcass of average weight of 42 kg, as follows: 14 carcasses of F1 generation crosses of Domestic Spotted breed with Charolais (G1),

18 carcasses of crosses of Domestic Spotted breed and Limousine (G2) and 25 Domestic spotted cattle carcasses (G3).

Slaughter and primary processing was performed in the experimental slaughterhouse of the Institute of Animal Husbandry. After cooling, and in the same conditions for all tested carcasses (+4°C), in the next 24 hours, three rib cuts were taken from the carcass. The 9-10-11 rib cut was separated from the cooled left carcass sides, cut at the cranial edge of the 9th and 11th rib, and cut parallel to the spinal column (perpendicular to the ribs), where the 1/3 of the upper rib part remained on the rib cut.

Investigation of the physical-chemical and organoleptic properties of is always conducted on the sample of *M. longissimus dorsi* (MLD), which originates from the region between 10th and 12th vertebrae. Evaluation was done by 3 assessors, and final grades (1 to 5) were given after consideration of individual opinion and consensus. All three judges were given equal pieces of meat in regard to the weight and muscle fibre direction and at the same temperature with the instruction to chew the same piece of meat in small pieces first with incisors teeth and in the second stage to use molars with each of them, and using the clock, to determine the duration of mastication.

Processing of experimental data was performed by the method of Least squares (LSMLMW-Harvey, 1990) according the fixed model:

$$y_{ijk}=\mu+G_i+b_1(x_1-x_1)+e_{ijk}$$

where G_i is the fixed effect of genotype and b_1 linear regression effect of pre slaughter weight.

Table 5. Genotype influence on MLD and pH_{24} value

	Average values (μ), standard error (SE) and deviation from the average (ci) by genotypes					
Characteristics	μ	SE	G1	G2	G3	F exp.
Colour (1-5)	4.50	0.12	0.30	0.41	-0.71	*
Marbling (1-5)	4.74	0.06	-0.24	0.16	0.08	NS
Structure estimate (1-5)	4.35	0.11	0.22	0.55	-0.77	NS
pH_{24} value	5.89	0.03	-0.05	-0.06	0.12	*

The results on the impact of genotype on the properties of MLD showed significant deviations from the general average for the colour and value of pH_{24} of MLD. The greatest negative deviation from the average (-0.24), for marbling was assessed in MLD of group G_1 and the largest positive deviation (0.16), in experimental group G_2. The results concerning the evaluation of MLD grainy structure in Domestic Spotted breed crossed with French beef cattle breeds indicate the muscle fibre fineness, e.g. the tenderness of the meat. Meat colour of French

beef breeds crosses is brighter red colour than in Domestic Spotted cattle. The difference in colour of meat is also confirmed by the measured values of pH_{24}.

Table 6. Genotype influence on physical characteristics of meat

	Average values (μ), standard error (SE) and deviation from the average (ci) by genotypes					
Characteristics	μ	SE	G1	G2	G3	F exp.
Firmness (Volotkiewitsch)	5.80	0.12	-0.12	-0.7	0.82	**
Muscle fibre diameter , μm	46.66	0.61	-1.38	-1.84	3.22	**
Total pigment, ppm	86.23	1.18	0.46	3.33	-3.79	*
cm^2 Muscle eye	105.4	0.73	3.47	-0.52	-0.95	**

Table 6 shows that the highest negative deviation from the average for the softness (-0.12, -0.7) and the thickness of the muscle fibre (-1.38, -1.84) was recorded in crosses of Domestic Spotted breed with French beef cattle breeds. These results indicate that the meat of these genetic groups according to their physical properties, has the softest structure. Cross-section of MLD (+3.47) was the largest in genetic groups G_1 i.e. in crosses Domestic Spotted breed with Charolais.

Softness ("firmness" or texture) and juiciness/succulence ("dryness") of cooked or roasted meat, and to a certain extent also aroma and flavour, are important parameters of quality of meat and are best assessed in a organoleptic/sensory examination.

Table 7. Genotype influence on organoleptic traits

	Average values (μ), standard error (SE) and deviation from the average (ci) by genotypes					
Characteristics	μ	SE	G1	G2	G3	F exp.
(1-5) Cooking test - firmness	4.48	0.12	0.38	0.51	-0.89	**
(1-5) Cooking test - dryness	3.57	0.15	0.27	0.42	-0.69	**
(1-5) Cooking test - taste	4.02	0.06	0.02	0.11	-0.13	**
(1-5) Cooking test - aroma	4.06	0.08	-0.02	0.20	-0.17	NS
Roasting test - firmness	4.66	0.09	0.13	0.29	-0.43	*
(1-5) Roasting test - dryness	3.52	0.08	0.07	0.38	-0.45	*
(1-5) Roasting test - taste	4.05	0.07	-0.11	0.18	-0.06	*
(1-5) Roasting test - aroma	4.15	0.08	-0.01	-0.04	0.05	NS

Conclusion

The aim of this study was to point to the possibility of improving the quality of edible meat in Domestic Spotted cattle through industrial crossing with French beef cattle breeds. Based on the obtained results, the following can be concluded:

Colour of meat from crosses of Domestic Spotted breed with Charolais and Limousine is more prominent than the meat of Domestic Spotted cattle, and these differences ($p < 0.05$) were statistically significant.

The values of shear force determined by the Volotkiewitsch, indicate that meat from crosses was softer and smaller diameter muscle fibres compared to meat of Domestic spotted cattle.

Acknowledgement

This research was a part of the Bilateral Project No. 1-9, funded by the Ministry of education and science, Republic of Serbia and Ministry of science of the People Republic of China and part of the Project TR 31053 funded by the Ministry of education and science, Republic of Serbia.

Efekat ukrstanja domace sarene rase sa tovnim rasama na kvalitet mesa u NR Kini i u Republici Srbiji

S. Aleksić, Sun Fang, Liu Di, M.M. Petrović, V. Pantelic, N. Stanisic, D. Ostojić-Andrić, M. Petričević, D. Nikšić, N. Delić

Rezime

U radu su prikazani rezultati ukrštanja domaće šarene rase sa tovnim rasama u Narodnoj Republici Kini i u Republici Srbiji. Kina je zemlja sa velikom govedarskom proizvodnjom i potrošnjom. U 2012. godini, proizvodnja govedine u Kini bila je 5.540.000 tona, što čini 9,7% svetske proizvodnje goveđeg mesa, rangirana treća u svetu. Glavni izvori goveda u Kini su grla iz iz ukrštanja (nativne rase ukrštene sa stranim rasama goveda). Simentalska rasa goveda se najviše koriste u poboljšanim grlima tovne junadi u Kini. Kina je odgajila svoju simentalsku rasu, nakon više od 40 godina ukrštanja i poboljšanje. Potrošači u Kini uglavnom imaju tri zahteva koji se odnose na kvalitet mesa i to: skupi goveđi proizvodi dobijeni od meleza Vagyu sa boljom marmoriranošću; odlično, nemasno

goveđe meso grla simentalske, šarole i limuzin rase, najvredniji delovi trupa koji se isporučuju hotelima, a manje vredni delovi supermarketima; teletina koja se proizvodi od ženske teladi. Kineski istraživači su obavljali istraživanja koji su fokusirana na mramoriranost mesa, nemasnu govedinu i teletinu, na kompletne tehnike dobrof upravljanja u odgoju – rase i ishrana, klanje i rasecanje i klasifikaciju trupa. Trenutno, istraživači su su zainteresovani za genetiku funkcionalnih svojstava kvaliteta mesa goveda, oni očekuju da koriste ove metode za proučavanje osobine kvaliteta mesa, a zatim poboljšanje kvaliteta mesa. Poboljšane rase goveda koje se koriste u u Kini su uglavnom simentalska, zatim šarole, limuzin, Vagyu i angus. Ostale tri rase goveda, uključujući belgijsku plavu rasu goveda, pijedmont i žutu rasu goveda se retko koriste u govedarskoj proizvodnji.

Republika Srbija u budućnosti mora da brzo i efikasno obezbedi adekvatne količine najkvalitetnijeg mesa. Jedan od načina da se poveća prinos i kvalitet mesa je ukrštanje goveda domaće šarene rase nižih proizvodnih osobina sa francuskim tovnim rasama goveda. Govedarska proizvodnja u EU je prilagođena ukusu potrošača. Meso mora da ima svetlo crvenu boju, podjednako pristnim masnim tkivom i sa izraženim senzornim karakteristikama kao što su mekoća, sočnost, ukus i aroms. Rezultati o kvalitetu mesa junadi meleza F1 generacije (domaća šarena rasa sa francuskim tovnim rasama šarole i limuzin) ukazuju da se industrijskim ukrštanjem mogu poboljšati tovne, klanične i organoleptičke osobine mesa.

References

ALEKSIĆ S., LAZAREVIĆ R., MIŠČEVIĆ B., PETROVIĆ M.M., JOSIPOVIĆ S. (1995): Proizvodni i ekonomski aspekti ukrštanja domaće šarene rase sa francuskim tovnim rasama. Tehnologija mesa, 2-3, 77-79.

ALEKSIĆ S., LAZAREVIĆ R., MIŠČEVIĆ B., PETROVIĆ M.M., JOSIPOVIĆ S. (1998): Fiksni uticaj genotipa na kvalitet junećeg mesa. Biotechnology in Animal Husbandry, 1-2, 31-39.

ALEKSIĆ S., MILICA V., MIŠČEVIĆ B., PETROVIĆ M., PERKOVIĆ S. (1999): The correlation between the distribution of carcass fatty tissue traits of meat in different genotypes of young bulls. Biotechnology in Animal Husbandry, 5-6, 1-7.

ALEKSIĆ S., LAZAREVIĆ R., MIŠČEVIĆ B., PETROVIĆ M., TOMAŠEVIĆ D. (2001): The effect of Live Weight Prior to Slaughtering on Yield and Weight of Retail Carcass Cuts. Biotechnology in Animal Husbandry, 17, 5-6, 125-133..

ALEKSIĆ S., PETROVIĆ M.M., MIŠČEVIĆ B., PANTELIĆ V., TOMAŠEVIĆ D., OSTOJIĆ D. (2005): Proizvodnja kvalitetnog junećeg mesa u skladu sa evropskim trendovima. Biotechnology in Animal Husbandry, 21, 5-6, 49-54.

ALEKSIĆ S., PETROVIĆ M.M., MIŠČEVIĆ B., SRETENOVIC LJ., PANTELIĆ V., TOMAŠEVIĆ D. (2006): Production of Beef Carcass According to Consumers Demands. 52nd International Congress of Meat Science and Technology, 13-18. August 2006, Dublin, Ireland. Proceding. 541-543.

ALEKSIĆ S., PETROVIĆ M.M., PANTELIĆ V., Ž. NOVAKOVIĆ, D. TOMAŠEVIĆ , N. STANIŠIĆ, M. NOVAKOVIĆ, N. DELIĆ, M. VORKAPIĆ (2009): The effect of genotype on yield of main carcass part, Journal of Mountain Agriculture on the Balkans, 12, nu 4, p.689-70

BUCHTER: L. (1985): Danish experiences in developing and operating specifications for beef. The long term definitions of meat quality: Controlling the variability of quality in beef, veal, pig meat and lamb. Brussels, Luxemburg: Comm. Europ. Commun., 43-50.

DICKMAN M.E., POLLAK E.J., ZHANG Z., MOSER D., W., GILL C.A., DRESSLER E.A., (2005). Phenotypic ranges and relationships among carcasses and meat palatibility traits for fourteen cattle breeds, and heritability and expected progeny differences for Warne-r Bratzler shear force in three beef cattle breeds. Journal of Animal Science, 31, 75

HARRINGTON G. (1985): Review of relative importance of factor up controlling the variability of quality in beef veal, pig, meat and lamb. Brussels, Luxemburg: Comm. Europ. Commun 17-34.

ČEPIN S. (2001): Blagovne znamke govjega mesa. Meso in mesnine, 2, 17-20.

ČOBIĆ T., NENADOVIĆ M., MEDIĆ D., NOVAKOVIĆ M. (1990): Ispitivanje tovnih sposobnosti muških meleza F1 generacije šaroleske i limuzinske sa simentalskom rasom goveda. Biotehnologija u stočarstvu, 3-4, 3-13.

NIU LEI, ZHANG ZHISHENG, LI HAIPENG, SUN ZHIZHONG (2011): Assessment on beef qualities of different parts of China Simmental [J]. China Husbandry and Veterinary Medicine, 38, 3, 217-219

OSTOJIC-ANDRIC D., S. ALEKSIC, HRISTOV S., NOVAKOVIC Z., M.M. PETROVIC, D. NIKSIC, N.STANISIC (2012): Serbia in the implementation of SEUROP standard for beef carcass classification: Legislation, Parameters and Evaluation Criteria (Part A). Biotechnology in Animal Husbandry 28 (1), p 47-58

WEN LIZHENG, ZHAO YUMIN, JIANG HAO (2008): Analysis on SNP of H-FABP gene in improved red steppe cattle and its relation to meat quality traits. China Husbandry and Veterinary Medicine, 35 (7): 66-69

WEN LIZHENG, ZHAO YUMIN, ZHANG GUOLIANG (2008): SNP of H-FABP gene in red steppe cattle and its impact on meat quality traits. Chinese Agricultural Science Bulletin, 24 (5): 17-21

XIE XIANGXUE, MENG QINGXIANG, REN LIPING (2011): Research on features of muscle fibers of 6 beef cattle breeds in China [J]. Journal of China Agricultural University, 16 (1): 66-72
ZHOU GUOLI, ZHU QI, GUO SHANLI (2005): Analysis on polymorphism of H-FABP gene of Luxi cattle and its relation to meat quality traits [J]. Northwestern Agricultural Journal, 14:5-7
ZHOU LEI, YU QINGYUN, DU WEI (2007) Research on beef qualities of different cattle breeds, Xinjiang Agricultural Sciences, 44 (4): 534-538

Received 7 May 2013; accepted for publication 11 June 2013

Biotechnology in Animal Husbandry 29 (2), p 399-404, 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.09'62
DOI: 10.2298/BAH1302399V

STABILITY TEST FOR MASTITIS REAGENT ad us.vet.

V.Vuković[1], M.Vićentijević[1], N. Plav□a[2]

[1]Institute of Veterinary Medicine of Serbia, 11070 Belgrade, Auto put 3, Republic of Serbia
[2]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
Corresponding author: vujadin.vukovic@gmail.com
Communication

Abstract: In the stability test of the mastitis reagent ad us.vet., as the finished product, three production series were tested in quantities of 500 ml of the sample, under appropriate storage conditions. For the testing, the appropriate uniformity of temperature and relative humidity was provided. Also, the procedure of the stability test was determined, which included the initial state, then every three months until the end of the first trial and a final testing at the end of shelf life (0, 3, 6, 9, 12 and 18 months). Of the tested parameters the following were included: appearance, pH value of the solution, dry residue (in %) and microbiological purity.

Key words: mastitis reagent, stability, appearance, dry residue, microbiological purity

Introduction

Mastitis is one of the most widespread diseases in domestic dairy animals (cows, sheep and goats) and requires permanent activity in its suppression, including prevention, diagnosis, treatment (*Adams, 1995; Jezdimirović, 2000; Vukovic et al., 1988*).

Mastitis reagent is a diagnostic tool for the detection of udder secretion disorders in order to achieve accurate diagnosis of the mastitis in dairy cows, sheep and goats (*Batis, 1976; Miljkovic, 1977; Ruegg, 2002; Wilson and Gonzales, 1997*). The procedure is based on the indirect method of determining the number of somatic cells in milk (*Dohoo et al., 1984; Vukovic, 1998*). The reaction is performed in the milk testator composed of four separate parts and based on the effect of surface-active substance (surfactant) alkyl aryl sulfonate in reagent on leukocytes in the milk, releasing the DNA that polymerizes and produces visible gel. The complete reaction is accompanied by changes in the pH visible in indicator colour change (brome cresol red).

The result can be displayed as: negative (-), suspicious (±), weakly positive (+), positive (+ +) or strongly positive (+ + +) and is in proportion to the respective range of somatic cell count (*Miljkovic, 1977*). In this way, the assessment of udder health status ranges from healthy to clinically manifested mastitis. Further procedure is taking samples of milk for bacteriological examination to determine the cause and to determine the appropriate therapy, therapy.

Mastitis reagent is not used in advanced pregnancy, and two weeks after birth, due to obtaining of inadequate results.

Stability testing of active and auxiliary substances and finished products includes the chemical, physical and microbiological stability. This stability is under the influence of external factors (temperature, humidity, storage method and conditions, etc.) and internal factors (characteristics of active and auxiliary substances: pH, particle size, pharmaceutical/dosage form, the characteristics of the technological process, etc.). Also, the shelf life of the product determines the procedure of the stability test in order to obtain sufficient data for parameter testing at specified intervals, with a final examination at the end of shelf life.

Materials and Methods

Mastitis reagent stability test, as the finished product, includes examining the relevant parameters, appropriate procedures and methods and their compliance with the requirements of the test. Mastitis reagent in 1 ml of solution contains 50.0 mg of alkyl aryl sulfonate (*Valčić, 1995*) and each of the series that will be included in the stability test must meet quality specifications. Assessment of results for individual parameters is expressed with: adequate/compliant (+) or not adequate/non-compliant (-) with the requirement or numerically compared with the limits required.

The quality specification of the mastitis reagent, the appearance/exterior was tested sensory (*Ph.Jug.IV, 1984; Ph.Jug.V, 2000; Ph.Eur.VI, 2006).* At the same time, the shape and colour were observed, and the requirement is that the colour is red-purple clear liquid and foam of yellowish purple colour. Of general tests *(Ph.Eur.VI, 2006)* the verification of charging is provided for, and the requirement is 500ml ± 2,0%.The identification was made according to internal regulation *(QCC-P-162-1, 2004)* by adding in the surplus 10% NaOH and after the flakes deposition, addition in excess of 10% HCl leading to intense blurring of the solution with a milky-yellow discoloration.

Of the constants, the pH value was investigated by potentiometric titration *(Ph.Eur.VI, 2006),* where the claim for pH value (6.5 to 7.0 by), and the limits required for the dry residue ranged from 4.75 to 5, and 25% were investigated by

the internal regulation *(QCC-P-162-1, 2004)* by a 3-4 ml solution steamed and dried in an oven at 105^0C for 3 hours.

Microbiological purity test procedure was carried out according to regulation Ph.Eur.VI, 2006. Microbial limit was that in 1 ml sample up to 10^3 aerobic bacteria is allowed and up to 10^2 of fungi yeast and mould spores. On the other hand, in 1 ml of the sample there must not be any: *E. coli* and other *enterobacteria*, *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

After confirming that all testing parameters match or are within quality specifications, it was decided to the stability test will encompass three series of mastitis reagent. In addition, for each series the same storage conditions, the same procedure with the relevant test parameters investigated will be applied.

For adequate storage during the test, along with keeping out of reach of children and protection from light and moisture, the constant temperature (25^0C $\pm$ 2^0) and a relative humidity of 60 ± 5% were provided. Further, based on the shelf life, stability test procedure was determined. In addition, it included the beginning, then testing every three months until the end of the first year of shelf life, and a final examination at the end of the stability test aligned with the expiry of its shelf life (0, 3, 6, 9, 12 and 18 months).

Of the tested parameters the stability test included appearance/exterior, pH value, dry residue and microbiological purity.

Results and Discussion

Total of three series of mastitis reagent were tested in the study of stability within the shelf life of the finished product (18 months). Also provided were the storage conditions: adequate temperature and relative humidity, shielding from light and humidity and placed out of reach of children.

From Table 1, it is apparent that all three series examined, in all observed periods, and in terms of the parameter of appearance/exterior meet the requirements of the test. This means that it was clear liquid, red-purple in colour, the foam of yellowish-purple colour appeared when shaking and that these characteristics did not change during the experiment.

Also, the pH value from the test requirements (6.5 to 7.0) corresponded (+) in all three tested series and in all observed time intervals. In all three series, the pH value fluctuated slightly in the observed times, and after 18 months in the first and second series the lowest values were registered, which amounted to 6.75 and 6.54.

Table 1. Stability test for mastitis agents within the shelf life period
Temperature: $25^0C \pm 2^0$
Relative humidity: $60 \pm 5\%$
protected from the light, moisture and out of reach of children

Parameter	Series	Stability test time (months)					
		0	3	6	9	12	18
Appearance/ exterior	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+
pH (6,50-7,00)	1	6.85	6.88	6.85	6.82	6.80	6.75
	2	6.70	6.68	6.71	6.70	6.65	6.54
	3	6.62	6.60	6.60	6.61	6.62	6.64
Dry residue 4,75-5,25 (%)	1	5.00	4.96	4.95	4.98	4.94	4.88
	2	5.02	5.00	4.98	4.97	4.92	4.85
	3	5.10	5.06	5.10	4.98	4.99	4.90
Microbiological purity	1	+	+	+	+	+	+
	2	+	+	+	+	+	+
	3	+	+	+	+	+	+

*The + = compliant/corresponds

Also, the values obtained from the dry residue corresponded/compliant with the requirement (4.75 to 5.25%) in all three series examined in all periods of observation. In addition, these values generally showed slight continuous decrease from the start of the testing to the end of the shelf life period (18 months), from 5.00 to 4.88% for Series 1, also from 5.02 to 4.85% for the Series 2 and from 5.10 to 4.90% for the third series (Table 1).

Microbiological purity was compliant/corresponding (+) in all three series examined and all tested periods of observation.

According to reports on sensory evaluation of the active and auxiliary substances and finished products ad.us.vet. *(NIVS, 2012),* of the total of 35 samples examined, the parameter appearance/exterior for all samples was compliant/corresponded with the requirements and the Law on Medicines and Medical Devices (*Official Gazette of RS 30/2010 and 107/2012).* The same results were obtained in our stability test for mastitis reagent, for the tested parameter appearance/exterior.

Also, according to the manufacturer attesting analysis *(QCC-P-162-1, 2004)*: pH value of the mastitis reagent was 6.60, i.e. it was within the requirements of 6.50 to 7.00. Also, values obtained in the pH stability test for mastitis reagents ranged within the limits of the requirement in the observed periods of time within the shelf life. Identical data were obtained for microbiological purity.

According to some authors (*Gaćić and Orešković, 2008*), by determining the moisture content % of dry residue was calculated, and the data were used for correlation with the content of active substance for several finished products. Dry

residues of atrazine were 48.30 to 52.53%, for 475 to 525 g/l of active substance. In our experiment, the dry residue of mastitis reagent ranged within the limits of the requirement - from 4.75 to 5.25%.

Conclusion

Based on the results obtained in the test of stability of the finished product mastitis reagent, the following can be can be concluded:

- that the storage conditions, temperature and relative humidity were adequate;
- appearance/exterior of the reagent didn't change within the shelf life;
- pH value and the dry residue were within the limits of the requirement;
- microbiological purity was adequate also at the end of expiration time;
- the quality of mastitis reagent, in above-mentioned storage conditions, was ensured/provided within a period of 18 months.

Acknowledgment

Projects MNTR Government of Serbia no, 46009 and no. 31034 for the period (2011-2014).

Test stabilnosti za mastitis reagens ad us.vet.

V.Vuković, M.Vićentijević, N. Plavša

Rezime

U okviru testa stabilnosti za mastitis reagens ad us.vet., kao gotovog proizvod, ispitane su tri proizvedene serije, u količinama od po 500 ml uzorka, pri odgovarajućim uslovima čuvanja. Za to je obezbeđena ujednačenost odgovarajuće temperature ($25^0C \pm 2^0$) i relativne vlažnosti ($60 \pm 5\%$). Isto tako, određena je i procedura testa stabilnosti koja obuhvata početno stanje, zatim tromesečno ispitivanje do isteka prve godine i jedno završno ispitivanje na kraju roka trajanja (0, 3, 6, 9, 12 i 18 meseci). Od testiranih parametara obuhvaćeni su: izgled, pH vrednost rastvora, suvi ostatak (u %) i mikrobiološka čistoća. Za vreme ispitivanja uzorci su bili zaštićeni od uticaja svetlosti, vlage i bili su van domašaja dece.

References

ADAMS R.H. (1995): Veterinary pharmacology and therapeutics. 7th Edition, Iowa State Univ. Press/Ames, Iowa.
BATIS J. (1976): Problemi dijagnostike mastitisa krava. Veterinarski glasnik, 7,30
DOHOO I.R., MEEK A.H., MARTIN S.W. (1984): Somatic cell counts in bovine milk: Relationships to production and clinical episodes of mastitis. Can J Comp Med Rev Can Med Comp. 48, 130-135.
GA□IĆ S., ORE□KOVIĆ Z. (2008): Determination of active ingredient content based on correlation with dry content in suspension concentrates Određivanje sadr□aja aktivne materije u koncentrovanim suspenzijama na osnovu korelacije rezultata sa sadr□ajem suvog ostatka. Pestic.Phytomed, Belgrade, 23, 59-62.
JEZDIMIROVIĆ M. (2000): Veterinarska farmakologija. II prerađeno izdanje, Fakultet veterinarske medicine Beograd.
MILJKOVIĆ V. (1977): Higijena i tehnologija mleka. Fakultet veterinarske medicine Beograd.
NAUČNI INSTITUT ZA VETERINARSTVO SRBIJE (NIVS) (2012): Knjiga laboratorijskih pregleda Odeljenja za ispitivanje lekova.
PH.EUR.(2006): European Pharmacopoeia - 6th edition, EDQM, Strasbourg.
PH.JUG. IV (1984): Farmakopeja SFRJ. Savezni zavod za zdravstvenu za□titu, Beograd.
PH.JUG. V (2000) Farmakopeja. Savezni zavod za zdravstvenu za□titu, Beograd.
POUTREL B., RAINARD P (1981): California mastitis test guide of selective dry cow therapy. J Dairy Sci 64, 241-248.
PROPIS QCC-P-162-1 (2004): Mastitis reagens ad.us.vet. Farmaceutsko hemijsko biolo□ka dokumentacija.
RUEGG L. PAMELA, REINEMANN J. DOUGLAS (2002): Milk Quality and Mastitis Tests. University of Wisconsin, Madison
VALČIĆ J. (1995): Lekovi ad us.vet. i zakonska regulativa. Savezno ministarstvo za privredu.
VUKOVIĆ V. (1998): Određivanje broja somatskih ćelija u zbirnim uzorcima mleka sa većine otkupnih mesta u Srbiji. VII Kongres veterinara Jugoslavije, sa međunarodnim uče□ćem, Beograd, Zbornik radova, 203.
VUKOVIĆ V., ERSKI-BILJIĆ MILANKA, JAKIĆ DOBRILA (1988): Ra□irenost mastitisa na jednoj dru□tvenoj farmi krava u SR Srbiji. XXX Naučni sastanak mikrobiologije, epidemiologije i infektologa Jugoslavije, Pula, 963.
Sl. GLASNIK RS 30/2010: Zakon o lekovima i medicinskim sredstvima.
WILSON D.J., GONZALEZ R.N. (1997): Evaluation of milk culture, SCC and CMT for screening herd additions. Proceedings of the 36th Annual Meeting of National Mastitis Council, Albuquerque, NM. Natl Mast Coun. Madison WI, 127-131

Received 5 February 2013; accepted for publication 12 April 2013

Correction

In the paper "Correlation between protein to fat ratio of milk and chemical parameters and the yield of semi-hard cheese" M. Bojanić Ra□ović, N. Nikolić, A. Martinović, V. Katić, R. Ra□ović, **M. Walcer**, K. Domig**,** published in the last issue of Biotechnology in Animal Husbandry (Vol 29, 1, pp. 145-159), one of the coauthors□name was mistaken □instead of M. Walcer it should be M. Walzer. Our appologies to the authors.

There is correction in the paper published in the Biotechnology in Animal Husbandry (Vol, 29, 1, pp. 53-64)

The reference missing in the reference list

OBERST R. D., HAYS M.P., HENNESSEY, K.J., STINE L.C., EVERMANN J.F., KELLING CLAYTON L.(1993): Identifying Bovine Respiratory Syncytial Virus by Reverse Transcription-Polymerase Chain Reaction and Oligonucleotide Hybridizations. Journal of Clinical Microbiology, 31(5): 1237□1240.

Instruction for authors

Papers for publishing in the *Biotechnology in Animal Husbandry* journal should be submitted to the Editorial Board. Address: Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, P.O.box 23, Republic of Serbia (for *Biotechnology in Animal Husbandry*).

Original papers in English, (on a CD-ROM or by e-mail: biotechnology.izs@gmail.com) 6 pages of typed text using, Paper size: Custom size, Width 17 cm, Height 24 cm; formata (Portrait), normal spacing (Single Space). Margine: Top 2,0 cm, Left 2.0 cm, Bottom 2.0 cm, Right 2,0 cm, no pagination. Use font Times New Roman, size 11 (except where it is stated otherwise). Title of the paper should be Times New Roman, font size 14, **bold**:

Example 1

TABLE EGGS OF KNOWN ORIGIN AND GUARANTEED QUALITY - BRAND EGG

Authors, Times New Roman, font size 12, **bold**

Z. Pavlovski, Z. □krbić, M. Lukić

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: zlaticapav@yahoo.com
Invited paper

Example 2

THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

M. D. Petrović[1], Z. Skalicki[2], V. Bogdanović[2], M. M. Petrović[3]

[1]Faculty of Agronomy, Cara Du□ana 34, 32000, Čačak, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[3]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: petrovicm@tfc.kg.ac.yu
Original scientific paper

use [1,2, ...] numbers in suffix to refer to addresses of authors, under affilations of authors should be mentioned e-mail of corresponding author and category of paper, Times New Roman, font size 9

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: 250 words

Key words: state key words (not more than 6)

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

Materials and Methods - state methods applied in the paper.

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

Acknowledgment - for example:

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

After Acknowledgment 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:

Konzumna jaja poznatog porekla i garantovanog kvaliteta - brand jaja

Z. Pavlovski, Z. krbić, M. Lukić

Summary - should contain the most important issues of the paper. It should be in English, and Serbian for domestic authors (min. 250 words).

References - should be in alphabetical order. Names of the authors must be given in capital letters followed by the year of publicationin brackerts, titles in the language of the original, examples:

PAVLOVSKI Z. (2004): Novi propisi EU, dobrobit ivine, zahtevi potroača. ivinarstvo, 8-9, 49-58.

PAVLOVSKI Z., MAIĆ B. (1994): Odnos potroača prema ivinskim proizvodima. ivinarstvo, 7-9, 77-82.

PETROVIĆ D.M., GUTIĆ M., BOGOSAVLJEVIĆ-BO□KOVIĆ S. (2004): Masa teladi pri rođenju i njena varijabilnost kod krava simentalske rase. Agroznanje, 5, 1, 111-116.

Citations in the text are presented in *italic* form, examples: ...results of *Pavlovski (2004)...*; *(Pavlovski and Ma□ić, 1994)*; *(Petrović et al., 2004)*; *(Pavlovski, 2004; Pavlovski and Ma□ić, 1994; Petrović et al., 2004)*.

Authors are fully responsible for the contents of the papers.

Biotechnology in Animal Husbandry contains three categories of papers:

- Original scientific paper,
- Review paper, and
- Communication.

Review papers must have minimum 5 self-citations (by the first author).

All papers are published in English, and reviewed.

Abbreviation for journal *Biotechnology in Animal Husbandry* is:
Biotechnol Anim Husb

Editorial Staff

Institute for Animal Husbandry, Belgrade-Zemun
10th International Symposium
"Modern Trends in Livestock Production"
2nd – 4th October 2013, Belgrade, Serbia

Hotel Park, Belgrade, Serbia

SECOND COMMUNICATION

The tenth International Symposium **"Modern Trends in Livestock Production"** will be organized from October 2-4 in Hotel Park, Belgrade, Serbia, in organization of Institute for Animal Husbandry, Belgrade-Zemun.

Full papers prepared for publication according to Instructions for authors for journal *Biotechnology in Animal Husbandry* (www.istocar.bg.ac.rs), should be submitted before **May 31st, 2013.** (Papers not prepared according to Instruction for authors will not be considered). Authors, kindly, state the method of presentation of the paper oral or poster.

Papers in English should be submitted to following e-mail address:
biotechnology.izs@gmail.com

or on CD, to the following address:
Institute for Animal Husbandry,
Autoput 16, P. Box 23,
11080 Belgrade-Zemun,
Serbia

For poster session - size of posters 60 x 90 cm (width x height).

Official language
Official language of Symposium is **English**. All oral presentations shall be interpreted.

Registration fee

Registration fee is obligatory for all Symposium participants except participants with invited plenary presentations.

- Registration fee which includes: publishing of paper in journal *Biotechnology in Animal Husbandry*, Symposium material, participation in all sessions of the Symposium, coffe/tea break, is **100 EUR** (for domestic participants in dinar value on the day of payment according to the exchange rate). Papers shall not be published without the payment of registration fee.
- Registration fee which includes: publishing of paper in journal *Biotechnology in Animal Husbandry*, Symposium material, participation in all sessions of the Symposium, coffe/tea break,cocktail, tourist programme and gala dinner, is **150 EUR** (for domestic participants in dinar value on the day of payment according to the exchange rate).
- **Deadline for payment of registration fee June 30th 2013**.

If authors need proforma invoice they can contact us by phone, +381 11 2670121, extensions 220, 203 (Vesna Krnjaja or Olga Devečerski) or e-mail address: biotechnology.izs@gmail.com

All papers presented (oral and posters) will be published in journal *Biotechnology in Animal Husbandry* (with two reviews)

Payment of registration fee before stated deadline is to be made on the following account :

INSTRUCTION FOR EUR PAYMENTS
AIK BANKA AD NIS

Please pay as pre instruction given below:

56A: Intermediary bank:	**COBADEFF** **COMMERZBANK AG** **60301 FRANKFURT AM MAIN** **GERMANY**
57A: Account with institution:	**AIKBRS22** **Acc.No. 400876864000EUR** **AIK BANKA AD** **NIKOLE PASICA 42** **18000 NIS** **SERBIA**
59: Beneficiary customer:	**INSTITUT ZA STOCARSTVO** **ZEMUN** **Autoput Beograd-Zagreb 16** **Zemun** **IBAN: RS35105050120000062319**

Symposium location and accommodation

Symposium will be held in **Hotel Park, Belgrade** Njego□eva street 2, 11000, Belgrade, Serbia

- Symposium participants can book accommodation at significantly lower prices :

Single room (bed and breakfast) **50 EUR**
Double room (bed and breakfast, 2 persons) **70 EUR**

- Tourist tax is not included in the price.

Deadline for accommodation booking at lower prices is **31st August 2013**

Address: Hotel Park, Njego□eva 2, 11000 Beograd
Tel. +381 11 3640385; +381 11 3640393
Fax. +381 11 3640393

E mail: **reception@hotelparkbeograd.rs**
www.hotelparkbeograd.rs

Chairman
Organizing Committee

Dr Milo□Lukić
Serbia

Chairman
International Scientific Committee

Prof.dr Martin Waehner
Germany

Институт за сточарство, Београд-Земун
10. међународни симпозијум о сточарству
Савремени трендови у сточарству
2.-4. октобар 2013. године, Београд, Србија

Хотел Парк, Београд, Србија

ДРУГО ОБАВЕШТЕЊЕ

Десети међународни симпозијум о сточарству **Савремени трендови у сточарству** одржаће се од 2. до 4. октобра 2013. године у Хотелу Парк, у Београду, Србија, у организацији Института за сточарство, Београд-Земун.

Радове, припремљене за штампу према упутству часописа *Biotechnology in Animal Husbandry* (www.istocar.bg.ac.rs), послати до **31. маја 2013.** године. (Радови који нису припремљени према упутству, биће враћени на измену или неће бити разматрани). Аутори би требало да наведу начин презентације рада, усмено предавање или постер.

Радове послати на енглеском језику на CD-у, на e-mail адресу:
biotechnology.izs@gmail.com

или поштом на адресу**:**

Институт за сточарство,
Аутопут 16, П. фах 23,
Земун-Београд 11080,
Србија

За секцију постера прилози се припремају у величини 60 x 90 cm (ширина x висина).
Службени језик
Службени језик симпозијума је **енглески**. Сва усмена излагања се симултано преводе.

Котизација

Котизацију уплаћују сви учесници симпозијума изузев учесника са пленарним предавањем

- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал симпозијума, учествовање у свим секцијама симпозијума, кафу/чај у паузама, износи **100 EUR** (за домаће учеснике у динарима на дан уплате по важећем средњем курсу). Ниједан рад неће бити штампан без уплаћене котизације.
- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал симпозијума, учествовање у свим секцијама симпозијума, кафу/чај у паузама, коктел, туристички програм и свечану вечеру, износи **150 EUR**.

  Рок за уплату котизација је **30. јун 2013.** године.

Уколико Вам је потребна профактура за уплату котизације можете нам се обратити телефоном, централа 011 2670 121, локали 220, 203 (Весна Крњаја или Олга Девечерски) или на e-mail адресу: biotechnology.izs@gmail.com

Међународни научни комитет симпозијума ће након пријема радова извршити две рецензије по раду. Рецензирани и прихваћени радови за штампу биће штампани у целости на енглеском језику у часопису *Biotechnology in Animal Husbandry*.

Уплату котизације потребно је у наведеном року извршити на рачун:

Институт за сточарство, Београд-Земун
11080 Земун, Аутопут 16
Комерцијална банка
Рачун бр. 205-65958-94

Место одржавања симпозијума и смештај

Симпозијум ће се одржати у **Хотелу Парк**, Његошева 2, 11000, Београд, Србија.
За учеснике симпозијума резервација смештаја до **31. августа 2013.** године је по знатно нижим ценама:

Једнокреветна соба (ноћење са доручком) **50 EUR**
Двокреветна соба (ноћење са доручком) **70 EUR**

Цена боравишне таксе није укључена у наведене цене.

Адреса: Хотел Парк, Његошева 2, 11000 Београд
Тел. 011/3640-385; 011/3640-393
Фах. 011/3640-393

E mail: **reception@hotelparkbeograd.rs**
www.hotelparkbeograd.rs

Председник
Организационог одбора

Др Милош Лукић
Србија

Председник
Међународног научног комитета

Проф. др Martin Wahner
Немачка