UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

VOL 29, 4
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 (4), p 581-749, 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, - M51 category

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

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

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

Biotechnology in Animal Husbandry 29 (4), p 581-589 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.09'62
DOI: 10.2298/BAH1304581J

THE EFFECT OF ANTIOXIDANTS ON PREVENTING THE RETAINED PLACENTA IN DAIRY COWS

M. Joksimović Todorović, V. Davidović

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

Abstract: Retaining of placenta is one of the most common ailments in dairy cows and it occurrs if placenta is not removed in the period of 12-14 hour after calving. The ailment is of multifactorial etiology and a primary cause is often unknown. During periparturient period in cows the metabolical, hormonal and biochemical changes are observed. Immunosuppression also characterizes this period and it is associated with endocrinal changes and decreased feed intake. Reduced function of neutrophiles before parturition is in correlation with increased frequency of incidence of postparturition ailments. Selenium and vitamin E are the most important antioxidants which have a positive effect on neutrophiles function protecting them from oxidative damage.

Key words: dairy cows, placenta retaining, selenium, vitamin E

Introduction

Frequency of retained placenta (RP) in cows after calving is 4-18% (*Han and Kim, 2005*). Aetiology of this ailment is not completely confirmed but numerous factors are included: mechanical, nutritive, inadequate management and infectious diseases (*Gunay et al., 2011*). Some studies show that 30-50% dairy cows have some metabolical and infectious diseases in the period about parturition. Certain physiological changes that happen during a transitional period may affect a nutritive status and contribute to higher sensitivity to some ailments. In this period the needs for energy are increased and they are caused by the beginning of lactation and insufficient feed intake, what brings the individual into the state of negative energy balance (NEB). The consequences of negative energy balance are mobilisation of fat from body reserves and entering of non-esterified fatty acids (NEFA) into blood. Abundant fat mobilisation and excessive accumulation of NEFA leads to the occurrence of metabolical and inflammatory ailments. Negative energy balance may occur at the end of gestation (*Brydl et al., 2008*) and contribute to the development of ailments such as fat liver and ketosis (*Bertics et al., 1992*) or provoke immunosuppression (*Goff, 2003*).

Disturbances in energy balance and acidobased balance are key factors for the incidence of placenta retaining. Increased levels of NEFA and ketonuria observed two weeks before parturition are important indicators for possible occurrence of this ailment. Besides these factors an excessive weight of animal must not be neglected (*Hayirli et al., 2002*) along with numerous management oversights – uncomfortable conditions (*Grummer et al., 2004*) and heat stress (*De Rensis and Scaramuzzi, 2003*). Oxidative stress may also contribute to placenta retaining. A great number of studies show that addequate supplement of selenium, zinc, cooper, iron and vitamins A, C and E playing a role of antioxidant, can reduce the percent of individuals with retained placenta.

Hormonal and biochemical changes in cows with retained placenta

Biochemical, hormonal and electrolyte profiles can be used as prognosis criteria for the incidence of placenta retaining in dairy cows. In cows with retained placenta a substantially higher level of progesterone and cortisol, and low level of estrogene is observed in relation to healthy cows (*Kornmatitsuk et al., 2000*). The increased level of cortisol is associated with the stress in dairy cows. The process of liberation between cotyledons and caruncule depends on hystological changes that happen inside them. In cows with retained placenta the chemotactic activity and leukocytes migration are reduced. On the contrary, increased hemotactic activity and number of lekocytes in placenta enable the proces of ejection of placenta so that in such cases the percent of placenta retaining in cows is low (about 1.4%). Cortisol reduces the function of neutrophiles and it can completely prevent their activity and thus lead to development of retained placenta. Increased content of cytotoxical aldehyde (malonil aldehyde) in erythrocytes and increased concentration of cortisol are deemed as major indicators of the incidence of retaining placenta. Decreased activity of mieloperoxidasis in cotyledones indicates to a reduced function of neutrophiles while a high activity of lysosomes and acid phosphatasis in retained placenta indicate to an acute inflammatory reaction of fetomaternal combination (*Gupta et al., 2005*). It is believed that in the week prior parturition the level of estradiol reaches its maximum what helps uterus to get free from the remains of fetal membrane and thus prevent the incidence of endometritis. However, reduced level of estrogene is one of the main factors which increases the risk of the occurrence of retained placenta.

In individuals with this ailment the occurrence of fat liver has also been found. As a consequence thereof, increased concentration of some enzymes in blood (alkaline phosphatase – ALP, aspartate aminotransferase – AST and gamma-glutamyl transferase – GGT) (*Semacan and Sevinc, 2005*) has been observed as well. Infiltration of fat in the liver is associated with the increase in the level of liver

enzymes and the fall in the level of glucosis, total lpids, cholesterol, triglycerides and electrolytes in the serum of dairy cows. A fat liver leads to its disfunction with no distruction to hepatocytes and the consequence is the increase in the activity of the liver enzymes (*Bülent et al., 2006*). In cows with retained placenta it can be combined with accumulation of lipids in hepatocytes. Liberated endotoxines in infectious diseases, such as endometritis, may cause the destruction and necrosis of liver what results in different level of its disfunction (Semacan and Sevinc, 2005).
In preparturition cows a hypoglycemia has been observed what was caused by an increased needs of fetus and production of colostrum. Hypoglycemia can be also correlated with a high level of cortisol which is connected with the incidence of placenta retention. It has been determined that hypoglycemia in the last month of gravidity is one of the important indicators of the incidence of retained placenta and metritis (*Markiewicz et al., 2001*). A total level of lipids, cholesterol and triglyceride in serum is lower in cows with RP than in healthy ones. A low level of cholesterole is a consequence of the increased synthesis of progesterone. The fall in total lipids and triglycerides is in correlation with disturbances in the metabolism of lipids and/or increase in the level of tissual lipolytical enzymes (*Michal et al., 2006*). Disturbances in energy balance and acid-based balance are key factors for the incidence of RP. Dairy cows, during a longer time period, become adapted to the state in energy balance by way of physiological, metabolic and endocrine changes (*Ingvartsen, 2006*). This process of adaptation can be monitored by help of indicators such as NEFA, hormonal status and net acido-based excretion (*Jorritsma et al., 2003*). Due to reduced feed intake in periparturition period there occurs an excessive mobilisation of fat and releasing of NEFA from fat tissue. Metabolism of NEFA in liver is followed by a partial oxidation and creation of ketone bodies or reesterification into triglycerides (lipogenesis). Accumulation of ketone bodies in blood unables the maintaining of homeostasis glucosis. These alternations change the functions of immuno cells, what results in higher frequency of the incidence of RP. The level of proteins in the serum of cows with RP is mostly unchanged but hiperglobulinemia may occur as a consequence of bacterial infection.

The cows with RP are anaemic, the number of erythrocytes concentration of hemoglobine and the percent of hematocrit are significantly reduced. In addition, the leukocytosis associated with lymphopenia and monocytosis is observed.

Immunosuppression and postparturition ailments

Metabolical and infectious diseases during periparturition period are caused by diminished immuno function in cows (*Sharma et al., 2011*). Immunosuppression is a consequence of endocrine changes which may happen in this period and reduced feed intake. Insufficient intake of nutritive matters and

reduced level of vitamina E are one of many factors which lead to the fall in the activity of neutrophiles, a therefore to their possibility to take active part in the phagocytosis process. Vitamin E and selenium are the most important antioxidants which enhance the activity neutrophyles protecting them from oxidative damage after intracellular killing of bacteria (*Joksimović Todorović and Davidović, 2013*). In postparturition period the response of lymphocytes to stimulation by mitogens is reduced as well as the synthesis of antibodies and production of cytokines by immuno cells.

Reduced function of neutrophiles before parturition increases the bovine sensitivity to various postparturition diseases. Neutrophiles are found in greatest number at places with high concentration of interleukine-8 (IL-8) (*Elliott et al., 2000*). This interleukin is a strong hemoattractant and the activator of neutrophiles. IL-8 secreted by cotyledon and/or uterus enters systemic circulation, attracts neutrophiles and set them to action. It is known that it increases the secretion of colagenasis which accelerates separating of fetal cotyledons from materinal caruncles (*Luo et al., 2000*). In the period of two weeks before and two weeks after calving the concentration of IL-8 is lower in cows with RP, so that on the day of calving in cows without RP it was 134±11 pg/ml, and in cows with RP 51±12 pg/ml (*Kimura et al., 2002*). After calving, the concentration of IL-8 is considerably higher in cows with RP than in cows without RP but all the same its level remains lower in a considerable degree.

Numerous studies confirm a stimulative effect of Se and vitamine E on the immunological status, including the activity of neutrophile granulocytes. Their antioxidative capability is manifested in the protection of non-esterified fatty acids, other cellular macromolecules and membrane against peroxidation. Bovine neutrophile granulocytes contain very little catalase, but the activity of selenoenzyme GSH-Px has an important role in protecting cytosol. Reduced function of neutrophile granulocytes is associated with a high level of superoxid (O^-). Inadequate protection against autooxidants leads to reduction of the function of neutrophyle granulocytes what may cause frequent ailments. A primary role of these nutrients is to ensure immunological defence, increase the migration of neutophile granulocytes into mammary gland where they phagocyte and destroy present bacteria (*Hogan et al., 1993*).

The importance of selenium and vitamin E in preventing the retained placenta in dairy cows. The relationship between antioxidative nutrition, oxidative stress and the incidence of retained placenta is well-known in dairy cows. Although pathogenesis of this ailment connected with Se and E vitamin deficiency is not clear enough the participation of oxidative stress in its etiology indicates a reduced incidence after selenium treatment. By adding the vitamin E and Se into feed, the level of this vitamin in erythrocytes, neutrophiles and plasma is increased along with the activity of enzyme GSH-Px. Besides the level of selenium in blood plasma (*Todorović et al., 1999a*) the activity of glutathione peroxidase (GSH-Px) is also a

reliable indicator of biologically adoptable selenium. Optimal and suboptimal selenium levels in feed (0.1 and 0.15 mgSe/kg feed) result in linear increase of GSH-Px in plasma of studied individual animals. However, at certain concentrations of selenium in feed the level of GSH-Px reaches plateau so that further increase of the selenium level does not lead to the increase in the enzyme activity as well. High levels of Se (above 2 mgSe/kg feed) do not result in proportionate increase of this selenoenzyme whose activity increases at the start but fall significantly after 10th day (*Joksimović Todorović and Jokić, 2005; Joksimović Todorović et al., 2005*).

These nutrients reduce oxidative stress and lead to certain changes in placenta. Selenium and vitamin E possess immunomodulatory effects, in the sense of improving the function of neutrophiles, increase their migration and chemotactic activity (*Gupta et al., 2005*). Se deficiency affects unfavourably the function of polymorphonuclear neutrophiles and the changes in the level of GSH-Px (*Finch and Turner, 1996*). The absence of leukocytes in placenta leads to retention of placenta in 100% cows after calving. Se and vitamin E increase the number of leukocytes in placenta, leukocyte chemotaxis, assist the abating of the links between fetomaternal juncture and ejection of placenta.

Retained placenta can be prevented by the supplement of Se and vitamin E, unless it is provoked by mechanical and pathological factors (*Han and Kim, 2005; Joksimović Todorović and Davidović, 2007*). Dairy cows are fed by different forms of selenium: selenate, selenite (by adding the non-organic Se in feed) and Se-met and Se-cys (Se-yiest and basal food). Numerous studies show that the adding Se (usually in the inorganic form) ensures immunological function, soundness of mammary gland and prenvents placenta retention. Inorganic selenium (selenit and selenat) and selenized yeast (Se-yeast) represent the sources of selenium used in bovine nutrition. Predominant form of selenium in Se-yeast is selenomethionine (Se-met). The mechanism of intestinal resorption differs in inorganic and in Se-met. Factors which reduce the resorption of inorganic selenium probably do not affect the absorption of Se-met. Metabolism of inorganic and organic selenium in the cell also differ. Inorganic Se is used exclusively for the synthesis of seleno specified enzymes while Se-met can be used for the synthesis of those proteins, but it can be built into any protein which contains methionine (*Weiss and Hogan, 2005*). Organic selenium is less toxic than inorganic (*Mihailović et al., 1996a,b, 1997*), can be more rapidly stored and retained in tissues (*Todorović et al., 1999b; Joksimović Todorović et al., 2006*).

Besides selenium and vitamin E other minerals and vitamins (zink, copper, iron, vitamins A, C and E) have also been found to have important role in preventing RP. Low levels of these nutrients in dairy cows in preparturition and postparturition perioda predispose occurence of RP (*Tillard et al., 2008*). Insufficent content of these minerals and vitamins in food can result in abortion in

dairy cows and as a consequence thereof in majority of cases the retained placenta is perceived.

Conclusion

Selenium and vitamin E as natural antioxidants have an important role in preventing the occurence of retained placenta. These nutrients increase the activity of neutrophiles, enhance their chemotactic effect and phagocytosis of opsonised pathogenic microorganisms. Adequately balanced rations with sufficient content of selenium, vitamin E and other antioxidants in food, appropriate housing of animals and good management lead to reducing the incidence of one of the most often ailments in dairy cows in postparturition period.

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.

Efekat antioksidanasa na sprečavanje pojave zadržavanja posteljice kod mlečnih krava

M. Joksimović Todorović, V. Davidović

Rezime

Zadržavanje posteljice je jedno od najčešćih oboljenja mlečnih krava, a nastaje ukoliko se posteljica ne izbaci u periodu od 12-14h nakon teljenja. Oboljenje je multifaktorijalne etiologije, a primarni uzrok često ostaje nepoznat. U toku peripartalnog perioda kod krava dolazi do metaboličkih, hormonalnih i biohemijskih promena. Imunosupresija takođe karakteriše ovaj period, a u vezi je sa endokrinim promenama i smanjenim unosom hrane. Smanjena funkcija neutrofila pre porođaja u korelaciji je sa povećanom učestalošću nastanka postpartalnih oboljenja. Selen i vitamin E su najvažniji antioksidansi koji pozitivno utiču na funkciju neutrofila štiteći ih od oksidativnog oštećenja.

References

BERTICS S.J., GRUMMER R.R., CADORNIGA-VALINO C., STODDARD E.E. (1992): Effect of prepartum dry matter imtake on liver tryglyceride concentration and early lactation. J. Dairy Sci., 75, 1914-1922.
BRYDL E., KÖNYVES L., TEGZES L., JURKOVICH V., TIRIAN A. (2008): Incidence of subclinical metabolic disorders in Hungarian dairy herds during the last decade. Mady Allatorv Lapja, 130, (suppl. I), 129-134.
BÜLENT E., MUSTAFA K., ÖZGÜL M.E. (2006): Evaluation of liver function tests in cows during periparturient period. Frat Üniversitesi Sal Bilimleri Dergisi (Veteriner), 20, 3, 205-209.
DE RENSIS F., SCARAMUZZI R.J. (2003): Heat stress and seasonal effects on reproduction in the dairy cow – a review. Theriogenology, 60, 1139-1151.
ELLIOTT C.L., SLATER D.M., DENNES W., POSTON L., BENNETT P.R. (2000): Interleukin-8 expression in human myometrium: Changes in relation to labor onset and with gestational age. Am. J. Reprod. Immunol., 43, 272-277.
FINCH J.M., TURNER R.J. (1996): Effects of selenium and vitamin E on the immune responses of domestic animals. Res. Vet. Sci., 60, 97-106.
GOFF J.P. (2003): Managing the transition cow-considerations for optimising energy and protein balance and immune functions. Cattle Pract., 11, 51-63.
GRUMMER R.R., MASHEK D.G., HAYIRLI A. (2004): Dry matter intake and energy balance in the transition period. Vet. Clin. NAm-Food Anim. Pract., 20, 447-470.
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.
GUPTA S., HARENDRA K.G., JYOTI S. (2005): Effect of Vitamin E and selenium supplementation on concentrations of plasma cortisol and erythrocyte lipid peroxides and the incidence of retained fetal membranes in crossbred dairy cattle. Theriogenology, 64, 1273-1286.
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.
HAYIRILI A., GRUMMER R.R., NORDHEIM E.V., CRUMP P.H. (2002): Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci., 85, 3430-3443.
HOGAN J.S., WEISS W.P., SMITH K.L. (1993): Role of Vitamin E and Selenium in Host Defense Against Mastitis. J. Dairy Sci., 76, 2795-2803.
INGVARTSEN K.L. (2006): Feeding – and management – related diseases in the transition cow. Physiological adaptations around calving and strategies to reduce feeding – related diseases. Anim. Feed Sci. Technol., 126, 175-213.

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. (2005): Uticaj visokih nivoa organskog selena na aktivnost glutation peroksidaze (GSH-Px) u krvnoj plazmi brojlera. Veterinarski glasnik, 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, 527-530.
JOKSIMOVIĆ TODOROVIĆ M., DAVIDOVIĆ V. (2013): Immunosuppression - postpartum diseases of dairy cows. Biotechnology in Animal Husbandry, 29, 2, 211-222.
JORRITSMA R., WENSING T., KRUIP T.A.M., VOS P.L., NOORDHUIZEN J.P.T.M. (2003): Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet. Res., 34, 11-26.
KIMURA K., GOFF J.P., KEHRLI M.E., REINHARDT T.A. (2002): Decreased Neutrophil Function as a Cause of Retained Placenta in Dairy Cattle. J. Dairy Sci., 85, 544-550.
KORNMATITSUK B., KONIGSSON K., KINDAHL H., GUSTAFSSON H. (2000): Clinical signs, body temperature, and hormonal changes in dairy heifers after induction of parturition with PGF2 α. 14th International Congress on Animal Reproduction, Stocholm, 1, 179.
LUO L., IBARAGI T., NOZAWA M., KASAHARA T., SAKAI M., SASAKI Y., TANEBE K., SAITO S. (2000): Interleukin-8 levels and granulocyte counts in cervical mucus during pregnancy. Am. J. Reprod. Immunool., 43, 78-84.
MARKIEWICZ H., KUMA K., MALINOWSKI E. (2001): Predisposing factors for puerperal metritis in cows. Bull. Vet. Inst. Pulawy, 45, 281-288.
MICHAL K., EDWARD M., HANNA M. (2006): Some hormonal and biochemical blood indices in cows with retained placenta and puerperal metritis. Bull. Vet. Inst. Pulawy, 50, 89-92.
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. 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. Program and Abstract, 125.
MIHAILOVIĆ M., TODOROVIĆ M., JOVANOVIĆ M., PALIĆ T., JOVANOVIĆ J., PEŠUT O. (1997): Toxicity of selenium to broilers. XIth International Congress of the World Poultry Association 18-22 August, Budapest, Hungary.
SEMACAN A., SEVINC M. (2005): Liver function in cows with retained placenta. Turk. J. Vet. Anim. Sci., 29, 775-778.
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.
TILLARD E., HUMBLOT P., FAYE B., LECOMTE P., DOHOO I., BOCQUIER F. (2008): Postcalving factors affecting conception risk in Holstein dairy cowsw in tropical and sub-tropical conditions. Theriogenology, 69, 4, 443-457.
TODOROVIĆ M., MIHAILOVIĆ M., HRISTOV S. (1999a): 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.
TODOROVIĆ M., MIHAILOVIĆ M., HRISTOV S. (1999b): Visoki nivoi selena u ishrani pilića i njihovo deponovanje u mišićima. Savremena poljoprivreda, 1-2, 257-260.
WEISS P.W., HOGAN S.J. (2005): Effect of Selenium Source on Selenium Status, Neutrophil Function, and Response to Intramammary Endotoxin Challenge of Dairy Cows. J. Dairy Sci., 88, 4366-4374.

Received 25 November 2013; accepted for publication 25 December 2013

Biotechnology in Animal Husbandry 29 (4), p 591-606 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.07'65
DOI: 10.2298/BAH1304591L

LAYER PERFORMANCES, EGGSHELL CHARACTERISTICS AND BONE STRENGTH IN THREE DIFFERENT HOUSING SYSTEMS

S. Lolli [1], A. Hidalgo[2], C. Alamprese[2], V. Ferrante[1], M. Rossi [2]

[1]DIVET-Department of Veterinary Science and Public Health, Università degli Studi di Milano, via Celoria 10, 20133 Milano, Italy
[2]DeFENS-Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy
Corresponding author: susanna.lolli@unimi.it
Original scientific paper

Abstract: The aim of this study was to determine the effects of three different housing systems for laying hens (cages, barn and organic) on layer performances, eggshell characteristics and bone strength. In each system, the same strain of laying hens (Hyline Brown®) was housed in agreement with current European regulation and the hens were fed on the same level of nutrition (2800 ME). The study was conducted over one year period in three typical farms in the north of Italy, from the 18th to the 70th weeks of layers age. The number of eggs collected and laid on the floor were recorded weekly, as well as the mortality and the feed consumption. At 27, 30, 35, 43, 53 and 68 weeks of layers age, the weight and the shell characteristics of eggs from the different systems were analysed. Bone breaking strength and stiffness were determined by three point bending test. The percentage of deposition was generally higher in comparison to the standard production of Hyline hens, probably, due to a high management standard and to the production persistence. The results indicated a clear relationship between the percentage of cracked eggs and the strength characteristics of the shells, with organic eggs showing the highest shell thickness, the most resistant shell and consistently the less cracked eggs. Considering the changes that occur during the laying cycle, shell strength and thickness in non-cage eggs were highly affected by hen age, while they were much stable in cage eggs. Organic hens also showed the strongest humerous, while their tibiotarsus were as robust as those of cage hens.

Keywords: layer performances, eggshell characteristics, bone strength, housing system

Introduction

In the last 20 years conventional cages for laying hens have been extremely

criticized in the European Community, because they restrict the birds' movement and prevent them from performing natural behaviours such as nesting, perching and dust bathing (*Duncan and Fraser, 1997; Leyendecker et al., 2005*). Moreover, birds housed in conventional cage may develop cage layer osteoporosis that leads to bone breakage and early death (*Wilson et al., 1992; McCoy et al., 1996*). Thus, osteoporosis induced by the high demands for calcium during eggshell production in layers has become a serious problem for the egg industry (*Fleming et al., 1996*). The costs associated with reduced productivity and increased mortality due to calcium deficiency have been recognized since the mid 1950s when battery cage housing for layers first became popular (*Leeson et al., 1995*). *Gregory and Wilkins (1989)* showed that skeletal damage was a major welfare problem in laying hens and more recent studies suggested that bone fractures caused by osteoporosis are an increasingly serious welfare issue (*Budgell and Silversides, 2004; Sandilands et al., 2005*). In these studies incidences of old healing fractures of up to 70% were recorded depending upon husbandry system and bird strain. Cage housing is associated with reduced bone strength compared with barn (*Barnett et al., 1998*), aviary (*Leyendecker et al., 2005*) or free-range (*Gregory et al., 1990*) systems. However, while more extensive housing can lead to increased bone strength, there are also increased opportunities for injuries such as feather pecking, peck injury and peck mortality (cannibalism). Many hen welfare concerns are not intrinsically linked to housing system although this is one factor out of more that influences the welfare of the birds. Other factors such as genetics, the environment the hen was raised in and the quality of human handling must also be considered (*Graml et al., 2008*). For instance, genetic selection may produce laying hens that are less prone to bone weakness (*Webster, 2004*). Bone strength is better when more space is available and there is the presence of equipment details promoting activity e.g. perches (*Fleming et al., 1994; Freire et al., 2003*). Especially hens in conventional cages may risk having their wing bones broken at the end of lay and hence experienced and gentle catching and handling methods are important (*Gregory et al., 1994*). Hens in aviaries however have a higher risk of breaking their fulculum and keel bones during the laying period by collisions with equipment or other birds (*Freire et al., 2003*). The perch design has been shown to have a considerable impact on the incidence of keel bone deviations mainly through differences in impact pressure on this part of skeleton (*Tauson and Abrahamsson, 1994*). The possibility to jump during rearing period reduces skeletal damage in the following production period (*Michel and Huonnic, 2003*). Although the limitations of space may vary according to stocking density, there are several reports on the negative effects on bone strength, which in turn may lead to bone fragility and eventually, breakage of parts of skeleton e.g. during depopulation at the end of the laying cycle (*Fleming et al., 1994*). Nutrition and management of the birds are important in maximizing the mineralization of skeleton, ultimately minimizing the severity of osteoporosis, which will occur as hen laying cycle progresses. However, it appears

that even with optimal calcium, phosphorus and vitamin D3 levels in the diet, osteoporosis is still likely to occur to some degree in the highly productive layers (*Whitehead, 2004; Leeson et al., 1995*). Egg quality is affected by a range of factors including the strain and the age of the hen, the diet and the housing system. The environmental conditions within the house can also influence the egg quality through a modification of the birds' physiological status (*Travel et al., 2011*).

A major impact has the bird age which affect the egg weight, the proportion of the different egg components and composition, and the eggshell quality. The egg weight increases with hen age varying between 50 and 70 g, however most modern commercial strains are now capable of achieving egg weights of 60 g by 26 weeks of age and 65.5 g by 50 weeks, and sustaining this until the end of production (*Nys et al., 2008*).

The influence of the bird age on the eggshell quality bears an increase in the percentage of cracked and broken shells, ranging from 2-5% at the beginning of laying to values up to 12-20% in older hens (*Nys et al., 2008*). This is consistent with the reduction in the eggshell percentage and breaking strength observed with the increasing egg weight during hen aging (*Casiraghi et al., 2005*).

Minor influence on eggshell quality is reported for the different housing systems. In a review by *Rossi and De Reu (2011)*, it was concluded that nest eggs of non cage systems are normally not more susceptible to cracks than those of cage eggs. However, general conclusions on the effect of cage and non-cage housing systems on egg weight, shell strength and thickness could not be drawn because of inconsistencies of results from different authors in the literature. As suggested by *Van den Brand et al. (2004)*, ambiguities or contrasts among the studies might be explained by differences in the amount of dietary Ca and available P, which can strongly affect shell quality.

The aim of the present study was to determine the effects of three different housing systems for laying hens (cage, barn and organic) on layer performances, eggshell quality and bone strength.

Materials and Methods

The study was carried out in three Italian layer farms over one year period, with hen aged from 18 to 70 weeks. A comparison was made among laying hens in three rearing technologies: conventional cage (C), barn (litter floor, B) and organic system (O), on the same level of nutrition, same season and bird flock. In each system, laying hens of the same strain (Hyline Brown®) were housed in agreement with Council Directive 1999/74 (*EU, 1999*) and Regulation (EC) No 834/2007 (*EU, 2007*) about organic production.

The experiments were carried out before the coming into force (1/1/2012) of the ban of conventional cages for laying hens in the European Union in favour of alternative housing systems, or furnished cages systems (*EU, 1999*).

The study involved 32429 Hyline Brown® layers. Birds were beak-trimmed, transferred from rearing to laying systems in July when 18 weeks old and slaughtered at week 70. Microclimate and lighting conditions for rearing followed the technological standard for the rearing of this hybrid.

Conventional battery cages of 20 cm x 40 cm x 40 cm were used, according to European Directive 1999/74/EC (*EU, 1999*), which stated that each hen should have at least 550 cm2 available space.

Farm B was a typical barn (12 m x 42 m) with 1/3 of litter and 2/3 of slat. The stocking density was 9.4 birds/m2. Sixteen hours of light/day was provided through lateral windows located along the two longest walls, and artificial lighting program.

In farm O a free-range area (9000 m2) was accessible through 16 doors (35 cm x 100 cm, in agreement with Regulation (EC) No 834/2007 (*EU, 2007*)). The indoor poultry farm (5 m x 75 m) was divided in three parts: litter (1/3 of whole area), slat with drinking and feeding areas, and nests. The stocking density was 5.4 birds/m2.

The hens were fed a standard layer diet containing 2800 ME. Food and water were provided ad libitum in all housing systems.

The total number of laid eggs were recorded weekly, as well as the number of second category eggs, hen mortality and feed conversion rate (FCR).

At 27, 30, 35, 43, 53 and 68 weeks of layers age, 100 eggs from each system were collected. The eggs were weighed and candled to measure the percentage of cracked eggs and to select intact eggs for successive analysis. Thirty intact eggs from the original 100 were manually broken and voided. The shells were wiped and weighed without removing the membranes. Shell thickness was then measured at the equator in triplicate, using a 550-501 digital micrometer (NSK, Japan).

Another group of 30 intact eggs were used to calculate the surface area of each egg (*Thompson et al., 1985*) and the shell index as the ratio between egg weight and its surface area. The same 30 eggs were used to perform mechanical analyses. Shell breaking strength (N) was measured using an Instron Universal Testing Machine 4301 (Instron Ltd, High Wycombe, England) supported by the Series IX Automated Material Testing System software. Compression tests were carried out on seven individual eggs per sample, at the constant cross-head speed of 20 mm/min using a 100 N load cell. A 35 mm diameter plate was used as a compression device. Strength (N), displacement (mm) and energy (mJ) at breaking point were determined.

Twenty hens per housing system were randomly chosen and slaughtered at 70 weeks of age. The right tibiotarsus and humerus were cleaned of flesh and stored at -20°C until analyses. After thawing, the major and minor diameters were measured in the centre of the bones with a calliper, and a mean centre diameter was calculated. Bone breaking strength and stiffness were determined by a three point

bending test, carried out using the Instron Universal Testing Machine 4301. The bones were placed on the support (30 mm apart for humerus and 65 mm apart for tibia), laying down on the major diameter and aligning the centre of each bone with the breaking probe. Analyses were carried out at room temperature, with a constant cross-head speed of 20 mm/min and a load cell of 1kN. Results are expressed as breaking strength (N), corresponding to the fracture load, and stiffness (N/mm), calculated as the slope of the linear portion of the force-deformation curve.

Statistical analysis. The data related to the characteristics of downgraded eggs at different ages were analysed using the non parametric analysis of variance of SPSS Version 18.0 software (test Wilcoxon) with housing system as the main effect. Shell and bone characteristics were analysed by ANOVA, considering housing systems and hen age as factors and the two-way interaction for shell data, while for bones the factor was only the housing system. The significance controls of the differences were determined by LSD test. Both ANOVA procedure and LSD test were performed using Statgraphics Plus Version 4.0 software (StatPoint Inc., Herndon, VA, USA). Correlation analysis among variables was performed using Systat software Version 5.0 (Systat Software Inc., Richmond, CA, USA) following the Pearson approach.

Results and discussion

Layer performances. Production performances of hens were recorded between 18 and 70 weeks of age (Figure 1). Hen-housed egg production (eggs laid*100/number of hens at time of recording) was similar among the housing systems being 84.9% (O), 86.4% (B) and 85.4% (C) ($P > 0.05$). After reaching the peak of lay at about 24 wk, the level remained steady, then as expected decreased overtime. As shown in Figure 1, in comparison to the standard production of Hyline (Hyline, 2007) at the same week of deposition, the percentage of deposition was generally higher, especially in the organic and barn systems, due probably to high management level and to the production persistence.

The number of laid eggs obtained per hen was identical in the different systems, with a mean weekly production rate of 6 eggs/hen in all systems.

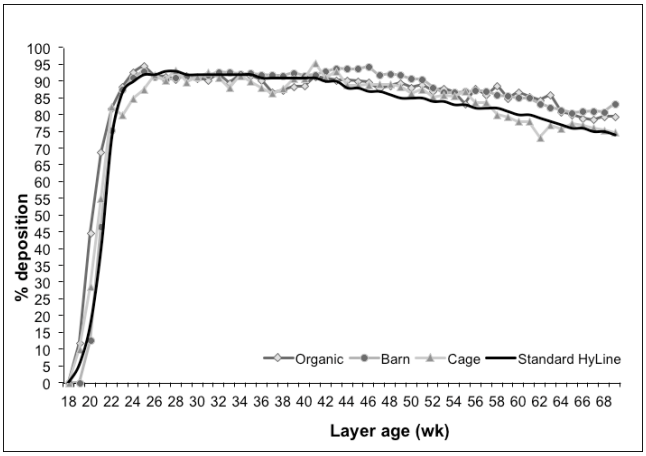


Figure 1: Egg deposition (%) in relation to layer age and housing system

Egg weight and eggshell characteristics. The mean values of egg weight and eggshell characteristics for the three housing systems and for different hen ages are reported in Tables 2 and 3, respectively. The ANOVA evidenced significant differences for all the variables and all factors (housing system (S), hen age (A) and S x A), with the exception of the displacement measurement obtained in the compression test that was not affected by housing system.

On average, barn system produced the heaviest eggs (Table 1) and consistently the highest number of cracked eggs, the greatest surface area and the lowest shell thickness and breaking strength.

Table 1. Mean values and pooled standard error (pooled SEM) of the shell characteristics of eggs from hens reared in three different housing systems during the whole production cycle

Variable	Housing system			Pooled SEM	*P*
	Cage	Barn	Organic		
Cracked eggs (%)	6.4 ^{b}	10.3^{c}	3.8 ^{a}	1.6	*0.033*
Egg weight (g)	62.9 ^{a}	66.0 ^{c}	63.9 ^{b}	0.4	*0.000*
Shell percentage (%)	10.9 ^{a}	11.0 ^{ab}	11.1 ^{b}	0.1	*0.004*
Egg surface area (cm^2)	73.5 ^{a}	75.8 ^{b}	74.2 ^{a}	0.5	*0.000*
Shell index (g/cm^2)	0.092 ^{a}	0.095 ^{b}	0.095 ^{b}	0.001	*0.001*
Shell thickness (mm)	0.463 ^{b}	0.456 ^{a}	0.476 ^{c}	0.005	*0.000*
Compression test					
Strength (N)	37.8 ^{b}	35.1 ^{a}	40.1 ^{c}	0.9	*0.000*
Displacement (mm)	0.27	0.27	0.27	0.0	*0.257*
Energy (mJ)	5.8 ^{b}	5.5 ^{a}	6.3 ^{c}	0.2	*0.000*

P represents the significance level of the "Housing system" factor for ANOVA; different letters in the same raw indicate significant differences at P≤0.05 following LSD test.

Table 2. Mean values and pooled standard error (pooled SEM) of the shell characteristics of eggs from hens of different ages (from 20 to 68 weeks) reared in different housing systems

Variable	Average hen age (weeks)			Pooled SEM			*P*	
	27	30	35	43	53	68		
Cracked eggs (%)	3.1 ^{a}	5.2 ^{a}	4.7 ^{a}	5.9 ^{a}	4.7 ^{a}	17.3 ^{b}	1.8	0.007
Egg weight (g)	60.7 ^{a}	62.1 ^{b}	64.1 ^{c}	66.0 ^{d}	66.0 ^{d}	66.6 ^{d}	0.8	0.000
Shell percentage (%)	10.4 ^{a}	10.9 ^{b}	10.8 ^{b}	11.6 ^{d}	11.4 ^{c}	10.8 ^{b}	0.2	0.000
Egg surface area (cm^2)	72.1 ^{a}	73.3 ^{b}	74.7 ^{c}	75.5 ^{cd}	75.4 ^{cd}	76.0 ^{d}	1.0	0.000
Shell index (g/cm^2)	0.088 ^{a}	0.093 ^{b}	0.092 ^{b}	0.100 ^{d}	0.098 ^{c}	0.093 ^{b}	0.002	0.000
Shell thickness(mm)	0.457 ^{ab}	0.466 ^{cd}	0.465 ^{bc}	0.480 ^{e}	0.476 ^{de}	0.449 ^{a}	0.009	0.000
Compression test								
Strength (N)	37.0 ^{b}	38.5 ^{bc}	38.9 ^{c}	40.0 ^{c}	38.4 ^{bc}	32.9 ^{a}	1.7	0.000
Displacement (mm)	0.26 ^{b}	0.27 ^{bc}	0.28 ^{c}	0.29 ^{d}	0.27 ^{bc}	0.25 ^{a}	0.01	0.000
Energy (mJ)	5.7 ^{b}	6.1 ^{c}	6.2 ^{cd}	6.5 ^{d}	5.8 ^{bc}	4.7 ^{a}	0.4	0.000

P represents the significance level of the "Hen age" factor for ANOVA; different letters in the same raw indicate significant differences at P≤0.05 following LSD test.

On average, cage eggs were the smallest and consistently presented the lowest shell percentage and surface area; shell index of cage eggs was also the lowest, although with values intermediate between barn and organic eggs for cracked eggs, shell thickness, strength and energy. These results indicate a clear relationship between the percentage of cracked eggs and shell breaking strength. Actually, a significant negative correlation ($P<0.001$; $R=0.997$) was observed between shell strength and cracked eggs. Moreover, shell resistance seems to be more dependent on shell thickness than on egg weight. In fact, significant correlations ($P < 0.001$) were found for shell thickness vs shell strength and energy ($R = 0.902$ and $R = 0.828$, respectively), while no correlation was found between shell strength and egg weight. However, an indirect correlation ($P<0.05$) between egg weight and shell breaking strength was previously reported by *Casiraghi et al. (2005)* for cage eggs of the size S, M, L, and XL and by *Clerici et al. (2006)* ($P<0.01$) for eggs from hens aged 28-64 wk reared in cage, free-range, barn and organic systems.

Bone strength. Mean values and standard errors of the bone characteristics of hens at the end of the production cycle are reported in Table 3. As evidenced by the ANOVA results, the housing system had a significant effect on the tibiotarsus and humerus breaking strength and stiffness. In particular, hens reared in the organic system showed tougher humerus with respect to cage and barn hens, whereas the tibiotarsus was stronger only if compared with barn hens.

Table 3. Mean values and pooled standard error (pooled SEM) of the bone characteristics at the end of the production cycle of hens reared in three different housing systems

Variable	Housing system			Pooled SEM	*P*
	Cage	Barn	Organic		
Tibiotarsus					
Breaking strength (N)	157 [ab]	146 [a]	171 [b]	9.2	*0.0072*
Stiffness (N/mm)	157 [b]	143 [a]	162 [b]	7.2	*0.0071*
Centre mean diameter (mm)	7.2 [a]	7.4 [a]	7.6 [b]	0.1	*0.0039*
Humerus					
Breaking strength (N)	150 [a]	182 [b]	211 [c]	13.7	*0.0000*
Stiffness (N/mm)	197 [a]	229 [b]	276 [c]	17.9	*0.0000*
Centre mean diameter (mm)	7.3 [a]	7.5 [b]	7.5 [ab]	0.1	*0.0039*

P represents the significance level of the factor "Housing system" for ANOVA; different letters in the same raw indicate significant differences at $P \leq 0.05$ following LSD test.

Layer performances. Our data were in contrast with several authors that found a better production in cage than in barn system. *Voslarova et al. (2006)* ascribed this fact to some eggs being mislaid outside the nests in the barn system. In addition, mislaid eggs are often damaged and broken and the number of laying hens is also decreased as a consequence of the higher incidence of mortality in the deep litter system. As expected, in the present study the second category eggs (dirty, misshapen or broken eggs) that are discarded during collection were higher in organic and barn than in cage system (4.6% O, 4.3% B, 3.8% C; $P<0.05$), however the mortality was the highest in cage. Some authors (*De Boer and Cornelissen, 2002; Petermann, 2003; Tauson, 2005*), documented in their studies that the replacement of traditional battery cage systems by alternative systems does not always have a positive impact on egg production, hen health and incidence of mortality. Accordingly, the LayWel database (*LayWel, 2006*) showed more dirty eggs in non cage systems, however this seemed to depend on the inclusion of floor eggs in the computation (*Rossi and De Reu, 2011*). Actually, *De Reu et al. (2009)* showed that the frequency of dirty eggs in nests of non-cage systems is not any higher than observed in cage systems.

The mortality observed in the present study for cage system was 6.8%, similar to the average value reported by *Hammershøj (2011)* for the 2008 Danish battery cages (6%). Surprisingly, lower mortality levels were obtained for organic and barn systems (2.4 and 4.2%, respectively). Differently, several authors reported that the incidence of mortality was greater in floor-reared laying hens than in laying hens reared in conventional cages (*Weitzenburger et al., 2005; Hammershøj, 2011*). The low levels of mortality observed in the present study are probably due to the high management level, good prevention practices and good health of animals. In fact, *Tauson et al. (1999)*, studying the mortality in cages compared

with aviary systems, detected higher levels of mortality (21 - 27%), caused mainly by bacterial infections due to pecking at naked skin by more aggressive laying hens.

Feed conversion rate (FCR) showed a value of 2.13 in cage system, lower than barn and organic values (2.20 and 2.30, respectively).

Usually FCR increases due to higher movement level in birds in systems with more activity, like organic and barn systems, in comparison to conventional cages (*Tauson et al., 1999; Michel and Huonnic, 2003*) but also due to higher heat lost in relation to feather cover and environmental temperature (*Peguri and Coon, 1993*).

Egg weight and eggshell characteristics. Considering the F values (data not shown), hen age had a great effect on all the variables, with major influence on egg weight, shell index, cracked eggs, shell percentage, displacement and energy in the compression test; the housing system was also important for the majority of variables but at levels lower than hen age. Housing system instead was the most influent factor for egg surface area, shell thickness and breaking strength. Differently, S x A interactions, even if significant, had minor effects.

Heavier eggs in barn system were observed also by *Pavlovski et al. (1981)*, *Hughes et al. (1985)* and *Voslarova et al. (2005)*; similarly, *Vits et al. (2005)* reported a higher egg weight in floor-reared laying hens. However, several authors reported a higher egg weight in cage system (*Mohan et al., 1991; Pavlovski et al., 1994a, 1994b, 2001*) and other researchers demonstrated no effect of housing system on egg weight (*Mostert et al., 1995; Van den Brand et al., 2004*).

In agreement with the lowest occurrence of cracked eggs, organic eggs showed the highest shell percentage, the highest shell thickness and the most resistant shell, as expressed by their strength and energy values.

In accordance to our results, *Leyendecker et al. (2001b)* observed thicker shells in free range eggs in a study including also cage and aviary (barn) systems, and *Mohan et al. (1991)* evidenced a thicker shell in cage eggs compared to deep-litter housing. Differently, *Pavlovski et al. (2001)*, analysing eggs from three different systems including cage, detected thicker shells in barn eggs and thinner shells in free range eggs.

Egg weight and egg surface area increased with layer age in all housing systems (Table 3), following the physiological development of the animals, as reported by different authors (*Hill and Hall, 1980; O'Sullivan et al., 1991; Peebles et al., 2000; Silversides and Scott, 2001; Van den Brand, 2004; Rizzi and Chiericato, 2005*).

Shell thickness and percentage as well as shell index, shell strength, displacement and energy of the compression test followed a common trend during the production cycle: they initially increased until the layers were 43-weeks-old, and then decreased reaching, at 68 wk, values generally lower than those of young hens (27 wk). These results agree with the sudden increase of cracked eggs

percentage at the end of the laying cycle. However, the common trend observed reflects mainly the fluctuation in the response variables evidenced when data are plotted on separate curves for the three housing systems. In fact, in Figure 2, almost steady breaking strength and shell thickness of cage eggs are observable as a function of hen age. On the contrary it is evident that the common trend described above was mainly due to the variations of non-cage eggs values, especially organic eggs.

Several authors observed a significant decrease of shell thickness with hen age (*Suk and Park, 2001; Rizzi and Chiericato, 2005*). A shell percentage decrease with age was reported by *Silversides and Scott (2001)*. *Rodriguez-Navarro et al. (2002)* attributed the low resistance of old hens shells to changes in structural properties of the eggshell associated with aging. However, *O'Sullivan et al. (1991)* and *Peebles et al. (2000)* found no clear effect of hen age on shell thickness and percentage. This inconsistency of the literature data are totally justified by our results, showing for instance completely different trends over hen aging for shell thickness of cage, organic and barn eggs (Figure 2).

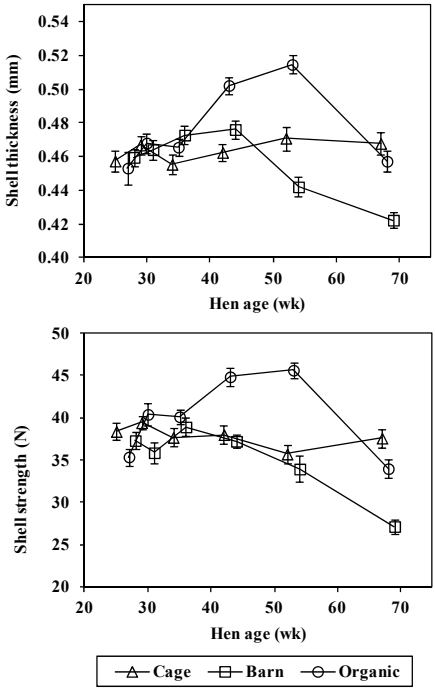


Figure 2: Shell strength and thickness of eggs from different housing systems throughout the complete hen laying cycle. Vertical bars represent the standard errors.

Bone strength. The differences among systems cannot be ascribed to the size of the bones, being the mean centre diameters very similar, although statistically different. In fact, no significant correlations were found between bone size and mechanical properties.

Several studies have shown a consistently higher incidence of bone fragility in caged laying hens compared to that of hens kept in alternative housing systems, mainly due to the limited opportunity to exercise (*Fleming et al., 1994; Van Niekerk and Reuvekamp, 1994; Leyendecker et al., 2001; Leyendecker et al., 2005*). In this research, only humeri of caged hens resulted weaker than those of hens reared in barn or organic systems. No significant differences in tibia breaking strength and stiffness were observed between cage and organic systems. Also previous studies comparing bone characteristics of hens reared in different housing systems reported significant differences in humeri mechanical indices and the lack of differences in tibiae properties (*Hughes et al., 1993; Abrahamsson and Tauson, 1997; Leyendecker et al., 2005*).

A great space availability and the presence of perches and sand baths increased humerus strength, probably because hens performed behaviours such as wing stretching, wing flapping, and sand bathing that have a positive effect on the bone mechanical properties (*Leyendecker et al., 2005*).

Conclusion

In conclusion the results suggested that the barn and the organic systems, as alternative systems to replace cage, showed layers performance similar to the cage system.

The study demonstrated that shell strength and thickness in non-cage eggs are highly affected by hen age, while they are much stable in cage eggs. Organic hens produce eggs with the most resistant shell and have the strongest humerous, while their tibiotarsus are as robust as those of cage hens.

Proizvodni rezultati, osobine ljuske i čvrstoća kostiju kokoši nosilja u tri različita sistema držanja

S. Lolli, A. Hidalgo, C. Alamprese, V. Ferrante, M. Rossi

Rezime

Cilj ovog istraživanja je bio da se utvrde efekti tri različita sistema držanja kokoši nosilje (u kavezima, živinarniku i organsko držanje) na proizvodne rezultate kokoši nosilja, osobine ljuske i čvrstoću kostiju. U svakom sistemu, korišćen je isti

hibrid nosilja (Hyline Braun ®) koje su bile smeštene u skladu sa trenutnim važećim evropskim propisom i kokoške su bile na istom nivou ishrane (2800 ME).

Istraživanje je sprovedeno u periodu od godinu dana na tri tipične farme na severu Italije, od 18. do 70. nedelje starosti kokoši. Broj jaja prikupljenih i izleženih na podu beležen je nedeljno, kao i mortalitet i potrošnja hrane. U 27, 30, 35, 43, 53 i 68 nedelji starosti nosilja, analizirani su težina i osobine ljuske jaja iz različitih sistema. Sila lomljena i čvrstoća su određivani testom tri tačke savijanja.

Procenat leženja je generalno veći u odnosu na standardnu proizvodnju Hyline nosilja, verovatno zbog visokog standarda upravljanja i proizvodne perzistencije. Rezultati su pokazali jasnu vezu između procenta ispucalih jaja i osobine čvrstoće ljuske, gde su organska jaja pokazala najveću debljinu ljuske, najotporniji ljusku i u skladu sa time manje slomljenih jaja. S obzirom na promene koje se dešavaju u toku ciklusa izlaganja, čvrstoća ljuske i debljina kod jaja koja ne potiču iz kavezne proizvodnje su pod snažnim uticajem starosti nosilja, dok su mnogo stabilniji kod jaja iz kaveza. Organske kokoši nosilje su takođe imale najjači humerus, dok je njihov tibiotarsus bio robustan kao i kod kokoši nosilja iz kaveza.

References

ABRAHAMSSON P., TAUSON R. (1997): Effects of group size on performance, health and birds' use of facilities in furnished cages for laying hens. Acta Agriculturae Scandinavica. 47:254-260.

BARNETT J.L. (1998): The welfare and productivity of hens in barn system and cages: a report for the Rural Industries research and Development Corporation. 1998. RIRDC Project No DAV 112°.

BUDGELL, K.L., SILVERSIDES F.G. (2004): Bone breakage in three strains of end of-lay hens. Canadian Journal of Animal Science, 84:745-747.

CASIRAGHI E., HIDALGO A., ROSSI M. (2005): Influence of weight grade on shell characteristics of marketed hen eggs. In: Proceedings of the 10th European symposium on the quality of eggs and egg products, pp 83–188 (Doorwerth: WPSA).

CLERICI F., CASIRAGHI E., HIDALGO A., ROSSI M. (2006): Evaluation of eggshell quality characteristics in relation to the housing system of laying hens. Proc. XII European Poult. Conf., pp 1-6 (Verona, Italy).

DE BOER I.J.M., CORNELISSEN A.M.G. (2002): A Method Using Sustainability Indicators to Compare Conventional and Animal-Friendly Egg Production Systems. Poultry Science. 81:173–181

DE REU K., RODENBURG B., GREIJSPEERDT K., HEYNDRICKX M., TUYTTENS F., SONCK B., ZOONS J., HERMAN L. (2009): Bacteriological contamination, dirt and cracks of eggshells in furnished cages and non-cage

systems for laying hens, an international on farm comparison. Poultry Science. 88: 2442-2448.
DUNCAN I.J.H., FRASER D. (1997): Understanding animal welfare. In: APPLEBY M.C. & HUGHES B.O. (Eds.) Animal welfare. pp. 19–31 (Wallingford, UK, CABI Publishers).
EU (1999) Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. OJ L203, 03/08/1999, p. 0053.
EU (2007) Council Regulation (EC) No 834/2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L189, 1-23.
FLEMING R.H., MCCORMACK H.A., MCTEIR H.A., WHITEHEAD C.C. (1996): The influence of medullary bone on humeral breaking strength. British Poultry Science. 37:30-32.
FLEMING R.H., WHITEHEAD C.C., ALVEY D., GREGORY N.G., WILKINS L.J. (1994): Bone structure and breaking strength in laying hens housed in different husbandry conditions. British Poultry Science. 35:651-662.
FREIRE L.J., WILKINS F., SHORT F., NICOL C.J. (2003): Behaviour and welfare of individual laying hens in a non-cage system. British Poultry Science. 44:22-29.
GRAML C., WAIBLINGER S., NIEBUHR K. (2008): Validation of tests for on-farm assessment of the hen-human relation in non-cage systems. Applied Animal Behaviour Science. 111:301-310.
GREGORY N.G., WILKINS L.J. (1989): Broken bones in domestic fowl: handling and processing damage in end-of lay battery hens. British Poultry Science. 30:555-562.
GREGORY N.G., WILKINS L.J., ELEPERUMA S.D. (1990): Broken bones in domestic fowls: Effect of husbandry system and stunning method in end-of-lay hens. British Poultry Science. 31:59-69.
GREGORY N.G., WILKINS L.J., KNOWLES T.G., SORENSEN P., VAN NIERKERK T. (1994): Incidence of bone fractures in European layers. In: Walker and Connell Proceedings of the Ninth European Poultry Conference Vol.2 (Glasgow, UK).
HAMMERSHØJ M. (2011): Organic and free-range egg production. In: NYS Y., BAIN M., VAN IMMERSEEL F. (Eds.): Improving the safety and quality of eggs and egg products. Volume 1, pp 463-486 (Woodhead Publishing Limited, Cambridge, UK).
HILLS A.T., HALL W. (1980): Effects of various combinations of oil spraying, washing, sanitizing, storage time, strain, and age of layer upon albumen quality changes in storage and minimum sample sizes required for their measurement. Poultry Science. 59:2237-2242.
HUGHES B.O., DUN P., MCCORQUODALE C.C. (1985): Shell strength of eggs from medium-bodied hybrid hens housed in cages or on range in outside pens.

British Poultry Science. 26:129-136.
HUGHES B.O., WILSON S., APPLEBY M.C., SMITH S.F. (1993): Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. Research in Veterinary Science. 54:202-206.
LAYWEL (2006): Work package 6 final report productivity and egg quality. Available from http://www.laywel.eu. Accessed March 2010.
LEESON S., DIAZ G., SUMMERS J.D. (1995): Poultry Metabolic Disorders and Nycotoxins, University Books, Guelph, Ontario, Canada.
LEYENDECKER M., HAMANN H., HARTUNG J. (2005): Keeping laying hens in furnished cages and an aviary housing system enhances their bone stability. British Poultry Science. 46:536-544.
LEYENDECKER M., HAMANN H., HARTUNG J., KAMPHENS J., RING C., GLUNDER G., AHLERS C., SANDER I., NEUMAN U., DISTL O. (2001): Analysis of genotype-environment interactions between layer lines and housing systems for performance traits, egg quality and bone breaking strength. 2nd communication: Egg quality traits. Zuchtungskunde. 73, 4:308-323.
MCCOY M.A., REILLY G.A.C., KILPATRICK, D.J. (1996): Density and breaking strength of bones of mortalities among caged layers. Research in Veterinary Science. 60:185-186.
MICHEL V., HUONNIC D. (2003): A comparison of welfare, health and production performance of laying hens reared in cages or in aviaries. British Poultry Science, 44/5:775-776.
MOHAN A., REDDY V.R., REDDY P.V., REDDY P.S. (1991): Utilization of pearl millet *(Pennisetum tynoides*) for egg production. British Poultry Science. 32:463–469.
MOSTERT B.E., BOWES E.H., VAN DER WALT J.C. (1995): Influence of different housing systems on the performance of hens of four laying strains. Suid-Afrikaanse Tydskrif vir Veekunde. 25:80-86.
NYS Y., BURLOT T., DUNN I. C. (2008): Internal quality of eggs: any better, any worse? XXIII World's Poultry Congress, Brisbane, Australia, June 30-July 4, 2008, World's Poultry Science Association (Australian branch). 113. (CD-Rom (papers\\wpc08Final00034).
PAVLOVSKI Z., HOPIĆ S., LUKIĆ M. (2001): Housing systems for layers and egg quality. Biotechnology in Animal Husbandry. 17:197–201.
PAVLOSKI Z., MASIC B., APOSTOLOV N. (1981): Quality of eggs laid by hens on free range and in cages. In: BEUVING, G., SHEELE, C.W. & SIMONS, P.C.M. (Eds) Quality of Eggs. Proceedings of the First European Symposium, pp. 231-235 (Apeldoom, the Netherlands).
PAVLOVSKI Z., VRACAR S., MASIC B. (1994b): The effects of housing system on external egg quality traits in small flocks of layers. Biotehnologija u Stočarstvu. 10(3/4):13-19
PAVLOVSKI Z., HOPIC S., VRACAR S., MASIC B. (1994a): The effect of

housing system on internal egg quality traits in small layer flocks. Biotehnologija u Stočarstvu. 10(5/6):37-43
PEEBLES E.D., ZUMWALT C.D., DOYLE S.M., GERARD P.D., LATOUR M.A., BOYLE C.R., SMITH T.W. (2000): Effects of breeder age and dietary fat source and level on broiler hatching egg characteristics. Poultry Science. 79:698-704.
PEGURI A., COON C. (1993): Effect of feather coverage and temperature on layer performance. Poultry Science. 72:1318-1329.
PETERMAN S. (2003): Laying hens in alternative housing systems – practical experiences. Dtsch. Tierarztl. Wochenschr. 110:220–224
O'SULLIVAN N.P., DUNNINGTON E.A., SIEGEL P.B. (1991): Relationships among age of dam, egg components, embryo lipid transfer, and hatchability of broiler breeder eggs. Poultry Science. 70:2180–2185.
RIZZI C., CHIERICATO G.M. (2005): Organic farming production. Effect of age on the productive yield and egg quality of hens of two commercial hybrid lines and two local breeds. Italian Journal of Animal Science. 4:160-162.
RODRIGUEZ-NAVARRO A., KALIN O., NYS Y., GARCIA-RUIZ J.M. (2002): Influence of the microstructure on the shell strength of eggs laid by hens of different ages. British Poultry Science. 43:395–403.
ROSSI M., DE REU K. (2011): Alternative hen housing systems and egg quality. In. NYS Y., BAIN M., VAN IMMERSEEL F. (Eds.): Improving the safety and quality of eggs and egg products. Volume 1, pp 351-375 (Woodhead Publishing Limited, Cambridge, UK).
SANDILANDS V., SPARKS, N. WILSON S., NEVISON I. (2005): Laying hens at depopulation: the impact of the production system on bird welfare. British Poultry Abstract. 1:23-24.
SILVERSIDES F.G., SCOTT T.A. (2001): Effect of storage and layer age on quality of eggs from two lines of hens. Poultry Science. 80:1240-1245.
SUK Y., PARK C. (2001): Effects of broiler breeder age and length of egg storage on albumen characteristics and hatchability. Poultry Science. 80:855-858.
SUPIĆ B., PERIĆ L., MILOŠEVIĆ N., PAVLOVSKI Z., CMILJANIĆ R. (1999): Uticaj ishrane na kvalitet konzumnih jaja. Savremena poljoprivreda. 1-2:57-64.
TAUSON R. (2005): Management and housing systems for layers – effects on welfare and production. Worlds Poultry Science Journal. 61:477–490.
TAUSON R., ABRAHAMSSON P. (1994): Effects on production, health and behaviour in three SCWL strains in an EMC model in comparison with other modified and conventional cage. In: SHERWIN C.M. Modified Cages for Laying Hens, pp 41-54 (ed. Univ. Fed. Anim. Welf., Potters Bar, UK).
TAUSON R., WAHLSTROM A., ABRAHAMSSON P. (1999): Effect of two floor housing systems and cages on health, production and fear response in layers. Journal Applied Poultry Research. 8:152-159.
THOMPSON B.K., HAMILTON R.M.G., GRUNDER A.A. (1985): The

relationship between laboratory measures of egg shell quality and breakage in commercial egg washing and candling equipment. Poultry Science. 64:901-909.
TRAVEL A., NYS Y., BAIN M. (2011): Effects of hen age, moult, laying environment and egg storage on egg quality. In: NYS Y., BAIN M., VAN IMMERSEEL F. (Eds): Improving the safety and quality of eggs and egg products. Volume 1, 300-329 (Woodhead Publishing Limited, Cambridge, UK).
VAN DEN BRAND H., PARMENTIER H.K., KEMP B. (2004): Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. British Poultry Science. 45:745–752.
VAN NIEKERK T.G.M.C., REUVEKAMP B.F.J. (1994): Husbandry factors and bone strength in laying hens, in: Proceedings of the 9th European Poultry Conference. 133-136 (Glasgow, UK).
VITS A., WEIZENBÜRGER HAMANN H., DISTL, O. (2005): Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes. Poultry Science. 84:1511-1519.
VOSLAROVA E., HANZALEK Z., VECEREK V., STRAKOVA E., SUCHY P. (2006): Comparison between laying hen performance in the cage system and the deep litter system on a diet free from animal protein. Acta Veterinaria Brinesis. 75:219-225.
WEITZENBÜRGER D., VITS A., HAMANN H., DISTL O. (2005): Effect of furnished small group housing systems and furnished cages on mortality and causes of death in two layer strains. British Poultry Science. 46:553-559.
WEBSTER A.B. (2004): Welfare implications of avian osteoporosis. Poultry Science. 83:184-192.
WHITEHEAD C.C. (2004): Skeletal disorders in laying hens: the problem of osteopororis and bone fractures in: PERRY G.C. (Ed.): Welfare of laying hen. Poultry Science Symposium Series. 27:259-278. Wallingford, Oxon: CABI Publishing.
WILSON S., DUFF S.R.I., WHITEHEAD C.C. (1992): Effects of age, sex and housing on the trabecular bone of laying genetic group domestic fowl. Research in Veterinary Science. 50:52-58.

Received 15 October 2013; accepted for publication 20 December 2013

Biotechnology in Animal Husbandry 29 (4), p 607-614 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.31'65
DOI: 10.2298/ BAH1304607M

EFFECT OF LIGHTING PROGRAM AND ENERGY LEVEL IN THE RATION ON THE SLAUGHTER TRAITS OF BROILERS

N. Milošević[1], M. Veljić[2], M. Đukić Stojčić[1], L. Perić[1], S. Bjedov[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000, Novi Sad, Republic of Serbia
[2]University of Montenegro, Faculty of Biotechnology, Mihajla Lalića 1, 81000, Podgorica, Montenegro
Corresponding author: niko.milosevic@stocarstvo.edu.rs
Original scientific paper

Abstract: Investigations were carried out to assess the effect of lighting program and energy levels in the diet on carcass characteristics of broilers. The experiment was performed on chickens Cobb 500 hybrids for up to 42 days. The setting was reflected by the principle of two factorial experiments (2x2) at the 40 broiler carcasses of both sexes (10 per replications). The first factor was the lighting program where a group represented by the application of broiler chickens lighting 23L: 1D and group B of the regime 1. wk – 23L: 1D; 2. wk – 12L: 12D, 3. wk –14L: 10D; 4. wk - 16L: 8D; 5.wk –18L: 6D, 6. wk –20L: 4D. Another factor was the energy level where meals are in the "A" group were carcasses of broiler chickens fed diets with standard protein and energy in group "B" were the carcasses of chickens fed diets with high energy content to 0.40 ME MJ / kg compared the standard mixtures. Tests have shown that a lighting program and energy level as factors have no significant effect on carcass yield of broiler chickens, but significant differences ($p < 0.05$) emerged as the interaction Axb and Bxa combinations of the parameters “ready to roast" and "ready to grill". The amount of abdominal fat was significantly higher ($p < 0.05$) in group A and Axb combination compared to Bxa and Bxb. Share weight edible offal expressed as % of “carcass cut classic " was significantly higher ($p < 0.05$) in group B. The combination of Axb had a significantly lower proportion of edible offal compared to the combination of Bxa.

Key words: broiler, light, energy level, carcass traits

Introduction

Broiler chickens are usually grown on a continuous or nearly continuous program of lighting (23 L: 1 D) in order to increase food intake and their growth

(*Kampo and Davila, 2002*). The downside of this is the accumulation of fat in the carcasses, higher incidence of metabolic disorders, and increased occurrence of skeletal deformities *(Kristensen et al., 2006; Olanrewaju et al 2006; Onbasilar et al., 2007; Škrbić et al. 2009b, 2012)*. More research was realized in order to examine different light programs in combination with a protein content and density of the ration for fattening broiler chickens. Tests have shown that, depending on the applied program length lighting can positively and negatively affect the performance of broiler chickens and on carcass quality *(Onbasilar et al., 2007; Archer et al., 2009)*. In addition to continuous lighting there were programs with discontinuous and achemeral light (*Li et al., 2007; Škrbić et al., 2009a)* and a wide range of light intensity *(Blatchford. et al, 2009; Li, et al., 2007, 2010)*.

Diet composition as a factor also is essential for the majority of production parameters. For maximum production and good quality meat, broiler chickens must be provided optimum ratio of protein and energy content of the meal. High-density meals may increase the price of food *(Brickett et al., 2007; Kamran et al., 2008; Huang et al., 2009)*, excretion of nitrogen *(Bregendahl et al., 2002)*, deposition of fat and the incidence of metabolic disorders *(Nahashon et al., 2005)*. Most studies show that feeding high energy meal increases body weight but reduced FCR *(Kampo and Davila, 2002; Nahashon et al., 2005; Archer et al., 2009)*, and produces hulls better conformations *(Nahashon al, 2005; Brickette et al, 2007)*, but significantly higher content of abdominal fat *(Sikur et al, 2004; Fan et al., 2008)* compared with the low-energy diet. Some researchers suggest that portions of low density will result in lower FCR *(Wu et al., 2007; Fanatico et al., 2008)*, and has no effect on carcass yield, breast meat yield, thigh and abdominal fat *(Kamran et al., 2008)*. Little research has focused on the interaction of light and the energy levels of a meal on production performance and carcass quality of broilers *(Buys et al. 1998)*. Testing was done in order to determine the effect of the length of the light period and the level of energy in food to carcass yield, abdominal fat content and edible offal of broiler chickens.

Materials and methods

The experiment was performed on chickens Cobb 500 hybrids that were grown in the floor system of holding up to 42 days of age. The tests were carried out two factorial experiments (2x2) with five repetitions per treatment with 20 chicks in each replication. The setting of the experiment is given in the attached schedule.

Treatment	Level of factor
Program of screen: 23L: 1D - standard	A

Program of screen: I week – 23L: 1D; II – 12L: 12D, III – 14L: 10D; IV – 16L: 8D; V – 18L: 6D, VI – 20L: 4D	B
Food with standard facilities in protein and energy	a
Food with a heightened energy facilities in 12:50 MJ ME / kg of a mixture	b

During the test it has been applied a standard technology for fattening broiler chickens. From the start experiment all relevant production parameters were monitored. Chickens were fed *ad libitum*. Feeding chickens was with three types of mixtures; starter mixture to 14 days of age that contained 21.22% CP and 12.3 MJ / kg ME grower to 35 days with a crude protein content of 20.2 and 15.5 MJ / kg ME and finisher to this end, with 18.6% crude protein and 12.7 MJ / kg of ME. In treatment "b" of the existing content meal added with the oil to increase the energy value of a 0:50 MJ ME / kg diet. At the end of the trial with 6 weeks of age all birds were measured individually in groups to calculate the averages of groups and then from each experimental group was allocated 10 birds (5 male and 5 female) to investigate the slaughter characteristics. Prior to slaughter chickens starved 6 hours, then measured their body weight, then hand-slaughtered carcasses and processed using conventional method.

Data obtained by records of carcass yield parameters, content of abdominal fat and weight of edible offal (liver, heart, stomach, total edible offal) were processed by a computer program STATISTICA 12., identified as average values and variability measures. Performed an analysis of variance (ANOVA), and in the expression of statistical significance in the analysis of variance was applied Duncan test (Duncan Multiple Range Test) at the probability level $p \leq 0.05$.

Results and Discussion

Analysis of carcass yield (Table 1) shows that there were statistically significant differences among all treatments and interaction treatment of processing the bodies (KO, SP and SR), which is due to different average body weight of broiler chickens selected sacrificed. This is an indicator that the lighting program and energy content in the diet of chickens factors that significantly affected ($P < 0.05$) on carcass yield in broiler chickens. When it comes to yields as the relative value of the carcass yield, individual treatments (light programs and density meals) had no significant effect, but there were significant spills ($P < 0.05$) interacting with slate SP combination between Axa and BXA. Statistically significant differences ($P < 0.05$) were noted in the SR yield combinations of Axa on the one hand and BXA and BXB. The available literature in other studies no significant difference in the effect of lighting program yields, but is the impact of the energy value of meals *(Brickette et al., 2007; Blatchford et al., 2009; Huang et al., 2009).*

Table 1. Carcass yield and dressing percentage

Effect	Fact.	N	BW[1]	Carcass yield, g			Dressing percentage, %		
				KO	SP	SR	KO	SP	SR
Total		40	2129	1889.6	1756.93	1583.57	82.41	76.64	67.92
Light	A	20	2215	1982.5[b]	1849.1[b]	1672.2[b]	82.46	76.93	68.45
	B	20	2042	1796.7[a]	1664.8[a]	1494.9[a]	82.36	76.35	67.40
Density meal	a	20	2158	1935.5[b]	1800.7	1622.6	82.62	76.89	68.26
	b	20	2099	1843.7[a]	1713.1	1544.5	82.21	76.39	67.59
Light x Density meal	Axa	10	2210	2019.8[b]	1885.7[b]	1705.7[b]	83.09	77,61[b]	69.19[b]
	Axb	10	2220	1945.1[bc]	1812,4[bc]	1638.8[bc]	81.83	76.26	67.71
	Bxa	10	2106	1851.2[ac]	1715.8[ac]	1539.6[ac]	82.14	76,17[a]	67.33[a]
	Bxb	10	1979	1742.3[a]	1613.9[a]	1450.2[a]	82.58	76.53	67.46[a]

a-c, Means in the same row with different letters are significantly different (P<0.05) KO - hull conventional tillage; Cup - hull ready to roast; FR - hull ready to grill
[1]Body Weight

Light as a factor of the environment (Table 2) were statistically significant (P <0.05) to both the absolute and the relative value of fat in carcass yield of broilers, which were confirmed by other researchers (*Sikur et al, 2004; Fan et al. 2008*). It was expected that the level of energy in the diet significantly influence the content of abdominal fat in carcass however, in this study it has not been confirmed. The reason for this is certainly the high variability in density FAKRA meals ("a" and "b"). The research conducted was in accordance with the research of *Wu et al.,* (2007a), but in contrast to most other studies *(Nahashon al, 2005; Brickette et al, 2007; Fanatico et al., 2008)*. Combinations of AXB and BXA gave a statistically significant difference (P <0 05) in the content of abdominal fat in broiler carcasses.

Table 2. Abdominal fat and edible offal

Effect	Factor	N	KO[1]	Mass in g					The share weight of the hull KO,%				
				Abdo. fat	Liver	Heart	Stomach	Edible offal	Abdo. fat	Liver	Heart	Stomach	Edible offal
Total		40	1889	22	45	10	35	90	1.20	2.39	0.53	1.88	4.80
Light	A	20	1982	24[b]	45	10	35	90	1.24	2.27[a]	0.52	1.77[a]	4.55[a]
	B	20	1796	20	45	9	35	91	1.16	2.52[b]	0.55	2.00[b]	5.06[b]
Density meal	a	20	1935	21	46	10	36	92	1.11	2.39	0.53	1.88	4.79
	b	20	1843	23	44	9	34	88	1.30	2.39	0.54	1.88	4.81
Light x Density meal	Axa	10	2019	22	46	10	35	91	1.11	2.29	0.50	1.74	4.53
	Axb	10	1945	26[b]	43	10	34	89	1.38[b]	2.25[a]	0.54	1.79	4.58[a]
	Bxa	10	1851	20[a]	46	10	37	94	1.11[a]	2.50	0.56	2.02	5.08[b]
	Bxb	10	1742	21[a]	44	9	34	88	1.210	2.54[b]	0.54	1.97	5.05

a-b, Means in the same row with different letters are significantly different (P<0 05)
[1] Mass carcass (Traditionally dressed carcass)

Light program "B" had significantly greater relative weight of the liver, stomach and a relatively larger share of supply of edible offal (P <0.05). The available literature on the impact of the program lighting values for these parameters is confirmed. The interaction of light meals programs and density in combination AXB and BXB showed significant differences (P <0.05) in the relative weight of the liver and stomach, and combinations composed between AXB and BXA in bulk edible offal (Table 2). These relationships also available literature could not be confirmed nor denied.

Conclusion

Overall conclusion in the shortest is the length of the light in broilers has a significant effect (P <0.05) the absolute value of all the carcass yield of broilers tested because of differences in average body weight at the time of sacrificing chickens. The impact of the light program and meals density did not give significant effects on yields of broilers, but the interaction of the light program and meals density combined Axa in relation to the combination of parameter BXA yield of SP was significantly better (P <0.05). The same was the case with the combination of Axa in relation to combination BXA and BXB the parameters in SR (P <0.05). The combination of lighting program B was significantly lower content of abdominal fat in broiler carcasses in relation to the light program A (P <0.05) combinations of light programs and density of meals BXA and BXB had a significantly lower share of abdominal fat in broiler carcasses in relation to the combination of AXB (P<0.05) light program B influenced the broiler chickens have a relatively larger mass of the liver and stomach as edible offal compared to the light program A (P<0.05). Liver weight was significantly greater in the combination of factors in relation to the BXB bxb and edible offal weight of this combination compared to BXA (P <0.05).

Acknowledgment

The study „ **Effect of lighting program and energy level in the ration on the slaughter traits of broilers** ” is part of research project TR 31033 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Uticaj svetlosnog programa i nivoa energije u obroku na klanična svojstva brojlera

N. Milošević, M. Veljić, M. Đukić Stojčić, L. Perić, S. Bjedov

Rezime

Istraživanja su izvedena kako bi se procenio učinak svetlosnog programa i nivoa energije u hrani na klanična svojstva brojlerskih pilića. Ogled je izveden na pilićima Cobb 500 hibrida u trajanju do 42 dana. Postavka ogleda je bila po principu dvofaktorijalnog ogleda (2x2) na ukupno na 40 brojlerskih trupova oba pola (po 10 ponavljanja). Prvi faktor je bio program osvetljenja gde su grupu A predstavljali trupovi brojlera iz program osvetljenja 23S:1M i grupu B iz režima I ned.- 23S:1M; II – 12S:12M; III – 14S:10M; IV – 16S:8M; V – 18S:6M; VI – 20S:4M. Drugi faktor je bio energetski nivo obroka gde su u grupi "a" bili trupovi iz grupe brojlerskih pilića hranjenih smešama sa standardnim sadržajem proteina i energije i u grupi "b" bili trupovi pilića hranjeni smešama sa povećanim sadržajem energije za 0.40 ME MJ/kg u odnosu na standardne smeše. Žrtvovani pilići su nasumično odabrani iz ispitivanih grupa mase ± 1 std. dev. od proseka grupe. Ispitivanja su pokazala da program svetla i gustina obroka kao faktori nemaju značajnog uticaja na randmane klanja brojlerskih pilića, ali su se značajne razlike ($p < 0,05$) javile kao interakcija kombinacije Axb i Bxa za randmane SP i SR. Količina abdominalne masti bila je značajno veća ($p < 0,05$) grupe A i kombinacija Axb u odnosu na Bxa i Bxb. Udeo mase jetre, želuca i jestivih iznutrica izraženih u % mase trupa KO bio je statatistički značajno veći ($p < 0,05$) za grupu B. Kombinacija Axb imala je značajno manji udeo jestivih iznutrica u odnosu na kombinaciju Bxa.

References

ARCHER, G. S., SHIVAPRASAD, H. L. MENCH, J. A. (2009): Effect of providing light on the health, productivity, and behavior of broiler chickens. Poult. Sci. 88:29-37.

BLATCHFORD, R. A., KLASING, K. C. SHIVAPRASAD, H. L. WAKENELL, P. S., ARCHER, G. S., MENCH, J. A. (2009): The effect of light intensity on the behavior, eye and leg health, and immune function of broiler chickens. Poult. Sci. 88:20-28.

BREGENDAHL, K., SELL, J. L. ZIMMERMAN, D. R. (2002): Effect of low-protein diets on growth performance and body composition of broiler chicks. Poult. Sci. 81:1156-1167.

BRICKETT, K. E., DAHIYA, J. P., CLASSEN, H. L., GOMIS, S. Influence of Dietary Nutrient Density, Feed Form, and Lighting on Growth and Meat Yield of Broiler Chickens. Poultry Science 86:2172–2181. 2007.
BUYS, N., J. BUYSE, HASSANZADEH-LADMAKHI, M. 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.
CAMPO, J. L. DAVILA, S. G. (2002): Effect of photoperiod on heterophil to lymphocyte ratio and tonic immobility duration of chickens. Poult. Sci. 81:1637-1639.
FAN, H. P., XIE, M., WANG, W. W., HOU, S. S. HUANG, W. (2008): Effects of dietary energy on growth performance and carcass quality of white growing Peking ducks from two to six weeks of age. Poult. Sci. 87:1162-1164.
FANATICO, A. C., PILIAI, P. B., HESTE, P. Y., FALCONE, C., OWENS, C. M. d EMMERT, J. L. (2008): Performance, livability, and carcass yield of slow-and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 87:1012-1021.
HUANG, Y., YOO, J. S., KIM, H., WANG, J. Y., CHEN, Y. J., CHO, J. H. KIM, I. H. (2009): Effect of bedding types and different nutrient densities on growth performance, visceral organ weight, and blood characteristics in broiler chickens. J. Appl. Poult. Res. 18 :1–7.
KAMRAN, Z., SARWAR, M., NISA, M., NADEEM, M. A., MAHMOOD, S. AHMED, S. (2008): Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poult. Sci. 87:468-474.
KRISTENSEN, H. H., PERRY, G. C., PRESCOTT, N. B., LADEWIG, J., ERSBOLL, A. K. d WATHES, C. M. (2006): Leg health and performance of broiler chickens reared in different light environments. Br. Poult. Sci. 47(3):257-263.
LI, W., GUO, Y., CHEN, J., WANG, R., HE, Y., SU, D. (2007): Influence of Lighting Schedule and Nutrient Density in Broiler Chickens: Effect on Growth Performance, Carcass Traits and Meat Qualit y. Asian-Aust. J. Anim. Sci. Vol. 20, No. 4 : 550 – 555.
LI, W., GUO, Y., CHEN, J., WANG, R., HE, Y., DONG-GE, S. (2010): Influence of Lighting Schedule and Nutrient Density in Broiler Chickens: Effect on Growth Performance, Carcass Traits and Meat Quality. Asian-Aust. J. Anim. Sci. Vol. 23, No. 11 : 1510 – 1518.
NAHASHON, S. N., ADEFOPE, N., AMENYENU, A. D. WRIGHT, D. (2005): Effects of dietary metabolic energy and crude protein concentrations on growth performance and carcass characteristics of French guinea broilers. Poult. Sci. 84:337-344.

OLANREWAJU, H. A., THAXTON, J. P., DOZIER, W. A., URSWELL, W. B., ROUSH, J. P. BRANTON, S. L. (2006): A review of lighting programs for broiler production. Int. J. Poult. Sci. 5(4):301-308.
ONBASILAR, E. E., EROLL, H., CANTEKIN, Z., KAYA, U. (2007): Influence of intermittent lighting on broiler performance, incidence of tibial dyschondroplasia, tonic immobility, some blood parameters and antibody production. Asian-Aust. J. Anim. Sci. 20(4):550-555.
SIKUR, V. R., ROBINSON, F. E., KORVER, D. R., RENEMA, R. A. ZUIDHOF, M. J. (2004): Effects of nutrient density on growth and carcass traits in fast- and slow-feathering female turkeys. Poult. Sci. 83:1507-1517.
STATSOFT, STATISTICA 12: 2012 Copyright © StatSoft. http://www.statsoft.com/
ŠKRBIĆ, Z., PAVLOVSKI, Z., VITOROVIĆ, D., LUKIĆ, M., PETRIČEVIĆ, V. (2009a): The effects of stocking density and light program on tibia quality of broilers of different genotype. *Archiva Zootechnica* 12:3, 56-63.
ŠKRBIĆ, Z., PAVLOVSKI, Z. LUKIĆ, M. (2009b): Stocking density – factor of production performance, quality and broiler welfare. Biotechnology in Animal Husbandry 25 (5-6), p 359-372.
ŠKRBIĆ, Z., PAVLOVSKI, Z., LUKIĆ, M., PETRIČEVIĆ, V. MILIĆ, D. (2012): The effect of lighting program and type of litter on production and carcass performance of two broiler genotypes. Biotechnology in Animal Husbandry 28 (4), p 807-816.
WU, G., BRYANT, M. M., GUNAWARDANA, P. ROLAND, D. A. (2007) Effect of nutrient density on performance, egg components, egg solids, egg quality, and profits in eight commercial leghorn strains during phase one. Poult. Sci. 86:691-697.

Received 19 June 2013; accepted for publication 30 November 2013

Biotechnology in Animal Husbandry 29 (4), p 615-624 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.05'65
DOI: 10.2298/ BAH1304615S

PRODUCTION PERFORMANCE AND CARCASS QUALITY OF COLOURED BROILERS DIFFERENTIATED GENETIC POTENCIAL FOR GROWTH

Z. Škrbić[1], Z. Pavlovski[1], M. Lukić[1], V. Petričević[1], D. Milić[2]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade, Republic of Serbia
[2]Perutnina Ptuj – Topiko a.d., Petefi Brigade 2, 24300 Bačka Topola, Republic of Serbia
Corresponding author: zdskrbic@gmail.com
Original scientific paper

Abstract: The study was conducted with the purpose of the comparative analysis of production performance and carcass quality of two coloured broiler genotypes of differentiated genetic potential for growth (Redbro and Redbro Naked Neck) in conditions of intensive feeding, as well as determining the effect of housing system (free range and poultry house) on fattening performance of Redbro broilers. Production performance is presented in the two control periods (days 42 and 84). Carcass quality was analysed at 84 days old broilers based on carcass conformation, yield, share of valuable parts and abdominal fat content. Redbro and Redbro Naked Neck broilers had different growth intensity according to the phases of the experiment, but at the age of 84 days, their body weight did not differ significantly (3382.44g to 3236.0g). Lower efficiency of food utilization (3.08 kg to 2.79 kg) and improved vitality (100 % vs. 97.33 %) was found in Redbro Naked Neck broilers. In terms of carcass quality, there were no significant differences except in the share of wings. The positive impact of growing Redbro broilers in the poultry house was determined on the body weight ($p< 0.05$), feed efficiency and broiler mortality, which resulted in higher production index (169.83 vs. 140.37) compared to free range reared Redbro chickens. The differences in carcass quality were determined on the basis of better conformation, a small share of the wings but also lower yields of classically dressed carcasses of broilers Redbro reared in the poultry house.

Key words: coloured broilers, production performance, carcass quality

Introduction

The largest part of the total market oriented production of chicken meat still is in intensive broiler production conditions and in this respect the continuous

increase of the genetic potential for growth of fast-growing broiler genotypes is important. In addition to other necessary technological prerequisites, the maximum production efficiency is provided, which guarantees the lowest price of the product. On the other hand, demands for high quality, safe and healthy food that is produced in accordance with the principles of animal welfare, have caused numerous studies with the aim of comparative analysis of commercial and slow growing genotypes in terms of unconventional production *Ristić and Damme, 2002; Grashorn and Serini 2006; Owens et al., 2006*) and the development of technological processes to produce chicken meat of special quality (*Škrbić et al., 2010*). Previous studies of chicken meat production in the revitalized traditional production system in our country, based on the available, few indigenous chicken breeds, pure breeds and some imported slow growing genotypes, resulted in significantly lower final body weight and meat yield (*Pavlovski et al., 2009b; Pavlovski et al., 2012; Blagojević et al., 2009*) in relation to the conditions of intensive production, which significantly raises the price of the product. Taking into account the results of a survey on consumer willingness to allocate more money for products from non-intensive production systems (*Rodić et al., 2003*), there is a need to balance between productivity and product quality, and in this regard selection of the appropriate genotype and adjustment of technological process are very important. To this end, a study was performed of the effect of extended fattening of fast growing broiler genotypes in extensive production conditions (*Bogosavljević-Bošković et al., 2007*) and an analysis of the production performance of our autochthonous breeds in meat production (*Pavlovski et al., 2009a*). Owing to the significant development of the part of the world poultry meat market originating from non-intensive housing systems, some breeding companies have created several different genotypes of broilers of slow and moderate growth, i.e. of differentiated genetic potential for growth, with feathers in colour and adapted to different conditions and production targets.

The objective of the study was a comparative analysis of production performance and carcass quality of two coloured broiler genotypes with differentiated genetic potential for growth in conditions of intensive feeding, and to determine the impact of housing system on fattening performance of medium-fast growth broilers.

Materials and methods

The experiment was conducted using the Hubbard coloured broilers, genotypes Redbro and Redbro Naked Neck, genetically predisposed to different intensity and speed of growth. In the first phase of the experiment, up to 42 days of age, broilers were reared in floor system in a box with a stocking density of 12 birds/m^2, 50 heads/box and 3 repetitions, in total 150 birds per genotype. In addition, another 150 Redbro broilers were included with the same housing

conditions in the facility until the slaughter age (84 days). Intensive feeding conditions were used in order to maximize growth and achieve better fattening performance (starter 22.2% CP and 3100 Kcal/kg; Grower 19.4 % CP and 3110 Kcal/kg and finisher, containing 17.3 % CP and 3170 Kcal/kg). The light program was following: the period 0-7 days 23L:1D and thereafter 16L:4D:2L:2D. In the second phase of the experiment, in the period from 43-84 days of age, broilers were reared in the free range system. Also at this stage of the experiment, the diet was based on the complete mixture (17 % CP, 3170 Kcal/kg) and broilers in the poultry house with range had free access to pasture 10m^2/bird. Control of body weight was performed by measuring individual broilers at the age of 42 and 84 days, while group food consumption was recorded for genotypes and therefore only the average feed conversion is presented. Mortality were recorded daily by the box, i.e. genotype. The production index was calculated according the following formula:

$$PI = \frac{\text{Body weight, kg} \times (100 - \%\ \text{mortality}) \times 100}{\text{Fattening duration, days} \times \text{feed conversion, kg}}$$

The investigation of the carcass quality, based on measures of carcass conformation (*Pavlovski et al., 2006*), yields of classically dressed carcass, carcass ready to roast, carcass ready to grill, share of valuable carcass parts (breasts, thighs, drumsticks, wings) and abdominal fat content in the carcass, which are expressed relative to body weight of broilers at slaughter, was performed on a random sample formed of 12 broilers (6 per gender) per genotype, i.e. the housing system for Redbro broilers.

Statistical analysis was performed by analysis of variance in the software program Statistica (Stat.Soft, Inc. version 6). Data expressed in percentages were previously transformed to *arcsin*. The evaluation of significance of mean values was performed using the Tukey test.

Results and Discussion

Control of production performance of Redbro and Redbro Naked Neck broilers at the age of 42 days (Table 1) showed significantly higher body weight and lower food efficiency of Redbro chickens. Intensive growth of Redbro Naked Neck broilers in the second phase of the experiment resulted in final body weight without significant statistical difference compared to Redbro broilers in free range system of rearing, at the age of 84 days (Table 2). Feed conversion ratio was worse in Redbro Naked Neck, as opposed to the vitality of these chickens.

Table 1. Production performance of Redbro and Redbro Naked Neck broilers at the age of 42 days

Genotype Production performance	42 days	
	Redbro	Redbro Naked Neck
Body weight, g	$1581.61^{a} \pm 261.54$	$1156.00^{b} \pm 168.96$
Feed conversion, kg	$2.14^{a} \pm 0.07$	$1.78^{b} \pm 0.05$
Mortality, %	0.34	0

*a-b means in the same row with different letters are significantly different ($p<0.05$)

Table 2. Production performance of broilers at the age of 84 days

Genotype/housing system Production performance	84 days		
	Redbro/ free range	Redbro Naked Neck/ free range	Redbro/ poultry house
Body weight, g	$3382.44^{b} \pm 494.51$	$3236.00^{b} \pm 436.56$	$3626.87^{a} \pm 645.54$
Feed conversion, kg	2.79	3.08	2.51
Mortality, %	2.67	0	1.36
Production index	140.37	125.23	169.83

*a-b means in the same row with different letters are significantly different ($p<0.05$)

Growing broilers at constant high temperatures can have an adverse effect on the growth rate and the differences between the genotypes associated with the genetic potential for growth (*Yalcin et al., 1997*). In conditions of summer temperature and free range rearing, Redbro Naked Neck broilers had more intensive growth thanks to favourable expression of gene for naked neck (Na), which provides better adaptability to conditions of high ambient temperature. A hundred percent vitality of these birds can be correlated with the results of *Rajkumar et al. (2010)* which have established the superiority of naked neck genotypes of chickens (*NaNa and Nana*) in immune status, all production traits and carcass quality in tropical climatic conditions. The results of our experiment indicated a tendency of better adaptation Redbro Naked Neck broilers in free range growing conditions, and complete concordance with the results of other studies is lacking as a result of moderate climatic conditions.

Similarly, the effect of housing system on performance of Redbro broiler was manifested largely through environmental conditions, primarily temperature. The positive impact of rearing in the poultry house was found on body weight ($p<0.05$), feed efficiency of broiler and mortality, which resulted in higher production index compared to free range reared Redbro broilers. Research results confirmed earlier studies (*Škrbić et al., 2007*) regarding the manifestation of the positive effect of growing conditions in the poultry house on body weight of Redbro broilers. Thanks to an intensive program of feeding during the experiment, significantly higher final body weights of Redbro broilers were achieved in both rearing systems in relation to the results of previous research.

Table 3. Conformation, yield of dressed carcass and share of abdominal fat in broilers Redbro and Redbro Naked Neck reared at the free range

Parameter	Genotype		
	Redbro	**Redbro Naked Neck**	**Significance**
Index of carcass confirmation measures (mean ± SD)			
BW/SL, g/mm	34.55±3.57	32.24±2.59	ns
BW/KL, g/mm	25.72±3.88	24.20±2.88	ns
BW/BD, g/mm	28.34±3.98	27.04±2.96	ns
BW/TG, g/mm	20.08±2.55	18.82±1.96	ns
BA, degree	122.83±7.40	121.42±6.50	ns
Yield of dressed carcass (mean ± SD)			
Body weight, g	3207.5 ± 601.65	3055.8 ± 462.92	ns
Conventional dressing,%	88.30 ± 1.23	87.95 ± 1.71	ns
Ready to roast,%	82.82 ± 1.42	82.3 ± 1.72	ns
Ready to grill,%	73.93 ± 1.38	74.44 ± 1.65	ns
Abdominal fat, %	2.55 ± 0.73	2.57 ± 0.92	ns

BW-body weight; SL-shank length; KL-keel length; BD-breast depth; TG-thigh girth; BA-breast angle

Based on index values of carcass conformation measures shown in Table 3, higher values in Redbro broilers can be stated, which are the result of higher body weight, but also the lack of statistical confirmation of the established differences between genotypes. Similar situation was regarding the data for pre slaughter body weight, carcass yields – conventional dressed carcass, carcass ready to roast and ready to grill and the share of abdominal fat. Uniform relative carcass yields of two genotypes of moderate growth were recorded in the survey by *Blagojević et al. (2009)*, whereas significant differences were determined only relative to slow growing genotype.

Significant influence of the rearing system was exhibited in all index values of carcass conformation measures (Table 4) through a positive effect on body weight of Redbro chickens reared in the poultry house. However, in regard to carcass yield of conventionally dressed carcass, significantly higher value ($p<0.05$) was found in the free range system of rearing Redbro broilers. Other indicators of carcass yield were very similar for both systems of rearing, and consistent with the research results *Mikulski et al. (2011), Wang et al. (2009)* who have showed that free range rearing system has not adversely affected the carcass yield of broiler chickens. Share of abdominal fat as expected was higher ($p>0.05$) in the carcass of chickens reared in the poultry house, as a result of less physical activity.

Table 4. Conformation, yield of dressed carcass and share of abdominal fat in broilers Redbro reared in the poultry house and at the free range

Parameter	Rearing system		
	Poultry house	**Free range**	**Significance**
Index of carcass conformation measures (mean ± SD)			
BW/SL, g/mm	39.35±3.77	34.55±3.57	**
BW/KL, g/mm	29.05±2.67	25.72±3.88	*
BW/BD, g/mm	32.49±2.67	28.34±3.98	**
BW/TG, g/mm	22.05±2.16	20.08±2.55	*
BA, degree	128.25±2.56	122.83±7.40	*
Yield of dressed carcass (mean ± SD)			
Body weight, g	3681.7±490.99	3207.5 ± 601.65	*
Conventional dressing,%	87.30±1.13	88.30 ± 1.23	*
Ready to roast,%	82.37±1.04	82.82 ± 1.42	ns
Ready to grill,%	73.38±1.20	73.93 ± 1.38	ns
Abdominal fat, %	3.03±0.95	2.55 ± 0.73	ns

* $p<0.05$; ** $p<0.01$

BW-body weight; SL-shank length; KL-keel length; BD-breast depth; TG-thigh girth; BA-breast angle

Shares of major carcass parts in pre-slaughter body weight are shown in Table 5. Based on data for genotypes Redbro and Redbro Naked Neck can be stated the difference ($p < 0.05$) in the share of wing can be concluded, as well as statistically unconfirmed but highly significant difference in the share of the breast. Effect of rearing system also exhibited a statistically significant difference in the share of wings. In comparison with the results of *Škrbić et al. (2007)*, a significantly higher share of the breast was identified, indicating a possible genetically predisposed conformational changes in Redbro broilers, which are important because the slower growing broiler genotypes are characterized mainly by the higher share of less valuable carcass parts compared to the fast-growing broilers (*Mikulski et al. 2011*).

Table 5. The shares of individual major broiler carcass parts

Share, %TM	Genotype		
	Redbro	**Redbro Naked Neck**	**Significance**
Free range			
Breast	19.72 ± 1.95	20.57 ± 1.67	ns
Drumsticks	10.32 ± 0.89	10.31 ± 0.67	ns
Thighs	12.80 ± 0.64	12.84 ± 0.48	ns
Wings	8.98 ± 0.52	8.43 ± 0.53	*
		Rearing system	
	Poultry house	**Free range**	**Significance**
Redbro			
Breast	19.44 ± 1.51	19.72 ± 1.95	ns
Drumsticks	9.78 ± 0.99	10.32 ± 0.89	ns
Thighs	12.94 ± 0.73	12.80 ± 0.64	ns
Wings	8.28 ± 0.49	8.98 ± 0.52	**

* $p<0.05$; **$p<0.01$

Conclusion

Based on the presented results, it can be concluded that the studied types of Redbro broilers (with plumage and naked neck) had different intensity of growth in certain phases of the experiment, which finally resulted in non-significant differences in body weight. There is a tendency of better adjustment of Naked Neck-type broiler chickens to free range growing conditions, which would probably be more pronounced in the extreme summer environment conditions. In regard to carcass quality, there were no significant differences except in the share of wings and non-significant but important difference in the share of the breast.

Controlled conditions of rearing in the poultry house positively affected the production performance through body weight and carcass conformation of Redbro broilers.

Acknowledgment

This research is part of the Project EVB: TR-31033 financially supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Proizvodne performanse i kvalitet trupa brojlera diferenciranog genetskog potencijala za porast

Z. Škrbić, Z. Pavlovski, M. Lukić, V. Petričević, D. Milić

Rezime

Istraživanje je sprovedeno sa ciljem uporedne analize proizvodnih performansi i kvaliteta trupa dve provenijence brojlera diferenciranog genetskog potencijala za porast (Redbro i Redbro Naked Neck) u uslovima intenzivne ishrane, kao i utvrđivanja efekta sistema gajenja (na ispustu i u objektu) na tovne performanse Redbro brojlera. Proizvodne performanse su prikazane u dva kontrolna perioda (42 i 84 dana). Kvalitet trupa analiziran je na osnovu konformacije, prinosa, udela vrednijih delova i sadržaja abdominalne masti. Redbro i Redbro Naked Neck brojleri su imali različit intenzitet porasta po fazama ogleda ali se u starosti 84 dana njihove telesne mase nisu značajno razlikovale (3382.44g prema 3236.0g). Manja efikasnost korišćenja hrane (3.08kg prema 2.79kg) i bolja vitalnost (100% prema 97.33%) utvrđena je kod Redbro Naked Neck brojlera. U pogledu kvaliteta trupa, nisu utvrđene značajne razlike osim u udelu krila. Pozitivan uticaj gajenja Redbro brojlera u objektu utvrđen je na telesnu masu ($p<0.05$), efikasnost korišćenja hrane i mortalitet brojlera, što je rezultiralo i većim proizvodnim indeksom (169.83 prema 140,37) u odnosu na Redbro piliće gajene na ispustu. Razlike u kvalitetu trupa su utvrđene na osnovu bolje konformacije, manjeg udela krila ali i manjeg prinosa klasično obrađenog trupa Redbro brojlera gajenih u objektu.

References

BLAGOJEVIĆ M., PAVLOVSKI Z., ŠKRBIĆ Z., LUKIĆ M., MILOŠEVIĆ N., PERIĆ L. (2009): The effect of genotype of broiler chickens on carcass quality in extensive housing system. Acta Veterinaria, 59, 1, 91-97.

BOGOSAVLJEVIĆ-BOŠKOVIĆ S., MITROVIĆ S., RADOVIĆ V., DOSKOVIĆ V. (2007): The age and housing system effects on the growth of broilers. Biotechnology in Animal Husbandry 23, 5-6, 519- 525.

GRASHORN M.A., SERINI C. (2006): Quality of chicken meat from conventional and organic production. XII European Conference, Verona, Italy. World's Poultry Science Journal. Book of abstracts, 62, 268. CD-room

MIKULSKI D., CELEJ J., JANKOWSKI J., MAJEWSKA T., MIKULSKA M. (2011): Growth Performance, Carcass Traits and Meat Quality of Slower-growing

and Fast-growing Chickens Raised with and without Outdoor Access. Asian-Aust. J. Anim. Sci., 24, 10: 1407-1416.
OWENS C., FANATICO A., PILLAI P., MEULLENET J., EMMERT J. (2006): Evaluation of alternative genotypes and production systems for natural and organic poultry markets in the US. XII European Conference, Verona, Italy. World's Poultry Science Journal. Book of abstracts, 62, 246. CD-room
PAVLOVSKI Z.**,** LUKIĆ M., CMILJANIĆ R., ŠKRBIĆ Z. (2006): Konformacija trupova pilića. Biotehnologija u stočarstvu, 3-4, 83-97.
PAVLOVSKI Z., ŠKRBIĆ Z., LUKIĆ M. (2012): Autochthonous breed of chicken in Serbia: research or development. Proceedings of XV Feed Technology Symposium "Feed-to-Food", Novi Sad, Republic of Serbia, October 3-5, 127-133.
PAVLOVSKI Z., ŠKRBIĆ Z., LUKIĆ M., VITOROVIĆ D., PETRIČEVIĆ V. (2009a)**:** Naked Neck – Autochthonous Breed of Chicken in Serbia: Carcass Characteristics. Biotechnology in Animal Husbandry, 25, 1-2, 1-10.
PAVLOVSKI Z., ŠKRBIĆ Z., LUKIĆ M., VITOROVIĆ D., PETRIČEVIĆ V., MILOŠEVIĆ N. (2009b): Naked Neck Chicken of Serbian and Foreign Origin: Carass Characteristic**.** 9th International Symposium "Modern Trends in Livestock Production", October 2009, Belgrade. Biotechnology in Animal Husbandry, 25, 5-6, Book 2, 1023-1033.
RAJKUMAR U., REDDY B.L.N., RAJARAVINDRA K.S., NIRANJAN M., BHATTACHARYA T.K., CHATTERJEE R.N., PANDA A.K., REDDY M.R., SHARMA R.P. (2010): Effect of Naked Neck Gene on Immune Competence, Serum Biochemical and Carcass Traits in Chickens under a Tropical Climate. Asian-Aust. J. Anim. Sci., 23, 7: 867 - 872.
RISTIĆ M., DAMME K. (2002): Carcass value and meat quality of slow growing broiler lines upon feeding with rations according to organic conditions. Mitteilungsblatt BAFF 41, 146, 89-94.
RODIĆ, V., PERIĆ, L., MILOŠEVIĆ, N., SUPIĆ, N. (2003). Konkurentnost pilećeg mesa iz ekstenzivnog sistema držanja. Agroekonomika 32, Poljoprivredni fakultet, Novi Sad, 119-125.
ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M. (2007): Slaughter traits of slow growing broiler genotypes in different housing systems. Proceedings XVIII European Symposium on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs and Egg Products, Prague, 323-325.
ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M., TOMAŠEVIĆ D. (2010): Tehnologija proizvodnje pilećeg mesa u sistemu gajenja sa ispustom. Biotechnology in animal husbandry, 26, spec issue, 67 – 81.
WANG K.H., SHI S.R., DOU T.C., SUN H.J. (2009): Effect of a free-range raising system on growth performance, carcass yield and meat quality of slow-growing chicken. Poultry Science 88: 2219–2223.

YALCIN S, SETTAR P., OZKAN S., CAHANER A. (1997): Comparative Evaluation of Three Commercial Broiler Stocks in Hot Versus Temperate Climates. Poultry Science 76:921–929.

Received 30 November 2013; accepted for publication 15 December 2013

Biotechnology in Animal Husbandry 29 (4), p 625-630 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.31'65
DOI: 10.2298/ BAH1304625B M

EFFECT OF DIFFERENT LITTER TREATMENTS ON PRODUCTION PERFORMANCE OF BROILER CHICKENS

S. Bjedov, D. Žikić, L. Perić, M. Đukić Stojčić, N. Milošević

University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000, Novi Sad, Republic of Serbia
Corresponding author: sinisa.bjedov@stocarstvo.edu.rs
Original scientific paper

Abstract: In modern poultry production, foot pad dermatitis (FPD) represents one of the main problems on broiler chicken legs with significant affect on animal welfare and performance. This problem is not solved by the using of straw as litter. Different treatments of straw are needed to eliminate the adverse effects of this material as litter. The most widely used method to improve the quality of litter is chopping straw and application of microbial products on that. These procedures can decrease the moisture in the litter and reduce the population of microorganisms whose activity produces ammonia, and hence improving environmental conditions in facilities. The experiment involved four treatments with four replications with a total of 300 broilers per treatment. The treatment I was unchopped straw, treatment II was chopped straw, treatment III unchopped straw with the addition of microbial products, and treatment IV was chopped straw with the addition of microbial preparation. Duration of trial was 42 days. During the experiment, the following production parameters were measured: body weight per week of the experiment, feed conversion ratio, mortality and FPD. Results of the trial point out that the best production results were in treatment IV with an average final body weight of 2.456g and feed conversion ratio of 1.71.

Key words: foot pad dermatitis (FPD), broiler chickens, microbial preparations, litter, production performance

Introduction

Foot pad dermatitis (FPD) is a type of contact dermatitis affecting the plantar region of the feet in poultry and other birds. The lesions are commonly named “ammonia burns” and are thought to be caused by a combination of moisture, high ammonia content, and other not yet specified chemical factors in the litter *(Berg, 2004)*. Broiler flocks affected with severe form of footpad dermatitis show leg abnormalities like limping and uncoordinated gait, which negatively

influences their activity. Birds may also show slower weight gain due to pain-induced reduction in feed intake *(Bilgili1 et al. 2006)*. Fast growth rate is generally accompanied by decreased locomotory activity and extended time spent sitting or lying. The lack of exercise is considered a main cause of leg weakness, and extreme durations of sitting on poor quality litter produces skin lesions at the breast and the legs. Management factors which slow down early growth alleviate many welfare problems (*Bessei, 2006)*. Locomotor activity is important for the ossification of the bones of growing animals. There is a causal interrelationship between fast growth, low locomotor activity and leg problems. Since growth is a main economical factor, there are problems of acceptability of these measures in the commercial broiler production. Production performance was significantly greater at 42 days of age in birds exposed to lower relative humidity compared with the higher regimen of relative humidity of litter *(William et al., 1991)*.

Both the incidence and severity of ammonia burns on the breast and infected foot pads were significantly higher with 75 versus 45% RH. Increases in RH significantly increased caking and litter moisture and reduced the percentage of dry matter and the percentage of nitrogen found in the litter.

Hough et al. (1984) indicate that the installation of the product consists of black sulphate, propionic acid, magnesium and copper sulfate in the litter cause significant weight gain without affecting feed conversion and mortality. Also, they found that the product did not significantly affect litter moisture.

Litter serves several functions that include thermal insulation, moisture absorption, protective barrier from the ground, and it allows for natural scratching behavior. Bedding material must not only be a good absorber of moisture but also have a reasonable drying time *(Grimes et al., 2002; Bilgili et al., 2009)*. Although litter refers to the mixture of bedding material, fecal droppings, and moisture, the term is used interchangeably with bedding materials. The most commonly used litter material is straw in Europe *(Grimes et al., 2002)*. Differences in particle size of straw were proposed to be the most important factor. The aim of this study was to determine the effect of litter on the performance of broiler chickens.

Materials and Methods

The experiment was conducted at the Experimental farm of Faculty of Agriculture in Novi Sad. A total of 1,200 day-old Ross 308 broilers were assigned to four treatments with four replicates. Each replicate consisted of 75 as-hatched birds per pen. Each floor pen measured 5 m^2, to give a stocking density of 15 birds/m^2. Air temperature was adjusted in accordance to the technological demands. Lighting program provided 23 hours of light + 1 hour of dark. Birds were vaccinated against Newcastle disease (NCD) and infectious bursal disease (IBD) as per commercial recommendations. Feed and water supply were *ad*

libitum. The treatment I was unchopped straw, treatment II was chopped straw, treatment III unchopped straw with the addition of microbial products (Micropan^{R}, Eurovix USA), and treatment IV was chopped straw with the addition of microbial preparation. Duration of trial was 42 days. During the experiment, the following production parameters were measured: body weight per week of the experiment, feed conversion ratio and mortality. These labeled birds were examined weekly for the presence of foot dermatitis and given a lesion score using the methodology described by (*Martrenchar et al. 2002)*. Briefly, the scores were 0: no lesion; 1: lesion on <25% of the pads; 2: lesion on 25 to 50% of the pads; 3: lesion on >50% of the pads. Data were analyzed by ANOVA using the GLM procedure and means separated by Duncan *post* hoc test using StatSoft computer package (STATISTICA 11, 2012).

Results and Discussion

In this paper, the results indicating that the decrease in litter moisture significantly affects the increase of body weight in the final fattening of chickens (Table 1). The results are consistent with the results reached by *Harn and Ellen (2009)*, which suggest that the reduction of the moisture content of the litter was a statistically significant increase in body weight and average daily gain in the fattening of broiler chickens.

Table 1. Production results reflected the proportional share of the lesion

Parameters/treatment	Unchoped straw	Unchoped straw + MP	Chopped straw	Chopped straw + MP
Body weight (g)	2,358.00^{ab}	2,322.00^{b}	2,381.00^{ab}	2,456.00^{a}
Body weight gain (g/b/d)	56.15	55.29	56.70	58.48
Mortality (%)	2.03	1.52	1.10	1.50
FCR	1.72	1.70	1.66	1.71
EPEF (PI)	320	321	338	337
Dry matter litter (%)	51.56	51.04	52.56	48.27
Footpad lesions	(%)	(%)	(%)	(%)
0	2.69	8.68	1.50	14.44
1	30.65	39.27	32.50	42.78
2	30.64	31.96	33.00	26.11
3	36.02	20.09	33.00	16.67

(^{a,b}) values in the table are statistically significant($p<0,01$)

By reducing the moisture in the ground cover can lead to decrease the occurrence and intensity of lesions. This results in a reduction in mortality, conversion and increase production indices in fattening of broiler chickens. A significant increase in the number of chicks without lesions *(Harn and Jong, 2012)*

come using acidifiers in the drinking water of broiler chickens. The results reached by *De Baere and Zoons (2004)* shows that the use of large quantities of chopped straw of 1.5 kg/m2, a significant increase in the intensity of the FPD in broiler chickens. In their work, they state that the use of chopped straw at a rate of 2.0 kg/m2 leads to a much larger increase in the number of lesions greatest degree of damage.

Conclusion

The emergence of the FPD is multifactorial problem that is influenced by a large number of factors the most significant ventilation, feeding, watering, microclimate and health status of animals. Consequences of the development are significant both in terms of animal welfare and in terms of economic feasibility of production. While reducing litter moisture was not significant it certainly contributed to a significant increase in body mass of chickens for fattening, reducing mortality and increasing conversion and production index in broiler chickens. Good knowledge of all factors can be occurrence of FPD significantly contribute to the prevention of these types of dermatitis and reduced intensity damage in flocks where the disease has occurred.

Acknowledgements

This work has been funded by the Ministry of Education, Science and Technology Development Republic of Serbia as a part of the Project No. TR 31033.

Efekat različitih tretmana prostirke na proizvodne rezultate brojlerskih pilića

S. Bjedov, D. Žikić, L. Perić, M. Đukić Stojčić, N. Milošević

Rezime

U savremenoj živinarskoj proizvodnji jedan od vodećih problema u tovu brojlerskih pilića jesu pododermatitisi. Upotrebom slame kao prostirke ovaj problem se ne rešava. Potrebni su različiti tretmani slame kao bi se negativni efekti ovog materijala, kao prostirke, eliminisali. Najčešće primenjivan način poboljšanja kvaliteta prostirke kod nas je seckanjem slame i aplikacijom mikrobioloških preparata po prostirci. Ovim postupcima je moguće sniženje vlage u prostirci i smanjenje populacije mikroorganizama čijom aktivnošću se stvara amonijak, a

samim tim se popravljaju i ambijentalni uslovi u objektima. U ogledu su bila uključena četiri tretmana sa po četiri ponavljanja sa ukupno 300 plilića po tretmanu. Tretmani su se sastojali od seckane i neseckane slame sa i bez dodatka mikrobiološkog preparata. Ogled je trajao 42 dana. U ogledu su praćeni sledeći proizvodni parametri: telesne mase po nedeljama tova, konverzija i mortalitet. Tretman I je činila neseckana slama, tretman II seckana slama, tretman III neseckana slama sa dodatkom mikrobiološkog preparata, a tretman IV seckana slama sa dodatkom mikrobiološkog preparata. Po završetku ogleda najbolje proizvodne rezultate je ostvario tretman IV sa prosečnom završnom telesnom masom od 2.456g i konverzijom 1,71.

References

DE BAERE, K., ZOONS, J. (2004): Strooisel bij vleeskukens hoveelheid houkrullen en stro. Plumivee nr. 39.

BERG, C. (1998): Footpad dermatitis in broilers and turkeys — prevalence, risk factors and prevention. PhD Thesis, Swedish Univ. Agric. Sci., Uppsala, Sweden. Acta Univ. Agric. Suecia,Vet. 36.

BERG, C. (2004): Pododermatitis and hock burn in broiler chickens. Pages 37–49 in Measuring and Auditing Broiler Welfare. C. A. Weeks and A. Butterworth, ed. CABI Publishing, Wallingford, UK.

BESSEI, W. (2006): Welfare of broilers: a review. World Poultry Sci J 62 (03): 455-466.

BILGILI, S. F., ALLEY, M. A., HESS, J. B. NAGARAJ, M. (2006): Influence of age and sex on foot pad quality and yield in broiler chickens reared on low and high density diets. J. Appl. Poult. Res. 15(3): 433-441.

BILGILI, S. F., HESS, J. B., BLAKE, J. P., MACKLIN, K. S., SAENMAHAYAK, B., SIBLEY, J. L.(2009): Influence of bedding material on footpad dermatitis in broiler chickens. J. Appl. Poult. Res. 18:583–589.

BRUCE, D. W., MCILROY, S. G., GOODALL, E. A. (1990): Epidemiology of a contact-dermatitis of broilers. Avian Pathol. 19:523–537.

EKSTRAND, C., ALGERS, B., SVEDBERG, J. (1997): Rearing conditions and foot pad dermatitis in Swedish broiler chickens. Prev. Vet. Med. 31:167–174.

GREENE, J. A., McCRACKEN, R. M., EVANS, R. T. (1985): A contact dermatitis of broilers — Clinical and pathological findings. Avian Pathol. 14:23–38.

GRIMES, J. L., SMITH, J., WILLIAMS, C. M. (2002): Some alternative litter materials used for growing broilers and turkeys. World's Poult. Sci. J. 58:515–526.

HARMS, R. H., and SIMPSON, C. F. (1975): Biotin deficiency as a possible cause of swelling and ulceration of foot pads. Poult. Sci. 54:1711–1713.

HARN, van J., ELLEN, H.H. (2009): Gebruik van vloerverwarming bij vleeskuikens. ASG Rapport 171.

HARN, van J., en JONG, de I. (2012): Zuur is zoet voor zolen (2). Pluimveehouderij 42/7. 1 juni 2012. P. 3031.
HESTER, P. Y. (1994): The role of environment and management on leg abnormalities in meat-type fowl. Poult. Sci. 73:904–915.
HUFF, W. E., MALONE, G. W., CHALOUPKA, G. W. (1984): Effect of litter treatment on broiler performance and certain litter quality parameters. Poult. Sci. 63(11): 2167-2171.
KESTIN, S. C., GORDON, S., SU, G., SORENSEN, P. (2001): Relationship in broiler between lameness, live weight, growth rate and age. Vet. Rec. 148:195–197.
MARTRENCHAR, A., BOILLETOT, E., HUONNIC, D. POL, F. (2002): Risk factors for foot pad dermatitis in chicken and turkey broilers in France. Prev. Vet. Med. 52:213–226.
MARTLAND, M. F. (1985): Ulcerative dermatitis in broiler chickens: The effects of wet litter. Avian Pathol. 14:353–364.
McFERRAN, J. B., McNULTY, M. S., McCRACKEN, R. M., GREENE, J. A. (1983): Enteritis and associated problems. Pages 129–138 in Proc. International Union of Immunological Societies: Disease Prevention and Control in Poultry Production. No. 66. University of Sydney, Australia.
MELUZZI, A., FABBRI, C., FOLEGATTI, E., SIRRI, F. (2008): Survey of chicken rearing conditions in Italy: Effects of litter quality and stocking density on productivity, foot dermatitis and carcass injuries. Br. Poult. Sci. 49:257–264.
WILLIAM, D., WEAVER, JR. MEIJERHOF R. (1991): The Effect of Different Levels of Relative Humidity and Air Movement on Litter Conditions, Ammonia Levels, Growth, and Carcass Quality for Broiler Chickens. Poult. Sci. 70 (4): 746-755.

Received 10 September 2013; accepted for publication 15 December 2013

Biotechnology in Animal Husbandry 29 (4), p 631-640 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'32
DOI: 10.2298/ BAH1304631S

EFFECT OF VEGETABLE OILS ON PRODUCTIVE PERFORMANCES AND LIPID FATTY ACID COMPOSITION OF CHICKEN ABDOMINAL FAT

V.Ž. Stanaćev[1], D. Milić[1], N. Milošević[2], V.S. Stanaćev[2*], Z. Pavlovski[3], Z. Škrbić[3] M. Vukić-Vranješ[4]

[1]Perutnina-Topiko, 24300 Bačka Topola, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.
[3]Institute for Animal Husbandry Belgrade - Zemun, Autoput 16, 11080 Zemun, Serbia.
[4]Institut for Science Application in Agriculture – Beograd, Bul. Despota Stefana 68b, Belgrade, Serbia
*Corresponding author: vidica.stanacev@stocarstvo.edu.rs
Original scientific paper

Abstract: This paper investigates the effect of soybean, flax and rapeseed oil on productive performance and lipid fatty acid composition of broiler chickens. Six groups of 40 one day-old chicks hybrid line Cobb 500, with five replications were formed. Three mixtures with 21, 20 and 18% protein were used. The first 14 days groups were fed with the starter mixture. The control group was based on the 4% and 8% soybean oil while in the experimental groups were included the same concentration of flax and rapeseed oil. The experiment lasted 42 days. At the end of the experiment 10 chickens from each group were sacrificed for examination of fatty acid composition of lipids. The control group achieved weight of 2704g and 2695g, and the experimental group in 2735, 2645, 2735 and 2670g, respectively. Feed conversion was improved with increasing the amount of oil in the diet. The usage of flax and rapeseed oil changed the fatty acid composition of lipids. Substituting soybean oil with rapeseed oil reduces the percentage of palmitic, stearic, and linoleic acids, and increases participation of oleic and linoleic acids in abdominal fat. The inclusion of flaxseed oil in the diet of chickens in the amount of 4% and 8%, increases the amount of linoleic acid by 63% and 203%, which is a statistically high and significant difference for the control group I and II, while the amount of linoleic acid is reduced by 14% and 33 %, which presents a statistically significant difference compared to group II.

Key words: nutrition, flax oil, rapeseed oil, chicken, fatty acids

Introduction

Studies have shown that increased energy intake, or dietary fat leads to decreased feed consumption and improved conversion in broilers (*Harms et al., 2000; Bryant et al., 2005*). Addition of fat in food exerts energy role and enhances the absorption of liposoluble vitamins, increases the palatability of meals and efficiency of energy use. It also leads to reduced passage of digestion of the gastrointestinal tract, allowing better absorption of nutrients in food. Today, most commonly used energy source in the diet of broiler chickens are oils and they are added in the amount of 5-7%. For this purpose the most often used one is soybean oil, although in recent years it has been replaced with flax and rapeseed oil, for desire to improve the nutritional value of chicken meat. Since the oil rich in ω-3 (α-linolenic) and ω-6 (linoleic) fatty acids significantly increased the content of lipids in chicken meat and hence improve anticholesterolemic and antisclerotic effect (*Goodnight, 1993*). However, exacerbate the sensory properties of the same, according to the *Osek et al. (2001); Osek et al. (2004); Rymer and Givens (2006); Ferrini et al. (2008); Zelenka et al. (2006).* The best source of polyunsaturated fatty acids among vegetable oils is flaxseed oil, which contains about 50% α-linolenic acid and 20% of linoleic acid of the total fatty acid amount (*Osek et al., 2005; Zelenka et al., 2003*). Addition of flaxseed oil to the chicken diet leads to a desirable relationship between polyunsaturated fatty acids and homologous ω-3 and ω-6 fatty acids, which in the human diet according to the World Health Organization (WHO) should be at 1:4 ratios. In addition, rapeseed oil is a good source of polyunsaturated fatty acids, although the linolenic acid is found in much smaller amounts (8%), which contributes to reduced oxidation of the oil. The most common is oleic acid (55%), and linoleic acid is approximately as in flaxseed oil. Such a composition of the oil significantly improves the nutritional quality of meat and performance optimization (*Sim, 1990; Yang et al., 2000; Bezard et al., 1994*).

Given the above, the aim of this study was to examine the broilers production parameters, when fed with different amounts of soybean, flaxseed and rape seed oil, and fatty acid composition of abdominal fat.

Materials and Methods

Experiments were conducted under production conditions on the experimental estate "Pustara" in Temerin, on the floor system posture. At the beginning, six groups of 40 one day-old chicks hybrid line Cobb 500 were formed with five replications. Three mixtures with 21, 20 and 18% protein were used for chicken nutrition. The first 14 days was a preparatory period of chicken, in which all groups were fed with starter mixture of standard composition and quality. Then in the next 21 days, grower mixture with different sources and amounts of oil were

used. The last 7 days chickens were fed with finisher diets with the same addition of oil (Table 1). The control group was based on the 4% and 8% soybean oil while in the experimental groups were included 4% and 8% flax, or 4% and 8% rapeseed oil (Table 2). In mixtures with lower oil amount was added 100 mg/kg tocopherol acetate as an antioxidant, and in mixtures with higher oil supplemented period, 200 mg/kg of antioxidants to prevent oxidation and maintain oil quality. During the experiment, which lasted 42 days, chicks were fed and watered *ad libitum* and micro-climatic conditions constantly monitored. Body weight control and feed consumption was carried out every seven days. At the end of the experiment, after 12 hours of fasting, 10 birds (5 males and 5 females), of average body weight, marked with tags were sacrificed for examination of fatty acid composition of lipids in abdominal fat. Then bleeding, scalding, plucking, evisceration and cooling were conducted. Analysis of fatty acid composition of abdominal fat was made by gas chromatography method. The same method was used to analyse fatty acid composition of soybean, rapeseed and linseed oils. Evaluations were conducted on the basis of yield and weight of certain body parts. For proper interpretation of the results appropriate statistical methods such as ANOVA and Tucky post-hoc test were used with the statistics software package 12.

Table 1. Composition of used mixtures

Feedstuffs	Starter	Grower		Finisher	
		4% oil	8% oil	4% oil	8% oil
Corn, grain	50,00	56,50	44,00	60,60	51,00
Wheat floor	4,00	1,00	6,00	5,00	5,50
Vegetable oil	0,00	4,00	8,00	4,00	8,00
Soybean meal (44%)	17,40	34,00	33,00	26,00	23,00
Soybean grits	21,00	0,00	0,00	0,00	0,00
Sunflower meal (33%)	0,00	0,00	4,50	0,00	8,00
Yeast, Torula	3,00	0,00	0,00	0,00	0,00
Monocalcium phosphate	1,50	1,50	1,40	1,50	1,50
Solt (NaCl)	0,30	0,30	0,30	0,25	0,30
Chalk	1,50	1,50	1.50	1,50	1,50
Lysine-L (78%)	0,10	0,00	0.10	0,00	0,00
Methionine-DL (99%)	0,20	0,20	0,20	0,15	0,00
B-Mikrovit-ŽKNB, premix	1,00	1,00	1,00	1,00	1,00
Total	*100,00*	*100,00*	*100,00*	*100,00*	*100,00*
Nutrient amount					
Proteins, %	22,01	20,04	20,90	18,00	18,46
Fat, %	6,09	6,54	10,33	6,79	10,53
Fibre, %	3,96	3,92	4,89	3,78	4,89
ME, MJ/kg	12,88	13,13	13,59	13,30	13,73

Table 2. Experiment design

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

Results and Discussion

Based on the obtained results it can be concluded that the introduction of various types and quantities of vegetable oils in the diet of broilers did not affect the intensity of growth (Table 3). During the preparatory period, chicks had a uniform body weight in all groups. However, the experimental period in the third and fourth week results shows statistically significant ($P<0.05$) and highly significant ($P<0.01$) difference in body weight between the experimental and control groups. In the fifth week of age it is observed a very small depression on treatment with 4% of rapeseed oil (V), while the other groups (III, IV, and VI) were superior to the control groups (I and II). In the sixth week, the body mass of chicks had no statistically significant differences ($P>0.05$) between the groups, but body weight in groups of 8% of the oil in the diet was lower in relation to the weight of chickens who were on treatment with a lower level of oil in the diet.

Table 3. Body weight of chickens, g

Chicken age (weeks)	**Group, treatment and oil amount**					
	I (T5)	**II (T6)**	**III (T1)**	**IV (T2)**	**V (T3)**	**VI (T4)**
	4%-soy	**8%-soy**	**4%-flax**	**8%-flax**	**4%-rape**	**8%-rape**
Preparation period (1 – 14 days)						
Initial weight	42	42	42	42	42	42
1	185	185	183	190	187	190
2	468	469	468	468	469	469
Index, %	*100*	*100*	*100*	*99,78*	*100,21*	*100*
Experimental period (15 – 42 days)						
3	986	967^{b}	989	997^{B}	995	977
4	1457^{bD}	1422^{ABCD}	1523^{A}	1532^{B}	1515^{C}	1575^{D}
5	2122^{E}	2053^{ae}	2164^{A}	2094	2121	2081^{a}
Index, %	*100*	*100*	*101,97*	*101,99*	*99,95*	*101,36*
6	2704	2695	2735	2645	2735	2670
Index, %	*100*	*100*	*101,14*	*98,14*	*101,15*	*99,07*

The same capital letters in the same row = highly significant ($P<0.01$); same capital and small letters in the same row = significantly ($P<0.05$)

The literature states that feed consumption is negatively correlated with the amount of oil in the feed, which was confirmed by this experiment (Table 4). Feed consumption in the experimental groups varied by periods compared to the control group, and at the end of the experiment the average consumption was lower in all groups for 0.76; 0.60; 1.46 and 1.47% compared to the group with soybean oil. Hypothesis comes to the fore in the sixth week of the fattening where feed consumption is lower by 20%. Consumption was lowest in group IV and VI, in the last seven days when the chickens consumed finisher mixture. Group IV on treatment with 8% flax oil, consumption was reduced by 19.34% and in group VI on treatment with 8% rapeseed oil is decreased by 12.93%, while in the group with 4% oil in diet was increased by 1.34% and was equal to the control group.

Table 4. Feed consumption, g/day

Period	Treatment and oil amount					
	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-flax	8%-flax	4%-rape	8%-rape
14-35 days	389,8	371,5	383,5	396,7	381,7	380,3
Index, %	*100*	*100*	*98,38*	*106,78*	*97,92*	*102,36*
35-42 days	186,3	208,9	188,8	168,5	186,2	181,9
Index, %	*100*	*100*	*101,34*	*80,66*	*99,94*	*87,07*
Average	572,1	566,9	567,8	563,5	563,8	558,6
Index, %	*100*	*100*	*99,24*	*99,40*	*98,54*	*98,53*

Table 5. Feed conversion, kg

Period	Treatment and oil amount					
	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-flax	8%-flax	4%-rape	8%-rape
Preparation period (1 – 14 days)						
1	1,13	1,16	1,14	1,12	1,14	1,08
2	1,35	1,34	1,30	1,35	1,36	1,33
Index, %	*100*	*100*	*96,29*	*100,74*	*100,74*	*99,25*
Experimental period (15 – 42 days)						
3	1,38	1,36	1,39	1,42	1,41	1,41
4	1,49	1,48	1,41	1,50	1,47	1,50
5	1,62	1,61	1,60	1,68	1,60	1,63
Index, %	*100*	*100*	*98,76*	*104,34*	*98,76*	*101,24*
6	1,87	2,09	1,85	1,83	1,83	1,85
Index, %	*100*	*100*	*98,93*	*87,55*	*97,86*	*88,51*
3-6	1,59	1,63	1,56	1,60	1,57	1,59
Index, %	*100*	*100*	*98,11*	*98,15*	*98,74*	*97,54*

Unlike consumption, feed conversion is improved with increasing the amount of oil in the diet. Thus, at the end of the sixth week in group IV with 8% flax oil there was 1.83 kg/kg gain, and in the sixth group with the same amount of rapeseed oil there was 1.85 kg/kg gain, which is 12.45% and 11.49%, compared to the control group with the same amount of soybean oil (Table 5). Average values of the conversion of the whole experiment were slightly lower compared to the

control group with soybean oil and amounted to 1.56 respectively; 1.60; 1.57; 1.59 kg/kg gain, while the control groups I and II ranged were 1.59 and 1.63 kg/kg gain.

When it comes to a fatty acid composition, eleven fatty acids were detected. Their percentage in abdominal fat lipids is shown in Table 6. Enrichment of chicken meat with linoleic and linolenic acid can be achieved using other types and levels of added oil (*Božić, 199*7), although not resulting in an increase level of mandatory and adequate conversion of fatty acids with 20 and 22 carbon atoms (*Moore et al., 1995; Sprecher et al., 1995; Lopez-Ferrer et al., 200*1).

These differences have a significant effect on the fatty acid composition of abdominal fat (Table 6). Flax oil is the richest in linoleic acid, which has as many as 7-8 times higher than soybean oil and rapeseed. Followed by linoleic acid 3 times a small amount compared to soybean oil, and stearic acid present in a lesser amount than in the same oil. The concentration of palmitic acid is close to rapeseed oil.

Based on data on fatty acid composition in abdominal fat, shown in Table 6, it can be noted that the use of flax and rapeseed oil changing the fatty acid composition of lipids. Substituting soybean oil with rapeseed oil at a rate of 4% in diets for chickens, reduces the percentage of palmitic, stearic, and linoleic acids, and increased participation of oleic and linoleic acids in abdominal fat. These changes are directly correlated with the fatty acid composition of the oil. Increase in linoleic acid in group with 5.89% to 6.83% is highly statistically significant ($P<0.01$). By increasing the amount of oil in diets for chickens to 8%, retains the same tendency acids exept stearic, whose participation has increased in group VI. Reduction of linoleic acid was significantly higher ($P<0.01$) in comparison to the second (control) group, and the increase of linoleic acid is a significant different ($P<0.05$) compared with II (control) group.

The inclusion of flaxseed oil in the diet of chickens in the amount of 4% and 8%, increases the amount of linoleic acid by 63% and 203%, with statistically significant difference compared to the control groups I and II, while the amount of other acids are decreased. From the above it can be determined that the linoleic acid is reduced by 14% and 33%, with statistically significant difference ($P<0.01$) compared to the second (control) group.

Reduced feed consumption in groups of 8% of the oil has had an impact on the amount of fatty acids in abdominal fat. All groups with higher oil amount showed a lower proportion of acid, but with the same oil, but the differences were not statistically significant ($P>0.05$).

It is well known that birds do not synthesize linoleic and linolenic acid (*Pinchasov and Nir, 1992*) and their presence in the body is result of their presence in the feed and was attended by oxidation in the tissues. Of total energy input in the diet of chickens, at least 3% must be essential fatty acids, of which 1/3 fatty acids should be linoleic acid. Similar results with the addition of other types of oil reached *Cherian et al. (1996) and Scaife et al. (1994)*.

Fatty acid composition in samples of abdominal fat (Table 6) is almost identical to the meat lipids only with different participation of stearic acid (C18). Its share has increased in all groups compared to the lipids of muscle tissue, according *Stanaćev et al. (2011).*

Analyses of variance and Tucky post-hoc test showed highly significant differences (P<0.01) between I, II and IV group of linolenic acid, then significant differences (P<0.05) between II and IV, II and VI group of linoleic acid.

Table 6. Fatty acid composition of chicken abdominal fat, 42 day

Fatty acids	Treatments and fatty acid composition of abdominal fat, %					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-Soy	8%-Soy	4%-Flax	8%-Flax	4%-Rape	8%-Rape
C14:0	$0,04^{d}$	$0,01^{abD}$	$0,07^{A}$	$0,07^{B}$	$0,03^{C}$	$0,10^{CD}$
C16:0	$17,75^{DE}$	$14,66^{AcE}$	$18,39^{A}$	16,35	$16,87^{C}$	$14,59^{AcD}$
C16:1	3,28	2,83	3,83	3,46	3,41	2,79
C18:0	5,23	4,75	5,06	4,72	4,67	5,34
C18:1	35,45	33,57	35,72	34,37	37,07	37,46
C18:2	$29,22^{E}$	$37,07^{ABCDE}$	$25,13^{A}$	$24,81^{B}$	$27,48^{Ac}$	$26,52^{cD}$
C18:3	$5,89^{B}$	$4,75^{aB}$	$9,61^{A}$	$14,54^{aB}$	$6,83^{B}$	$9,18^{b}$
C20:0	0,07	0,10	0,07	0,09	0,09	0,08
C20:1	0,46	0,41	0,42	0,33	0,49	0,56
C22:0	0,06	$0,00^{d}$	0,01	0,09	0,08	$0,09^{D}$
C24:0	$0,01^{D}$	$0,00^{cD}$	$0,00^{A}$	$0,00^{B}$	$0,05^{abC}$	$0,08^{ABD}$

The values in the table are the mean ± SD (n = 5); same capital letters in the same row = highly significant (P<0.01); same capital and small letters in the same row = significantly (P<0.05)

Conclusion

Based on these results it can be concluded that the use of 4% and 8% flax and rapeseed oil shows no significant differences in body weight in comparison to the control group that received diet with addition of 4% to 8% soybean oil. Groups with lower amount of oil had a higher body weight at the end of the experiment. The control group achieved weight of 2704g and 2695g, and the experimental group in a row 2735, 2645, 2735 and 2670g. Feed consumption was lowest in the last week, when the chickens consumed finisher mixture and feed conversion improved with increasing the amount of oil in the diet.

The usage of flax and rapeseed oil change the fatty acid composition of lipids. Substituting soybean oil with rapeseed oil at a rate of 4% in diets for chickens, reduces the percentage of palmitic, stearic, and linoleic acids, and increased participation of oleic and linoleic acids in abdominal fat. By increasing the amount of oil in the diets to 8%, it retains the same tendency of6acids.

The inclusion of flaxseed oil in the diet of chickens in the amount of 4% and 8% increases the amount of linoleic acid by 63% and 203%, which is high statistically significant difference from the control group I and II, while the amount of other acids decreases. From the above we can also conclude that linoleic acid is reduced by 14% and 33%, with high statistically significant difference ($P<0.01$) compared to group II.

Acknowledgment

This paper is a part of the project TR 31033 which is financed by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Efekat različitih izvora biljnih ulja na proizvodne performanse i masnokiselinski sastav abdominalne masti brojlerskih pilića

V.Ž. Stanaćev, D. Milić, N. Milošević, V.S. Stanaćev, Z. Pavlovski, Z. Škrbić, M. Vukić-Vranješ

Rezime

Za normalno odvijanje metaboličkih procesa u ljudskom organizmu, neophodne su esencijalne masne kiseline. Pošto organizam nije u mogućnosti da ih sintetiše, moraju se unositi hranom u dovoljnoj količini, obično konzumiranjem namirnica životinjskog porekla. Istraživanja su pokazala da se odabirom lipida u hrani za piliće može značajno uticati na masnokiselinski sastav lipida pilećeg mesa. Značaj ovog odabira je u toliko veći, jer se istovremeno postižu dva efekta, izuzetno važna sa nutritivnog i sa aspekta zdravstvene bezbednosti namirnica. Jedan efekat se odnosi na povećanje učešća polinezasićenih masnih kiselina, linolne i linolenske kiseline, koje zajedno sa oleinskom predstavljaju direktne prekursore za višestruko nezasićene masne kiseline sa 20 i 22 atoma ugljenika i jednom dvostrukom vezom u položaju n-3, n-6 ili n-3 i cis konfiguracijom, koje se takođe ubrajaju u esencijalne (Pokorn, 1990). A drugi, na smanjenje učešća masnih kiselina sa dokazanim štetnim efektima (C10:0, C12:0, C14:0).

Polinezasićene masne kiseline n-3 familije i njihove poznate prednosti sa aspekta zdravstvene bezbednosti, dovele su do razvoja proučavanje efekta masnokiselinskog sastava lipida u hrani za živinu, na deponovanje masnih kiselina u lipidima pilećeg mesa i konzumnih jaja (*Cherian and Sim, 1991; Scaife et al., 1994*). Glavni izvori polinezasićenih masnih kiselina dugog lanca su ulja morskih riba. Međutim, njihovo dodavanje u hranu za piliće narušava organoleptička svojstva proizvoda, stoga se pribegava obogaćivanju pilećeg mesa polinezasićenim

masnim kiselinama sa 18C atoma dodavanjem biljnih ulja bogatih ovim kiselinama (*Leeson i Summers, 1997*).

References

BEZARD, J., J.P., BLOND, A., BERNARD P., CLOUET (1994). The metabolism and availability of essential fatty acids in animal and human tissues. Reprod. Nutr. Dev. 34(6): 539-568.

BOŽIĆ, A. (1997). Influence of feed fatty acid origin on fatty acid composition and aterogenic potential of muscle and fat tissue of broiler chickens. Ph.D thesis. Deptt. of Animal Sci., Univ. Novi Sad, Serbia.

BRYANT, M., G., WU, and D.A., ROLAND (2005). Optimizing dietary energy for profits and performance of two strains of White Leghorns. Proc. Intter. Poultry Sci. Forum., 84:23.

CHERIAN, G., J.S., SIM (1991). Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, ambrosyos and newly hatched chicks. Poultry Sci. 70: 917-922.

CHERIAN, G., F.W., WOLFE, J.S., SIM (1996). Feeding dietary oils with tocopherols: effects on internal qualities of eggs during storage. J. Food Sci. 61: 15-18.

FERRINI, G., M.D. BAUCELLS, E. ESTEVE-GARCIA, A.C. BARROETA (2008). Dietary polyunsaturated fat reduces skin fat as abdominal fat in broiler chicken. Poultry Sci. 87(3): 528-535.

GOODNIGHT, S.H., (1993). The effects of n-3 fatty acids on atherosclerosis and the vascular response to injury. Arch. Pathol. Lab. Med. 117: 102.

HARMS, R.H., G.B., RUSSELL, D.R., SLOAN (2000). Performance of four strains of commercial layers with major changes in dietary energy. J. Appl. Poultry Res. 9: 535-541.

LEESON, S., J.D. SUMMERS (1997). Commercial poultry nutrition. 3rd Ed. University book, Guelph, Ontario (Canada). 406 p

LOPEZ-FERRER, S., M.D. BAUCELLS, A.C. BARROETA, J., GALOBRAT, M.A., GRASHORN (2001). n-3 enrichment of chicken meat. 2. Use of precursors of long chain polyunsaturated fatty acids: linseed oil. Poultry Sci. 80: 753-761.

MOORE, F.L., J.C. ROBINSON, C.F. BHARUCHA, S. BALA, M.G. RAIZEN (1995). Atom optics realization of the quantum kicked rotor. Physical Rev. 75: 4598-4601.

OSEK, M., A., JANOCHA, A., MILCZAREK, B., KLOCEK (2005). Wyniki produkcyjne i poubojowe oroz walory smakowe miesa kurczat brojlerow zywionych mieszankami natłuszczanymi roznymi olejami roslinnymi. Ros. Olei. 26(2): 541-550.

OSEK, M., A., JANOCHA, B., KLOCEK, Z., WASILOWSKI (2001). Wpływ mieszanek zawierjacych rozne tłuszcze na wskazniki produkcyjne i jakosc miesa kurczat rzeznych. Ros. Olei. 23(2): 515-530.
OSEK, M., Z., WASILOWSKI, A., JANOCHA (2004). Wpływ roznych olejow roslinnych na sklad podstawowy i profil kwasow tłuszczowych miesa kurczat brojlerow. Rocz. Nauk. Zoot. 20: 235-238.
PINCHASOV, Y., I., NIR (1992). Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition, and carcass fatty acid composition in broiler chickens. Poultry Sci. 71: 1504-1512.
POKORN, D. (1990). Omega-3 fatty acids in the diet. Bulletin Yug. Comm. Lipids 3: 25-27.
RYMER, C., D.I., GIVENS (2006). Effect of species and genotype on the efficiency of enrichment of poultry meat with n-3 polyunsaturated fatty acids. Lipids 41(5): 445-451.
SCAIFE, J.R., J., MOYO, H., GALBRAITH, W., MICHIE, V., CAMPBELL (1994). Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. Brit. Poultry Sci. 35(1): 107-118.
SIM, J.S. (1990). Flax seed as a high energy/protein/omega-3 fatty acid feed ingredient for poultry. Proc. Flax Inst. US, Fargo, ND. 53: 65-71.
SPRECHER, H., D.L., LUTHRIA, B.S., MOHAMMED, S.P., BAYKOUSHEVA (1995). Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J. Lipid Res. 36: 2471-2477.
STANAĆEV, S.V., S., KOVČIN, V.Ž., STANAĆEV, M., PUCAREVIĆ, N., PUVAČA (2011). Extruded canola seed in improving chicken fattening and fatty acid composition. Kuwait J. Sci. Eng. 38(1): 71-80.
YANG, W.S., W.J., LEE, T., FUNAHASHI, S., TANAKA, Y., MATSUZAWA, C.L., CHAO, C.L., CHEN, T.Y., TAI, L.M., CHUANG (2000). Weight reduction increases plasma levels of an adipose-derived anti-inflamatory protein, adiponectin. J. Clinical End. Met. 86(8): 3815-3819.
ZELENKA, J., D., SCHNEIDEROVA, E., MRKVICOVA (2006). Linseed oils with different fatty acid patterns in the diet of broiler chickens. Czech J. Anim. Sci. 51(3): 117-121.
ZELENKA, J., E., FAJMONOVA, T., KOMPRDA, D., KLADROBA, I., ŠARMANOVA (2003). Effect of dietary linseed and sunflower oil on cholesterol and fatty acid contents in rainbow trout (*Oncorhynchus mykiss*) fillets. Czech J. Anim. Sci. 48: 321-330.

Received 10 December 2013; accepted for publication 25 December 2013

Biotechnology in Animal Husbandry 29 (4), p 641-650 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082.453'64
DOI: 10.2298/ BAH1304641S

VARIABILITY OF EJACULATE VOLUME AND SPERM MOTILITY DEPENDING ON THE AGE AND INTENSITY OF UTILIZATION OF BOARS

R. Savić[1], M. Petrović[1], D. Radojković[1], Č. Radović[2], N. Parunović[3], M. Pušić[4], R. Radišić[4]

[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
[4] Institute of Science Application in Agriculture, 11000, Belgrade, Republic of Serbia
Corresponding author: savic@agrif.bg.ac.rs
Original scientific paper

Abstract: The main objective of this study was to evaluate the effect of age (A) and the intensity of the boars' utilization (s) on the phenotypic variability of ejaculate volume (VOL) and sperm motility (MO). The study included 274 ejaculates of Large White boars (LW). Boars were divided into six classes according the age when the ejaculate was taken (10-13, 14-17, 18-21, 22-25, 26-29 and ≥30 months). Semen samples were analyzed during four seasons (spring, summer, autumn, winter). The intensity of the use of boars was seen as the impact of the group (class) of intervals between two successful collections (I; ≤ 5 , 6, 7 , 8, 9-10, 11-13, 14-21 days). Data processing was performed using the GLM procedure using the statistical package *SAS 9.1.3 (SAS Inst. Inc., 2002-2003)*. Average values of VOL, MO, I, A, were: 178.94 ml, 90.35%, 9.37 days and 655.93 days. The effect of boars on both studied sperm traits was highly significant ($p<0.001$). The volume of ejaculate varied under the influence of boar age ($p<0.001$) and season ($p<0.01$). The effect of I on VOL and MO was not significant ($p>0.05$). Traits VOL and MO varied ($p<0.001$) between boars which indicated the need for individual control of each of boars used for artificial insemination. The highest VOL was determined in boars at the age of 26 to 29 months, and the difference to the boars aged 10-13 months was 62.70 ml ($p<0.001$). No statistically significant differences in regard toVOL between boars older than 17 months (from class 3 to 6) were recorded. The highest differences between winter and spring period were determined in mean values of VOL (32.15 ml; $p<0.01$). In the winter period, boar ejaculate volume was lower than in the autumn (-26.57 ml) and the difference determined was statistically significant ($p<0.05$).

Keywords: pigs, boar, ejaculate volume, motility

Introduction

In modern pig production, artificial insemination has completely suppressed the natural mating, so that the evaluation of quantitative and qualitative characteristics of ejaculate is a standard procedure in breeding technology. Rating quantitative and qualitative characteristics of semen has high economic importance to the pig breeders *(Smital, 2010)*.

In populations of pigs which are under continuous selection program there is a tendency for the better utilization of boars, good production performance, which is reflected in the preparation of a large number of doses per ejaculate of optimal fertile ability. In addition to sperm concentration, semen volume and sperm motility determine fertility of doses produced per ejaculate. Motility is the most important trait that affects fertilization capacity of sperm *(Feitsma, 2009)*. Rating motility of spermatozoa is the most important parameter of sperm *(Kunowska-Slósarz and Makowska, 2011)*.

Variability of ejaculate volume and sperm motility is influenced by various genetic and paragenetic factors. Properties of boar sperm vary under the influence of breed, age, season, intensity of use, and other factors *(Stančić et al., 2003; Frangež et al., 2005; Okere et al., 2005; Wolf and Smital, 2009a; Wolf and Smital, 2009b; Kondracki et al., 2009; Smital, 2010; Kunowska-Slósarz and Makowska, 2011)*.

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

Material and methods

The study included a total of 274 ejaculates from 6 boars of breed Large White (LW).

Ejaculates from boars grown under production conditions for a period of two years (October 2010 - September 2012) were examined. The data on the volume of ejaculate (VOL, ml) and sperm motility (MO %) of boars were used. Ejaculate volume was measured with an accuracy of ± 10 ml, and sperm motility evaluation was performed by a subjective method, observing the native sperm sample under a microscope with a standard magnification (visual score). The average number of ejaculates per boar was 45.67, and boars with less than five ejaculate were excluded from the analysis. Ejaculates with VOL below 50 ml or MO less than 70% were not included in the study. At each taking of ejaculate collection) the age of the boar was recorded, and the average age was calculated as the average of all ages at the individual collections of boars. Boars were divided into six classes according to their age when the ejaculate was taken (10-13, 14-17, 18-21, 22-25,

26-29 and ≥30 months). Semen samples were analyzed during four seasons of a year (spring, summer, autumn, winter). The intensity of the use of boars (I) was observed as the influence of groups (classes) of the interval between two successful collections (≤ 5 , 6, 7 , 8, 9-10, 11-13, 14-21 days). Ejaculates from boars that were preceded with the interval between two consecutive successful collections of 3 and 4 days, because of the small number, were attached to the group (class) of ≤5 days.

Data analysis was performed using the GLM procedure using the statistical package *SAS 9.1.3 (SAS Inst. Inc., 2002-2003)*. The following statistical model was used:

$$Y_{ijklm} = \mu + B_i + A_j + S_k + I_l + \varepsilon_{ijklm},$$

where: Y_{ijklm}- observed sperm property, μ- general population average, B_i- the effect of the boar (i=1,2,3,...,6), A_j- the effect of boar age class (j=1,2,3,…,6) , S_k- the effect of season (k=1,2,3,4), I_l- the effect of utilization intensity (l=1,2,3,..., 7) and ε_{ijklm} – random error. Testing (comparison) of Least Square Means (LSMeans) values was done using t- test.

Results and discussion

The mean age of studied boars was 655.93 days or 21.86 months (Table 1). The mean volume of ejaculates was 178.94 ml, sperm motility was 90.35%. The interval between successive collections of boars ranged from 3 to 21 days, or in average 9.37 days.

Table 1. The average values ($\overline{X}$) and variability of studied properties of boar ejaculates

Variable	NE	$\overline{X}$	SD	Min	Max
Volume of ejaculates (VOL, ml)	274	178.94	51.30	50	300
Motility (MO, %)	274	90.35	5.69	70	100
Interval between successive collections (I, day)	274	9.37	3.79	3	21
Age of boars of the collection (A, day)	274	655.93	239.33	289	1628

NE- Number of ejaculates; SD- Standard Deviations; Min- Minimum; Max- Maximum

The average value for VOL of ejaculate in this study was lower compared to the research by *Kunowska-Slósarz and Makowska (2011)* 178.94 compared to 255.25 ml) for the same breed of boars. In regard to MO, the average value in the present paper was higher (+11.20%) compared to the value obtained by aforementioned authors. The high average value of MO in this study was the result of exclusion of ejaculates with value below 70%, and subjectivity in the visual assessment of sperm motility in the ejaculate. In the study by *Sutkevičienė and*

Žilinkas (2004), the average age of the boars was 18.79±5.59 months, with an average volume of ejaculate from 261.60±134.25ml, and the percentage of subjective sperm motility of 71.49±6.42%.

The impact of factors on varying of sperm properties is presented in Table 2. Calculated coefficients of determination indicated that the variability of the VOL compared to the MO, can be explained to a greater extent as influenced by factors observed.

Both properties of sperm varied under the influence of boars (B, $p < 0.001$), indicating a need for individual control of each of boars used for artificial insemination. Unlike the MO whose variability was not influenced by other observed factors, the VOL varied under the influence of age (A, $p<0.001$), and the season (S, $p<0.01$). The intensity of use of boars (I, $p>0.05$) did not significantly affect the variability of VOL and MO in sperm.

Table 2. Effect of variables included in the model

Variable	Semen traits			
	VOL		MO	
	p	R^2	p	R^2
Boars (B)	***	0.361	***	0.143
Classes of age of boars (A)	***		ns	
Season (S)	**		ns	
Interval between successive collections (I)	ns		ns	

VOL- Volume of ejaculates; MO- Motility; R^2- Coefficient of determination;
- $p<0.01$; *- $p<0.001$

The results of this study pertaining to the significance of the factors included in the model are partly consistent with the research of *Smital (2010)* in which all the studied factors (breed-center, year, month, and year x month, boar age, interval between collections and boars) have influenced ($p<0.001$) all the analyzed properties of the sperm.

Both studied properties of sperm varied between boars (Table 3). Boar number 4 had the highest VOL, and the difference compared to the boars number 2 and 3, was 41.57 and 31.57 ml, respectively ($p<0.001$). The difference between boars with the highest and lowest MO (boars 6 and 5) was 8.86% ($p<0.01$). Boar 5 had lower average sperm motility than boars 4 and 2 (-8.38 and -8.69%). The differences established between the mean values of MO between stated boars were highly statistically significant ($p<0.01$).

Table 3. Effect of boars on semen properties

Boars	LSMeans±SE	
	VOL (ml)	MO (%)
1	169.03±9.31	90.19±1.20
2	156.79±7.06^{A}	91.69±0.91^{Aa}
3	166.79±5.05^{A}	88.71±0.65^{a}
4	198.36±4.63^{B}	91.38±0.60$^{b, Aa}$
5	170.42±16.29	83.00±2.09^{Bb}
6	179.81±8.09	91.86±1.04^{Aa}

VOL- Volume of ejaculates; MO- Motility; a, b- $p<0.05$; Aa, Bb- $p<0.01$; A, B- $p<0.001$

If both properties are observed, the highest number of doses with an average motility of spermatozoa of 91.38% was possible to produce from ejaculates taken from boar 4.

The lowest VOL of ejaculate (Table 4) was at the age of boars of 10-13 months (class 1). Lower mean values of the volume of ejaculate (from 134.59 to 169.97 ml) were observed in age classes 1 to 3 (boars at the age of 10 to 21 months), higher values (from 191.04 to 197.29 ml) were observed in the class of 4 to 6 (boars at the age of over 21 months).

The main reason for the increase of VOL with the age of boars is increase in the size and mass of the testes, causing increase in the production of sperm. According *Kanokwan (2011)* one of the reasons for increasing sperm production from a biological point of view is the increase in the number of Sertoli cells in the testes. According to *Ford et al. (2006)* the primary factor in daily sperm production is the number of Sertoli cells, which is associated with the testicular mass. The highest VOL was determined in boars at the age of 26 to 29 months, and the difference compared to the boars aged 10-13 months was 62.70 ml ($p<0.001$). Boars of age group 2 had lower average volume of ejaculate compared to boars in group 4 (-36.66 ml , $p<0.05$), group 6 (-39.55 ml, $p<0.05$) and group 5 (-42.91 ml, $p<0.001$). No statistically significant differences were established in VOL between boars older than 17 months (from class 3 to 6). In populations of pigs which are in a program of continuous selection and breeding, due to high selection pressure, boars are commonly used in the reproduction for 6-8 months, so they do not achieve maximum VOL values. In regard to MO, there were no statistically significant differences ($p>0.05$) in mean values between the age groups of boars.

Table 4. Least Square Means values and differences between classes of age of the boars

Age of the boars		LSMeans±SE	
Classes	Month	VOL (ml)	MO (%)
1	10-13	134.59±10.25^{A}	89.92±1.32
2	14-17	154.38±8.34$^{a, AA}$	90.46±1.08
3	18-21	169.97±7.05	87.87±0.91
4	22-25	191.04±7.30$^{B, b}$	88.27±0.94
5	26-29	197.29±8.53$^{B, BB}$	89.54±1.10
6	≥30	193.93±7.79$^{B, b}$	90.77±1.00

VOL- Volume of ejaculates; MO- Motility; a, b- $p<0.05$; A, B i AA, BB- $p<0.001$

There is a tendency of increase of ejaculate volume with increasing age of boars. Similar conclusions have been made in the study by *Stančić et al.(2003), Banaszewska and Kondracki (2012)*. Ejaculate volume increases until the age of about two years, and remains more or less constant *(Wolf and Smital, 2009a; Wolf and Smital, 2009b)*, so that our research was in accordance with the research of these authors, since the higher VOL values were observed in boars older than 21 months. The results of this study are consistent with research results of *Smital (2009),* in which an increase of sperm parameters with age boars was observed, but achieving of the maximum values of the parameters of boar semen was at the age of 3.5 years.

The results of the present study pertaining to the effect of boar age on MO are consistent with the research of *Šerniené et al. (2002)* in which the differences between the age groups of boars were recorded, but not significant.

The effect of the season on the variability of VOL was highly significant (Table 2, $p<0.01$), and LSMean values during different seasons, according the analyzed properties of the sperm, are presented in Table 5.

The highest VOL was in the summer period (187.34±7.75 ml), and lowest in the winter period (155.19±6.37 ml). The highest difference in mean values was recorded between these two annual seasons (32.15 ml; $p<0.01$). In winter the volume of ejaculates was lower than in the autumn (-26.57 ml) and observed difference was statistically significant ($p<0.05$). Low value of VOL during winter may be due to inadequate housing conditions caused by low temperatures. The lowest MO was during the winter period (88.58%), but when it comes to the variability of these properties, the differences between seasons were not significant.

Table 5. Effect of season on variability of the semen traits

Season	LSMeans±SE	
	VOL (ml)	MO (%)
Spring	169.85±6.33	89.50±0.81
Summer	187.34±7.75^{Aa}	89.10±1.00
Autumn	181.76±7.29^{a}	90.71±0.94
Winter	155.19±6.37$^{Bb, b}$	88.58±0.82

VOL- Volume of ejaculates; MO- Motility; a, b- $p<0.05$; Aa, Bb- $p<0.01$

The results of this study, which related to the value of the VOL in the spring period (169.85 ml), and which was below the annual average (178.94 ml), are partially similar to studies by *Okere et al. (2005), Kondracki et al. (2009), Wolf and Smital (2009a)*, who established, in the spring, the lowest volume of ejaculate in the studied breeds. Partial similarity is also with the results of research *Kunowska-Slósarz and Makowska (2011),* given that in the study of the authors in the period January-June VOL values determined were below the annual average, and above average values from August to December.

In Table 6, LSMean values are presented according to groups (classes) of the interval between two successful collections. There were differences between the groups (classes) but they were not significant ($p>0.05$). A small number of ejaculate that were preceded by the interval between two consecutive successful collections of three or four days contributed to a reduction in the variability of sperm properties influenced by this factor.

Table 6. Effect of the interval between successive collections (I)

Interval between successive collections (I, day)	LSMeans±SE	
	VOL (ml)	MO (%)
≤5	159.65±8.80	88.34±1.13
6	182.30±7.88	89.80±1.01
7	173.91±8.37	89.16±1.07
8	175.94±7.15	89.23±0.92
9-10	169.47±6.26	89.91±0.80
11-13	173.97±7.05	89.66±0.91
14-21	179.50±7.67	90.21±0.99

VOL- Volume of ejaculates; MO- Motility

Contrary to the present study, in the research carried out by *Frangež et al. (2005)*, the differences in the characteristics between the sperm of different frequencies of use of boars were established. The research by *Wolf and Smital (2009a)* has shown small increase in ejaculate VOL with extension of I from two to seven days, while for longer intervals changes were insignificant, and small changes have been observed when it comes to the variability of MO.

Conclusion

Variability in the ejaculate VOL compared to the MO can be largely explained by the influence of the analyzed factors .

Ejaculate volume varied under the influence of boars (B, $p< 0.001$), age (A, $p<0.001$), and the season (S, $p<0.01$). MO variability was only under a significant effect of boars (B, $p< 0.001$). The intensity of use of boars (I, $p>0.05$) in this study did not show a significant effect on the studied properties of semen.

Variability between boars in the studied traits of sperm is high, indicating the need for individual control of each of boars used for artificial insemination.

There is a tendency of VOL increase with the age of boars. No statistically significant differences were determined between VOL values of boars older than 17 months (from class 3 to 6). However, given that in the selected population of pigs, boars are used in the reproduction for 6 to 8 months, this tendency has no practical significance.

Given that there are differences in the VOL according to seasons, it is necessary to provide boars during the year with the adequate housing conditions and optimum microclimate parameters.

Acknowledgements

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

Varijabilnost volumena ejakulata i pokretljivosti spermatozoida u zavisnosti od starosti i intenziteta korišćenja nerasta

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

Rezime

Osnovni cilj istraživanja bio je da se oceni uticaj starosti (A) i intenziteta korišćenja nerasta (I) na fenotipsku varijabilnost volumena ejakulata (VOL) i pokretljivosti spermatozoida (MO). Istraživanjem je bilo obuhvaćeno 274 ejakulata nerasta rase veliki jorkšir (LW). Nerasti su prema starosti kada je uzet ejakulat podeljeni u šest klasa (10-13, 14-17, 18-21, 22-25, 26-29 i ≥30 meseci). Ejakulati su analizirani tokom četiri godišnje sezone (proleće, leto, jesen, zima). Intenzitet korišćenja nerasta posmatran je kao uticaj grupe (klase) intervala između dva uspešna skoka (I; ≤5, 6, 7, 8, 9-10, 11-13, 14-21 dan). Obrada podataka izvršena primenom GLM procedure uz pomoć statističkog paketa *SAS 9.1.3 (SAS Inst. Inc., 2002-2003)*.

Prosečne vrednosti VOL, MO, I, A bile su: 178,94 ml, 90,35%, 9,37 dana i 655,93 dana. Uticaj nerasta na obe ispitivane osobine sperme bio je vrlo visoko značajan ($p<0,001$). Volumen ejakulata varirao je pod uticajem starosti nerasta ($p<0,001$) i sezone ($p<0,01$). Uticaj I na VOL i MO nije bio značajan ($p>0,05$). Osobine VOL i MO varirale su ($p<0,001$) između nerasta što ukazuju na potrebu

individualne kontrole svakog nerasta koji se koristi za veštačko osemenjavanje. Najveći VOL utvrđen je kod nerasta pri uzrastu od 26 do 29 meseci, a razlika u odnosu na neraste uzrasta 10-13 meseci bila je 62,70 ml ($p<0,001$). Nisu ustanovljene statistički značajne razlike VOL izmedju nerasta starijih od 17 meseci (od klase 3 do 6). Izmedju zimskog i letnjeg perioda godine utvrdjena je najveća razlika srednjih vrednosti VOL (32,15 ml; $p<0,01$). U zimskom periodu volumen ejakulata nerasta bio je manji nego u toku jeseni (-26,57 ml) i utvrdjena razlika je bila statistički značajna ($p<0,05$).

References

BANASZEWSKA D., KONDRACKI S. (2012): An assessment of the breeding maturity of insemination boars based on ejaculate quality changes. Folia Biologica (Kraków), 60, 3-4, 151-162.

FEITSMA H. (2009): Artificial insemination in pigs, research and developments in the Netherlands, a review. Acta scientiae veterinariae, 37, 1, 61-71.

FORD J.J., McCOARD S.A., WISE T.H., LUNSTRA D.D., ROHRER G.A. (2006): Genetic variation in sperm production. Control of pig reproduction VII: Proceedings of the Seventh International Conference on Pig Reproduction, Kerkrade, the Netherlands, June 2005/ edited by C.J. Ashworth and R.R. Kraaling, Nottingham, U. K.: Nottingham University Press, 99-112.

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, 3**,** 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.
SUTKEVIČIENÉ N., ŽILINKAS H. (2004): Sperm morphology and fertility in artificial insemination boars. Veterinarija ir zootechnika, 26, 48, 11-13.
ŠERNIENÉ L., RIŠKEVIČIENÉ V., BANYS A., ŽILINKAS H. (2002): Effects of age, and season on sperm qualitative parameters in Lithuanian white and Pietrain 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 22 July 2013; accepted for publication 28 November 2013

Biotechnology in Animal Husbandry 29 (4), p 651-658 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.05'64
DOI: 10.2298/ BAH1304651S

THE EFFECT OF GENDER AND BREED ON SOME PROPERTIES OF PIG MEAT

N. Stanišić[1], M. Petrović[2], Č. Radović[1], M. Gogić[1], N. Parunović[3], S. Stajić[2], M. Petričević[1]

[1] Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
[2] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Serbia
[3] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000, Belgrade, Serbia
Corresponding author: nikola0135@yahoo.com
Original scientific paper

Abstract: This study was conducted to investigate differences in characteristics of muscles from male and female fatteners from Mangalitsa and Swedish Landrace pig breed. The research was carried out on three muscles: *m. longissimus thoracis et lumborum*, *m. gluteus medius* and *m. triceps brachii*. Compared to the Swedish Landrace, it was found out that the meat of the Mangalitsa had considerably higher intramuscular fat content ($p<0.05$). The highest intramuscular fat content was determined in male fatteners of Mangalitsa in *m. gluteus medius* (6.81%) and the lowest in female fatteners of Landrace pig breed in the *longissimus* muscle (1.12%). As for technological quality of meat, after cooking, meat from Mangalitsa pig had greater cooking weight loss compared to Landrace and also lower shear force (SF) values for *longissimus*, but higher SF values for *gluteus* muscle. Gender had no statistically significant effect on technological meat quality. It can be concluded, on the base of the results, that the Mangalitsa pig, had favourable meat quality traits, which are very desired for production of traditional meat products of high quality.

Key words: Mangalitsa, gender, meat, chemical composition, technological quality

Introduction

Quality of meat within species can be influenced by the origin of the animals, the feed materials, slaughter and chilling procedures, storage conditions, muscle type, etc. Meat and meat products from autochthonous pigs are highly appreciated by consumers because of their high sensory quality *(Živković et al., 2012; Stanišić et al., 2013)*.

In Republic of Serbia three native autochthonous pig breeds are registered: Mangalitsa, Moravka and Resavka. First one is the most present and the last one

the least *(Petrović et al., 2010)*. Mangalitsa is characterized by dark colour, robust constitutions and slower growth rate with higher adiposity and reduced lean deposition in comparison to modern white pigs. For this reason, producers have lost their interest in producing these pig breeds and their numbers have declined considerably to the point of extinction. However, fat is one of the most important raw material derived from slaughtered pigs and one of the main ingredients for the production of quality meat products *(Stanišić et al., 2012)*, especially in the production of high priced cured meat products, such as proscuito and dry-cured ham *(Antequera et al., 1992)*. Fat contributes to the juiciness of dry-cured hams and due to the lipolytic and oxidative processes that occurs during the curing treatment, it also influences the development of the aroma *(López et al., 1992)*.

The present study was undertaken to evaluate the differences in proximate composition, colour, technological and physicochemical characteristics of meat of Mangalitsa and Swedish Landrace pigs.

Materials and Methods

Trial was carried out on the farm, in slaughterhouse and laboratory of the Institute for Animal Husbandry (Belgrade, Serbia) on two different pig breeds: Mangalitsa (n=16) and Sweidish Landrace (n=16). Each group of pigs was divided into two equal groups according to gender. The diet of animals consisted of concentrated commercial feed administered "*ad libitum*" (Table 1). Water was provided using automatic feeding troughs. Pigs were slaughtered with average age of 12 months (Manglaitsa) and 6 months (Landrace), when they attained their target slaughter weight of 110 kg.

Table 1. Composition of feed used in the fattening of pigs

Mixture ingredients (%)	First 7 days of fattening	To the end of fattening
Corn - dry	67.50	68.75
Livestock flour	10.00	10.00
Soybean meal	14.80	14.80
Sunflower meal	2.00	2.00
Soybean oil	2.50	1.25
Lime	1.30	1.30
Monocalcium phosphate	0.80	0.80
Salt	0.45	0.45
Premix	0.50	0.50
Synthetic lysine	0.05	0.05

Animals were denied food 12h prior to slaughtering, but had free access to water. After slaughtering, pig carcasses were processed using standard techniques. After hair removal and evisseration, carcasses were cut into carcass sides and put in cooling chamber at temperature of 2-4°C for next 24 hours. After chilling, all muscle samples (*m. longissimus thoracis et lumborum*, *m. gluteus medius*, *m. triceps brachii*) were removed from the left carcasses side.

Analyses of pH value, water holding capacity, shear force and cooking loss, were performed on fresh meat samples, one day after slaughter and chilling of carcass sides. Chemical analyses were performed on defrosted meat samples, subsequent to freezing at -18°C.

In all samples, main proximate composition was determined: moisture content by drying of samples at temperature of 105°C to a constant mass, quantity of intramuscular fat by method of extraction according to Soxlet (with petrol-ether as solvent), quantity of proteins using method according to Kjeldahl and multiplying by factor 6,25 and quantity of mineral matters (ashes) by burning of samples at temperature of 550-600°C to a constant mass *(AOAC, 1990)*.

Water holding capacity (WHC) was done according to method by Grau and Hamm *(Grau et al., 1953)*. pH value of meat was measured using pH-meter Hanna, HI 83141 (Hanna Instruments, USA). Cooking loss was determined in the following way: sample of size of 3x4x2 cm is weighed and put into glass with boiling water and cooked for 10 minutes; difference in mass of sample before and after cooking represents loss of mass during heat treatment and it is expressed in percentages. Samples used for determination of cooking loss were also used for determination of meat shear force (kg): muscles have been cut to parts of size of 1x1 cm in the direction of muscle fibre extension; meat tenderness, expressed by shear force, was measured on Volodkevich apparatus *(Volodkevich, 1938)*; higher values read out on the apparatus marked higher values of shear force, i.e. firmer meat.

In order to determine the effect of gender and breed on meat quality characteristics, a 2x2 factorial design was performed using General Linear Model procedure of SPSS 15.0 software (IBM Statistics). Interactions between gender and breed were not significant and therefore are not shown in this paper. All results are expresed as mean ± standard deviation.

Results and Discussion

Proximate composition of meat samples from male and female fatteners of Mangalitsa and Swedish Landrace breed is presented in Table 2. From the literature data it is known that the percentage of intramuscular fat influence meat appearance (marbling) and also have an effect on meat tenderness, juiciness and flavour. Regarding this, for *longissimus* muscle, an optimum level of 2% of

intramuscular fat has been proposed *(Bejerholm and Barton-Gade, 1986)*. In all analysed meat samples, content of intramuscular fat was statistically significantly higher in Mangalitsa compared with Landrace. That tendency of autochotonous pig breeds to have higher intramuscular fat content than improved breeds is well known *(Estévez et al., 2003; Franci et al., 2005; Petrović et al., 2010; Parunović et al., 2013)*. Regarding gender of animals, in *longissimus* and *gluteus* muscle, male fatteners had higher share of fat compared with females, while in *triceps* muscle, female fatteners had statisticly higher content of intramuscular fat. The highest intramuscular fat content was determined in male fatteners of Mangalitsa in *m. gluteus medius* (6.81%) and the lowest in female fatteners of Landrace in the *longissimus* muscle (1.12%).

Effect of breed on reduction of fat content probably influenced the increased moisture content, which was statistically significantly higher in *gluteus* muscle of Landrace compared with Mangalitsa. The higher content of ash was established *longissimus* muscle of Landrace. Content of protein did not differ significantly between the analysed groups.

Table 2. Proximate composition of meat from two pig breeds

Content (%)	Mangalitsa		Swedish Landrace		Significance	
	Male	Female	Male	Female	G[1]	B[2]
M. longissimus thoracis et lumborum						
Moisture	69.41 ± 1.86	70.85 ± 1.84	73.52 ± 0.65	73.85 ± 1.65	ns	ns
Fat	6.73 ± 0.46	5.09 ± 0.29	1.71 ± 0.51	1.12 ± 0.31	*	*
Ash	1.10 ± 0.05	1.00 ± 0.10	1.16 ± 0.05	1.18 ± 0.03	ns	*
Protein	22.73 ± 0.94	22.33 ± 1.23	23.56 ± 0.24	23.81 ± 0.55	ns	ns
M. gluteus medius						
Moisture	70.96 ± 2.44	71.92 ± 2.22	74.29 ± 0.67	74.54 ± 0.79	ns	*
Fat	6.81 ± 0.38	5.76 ± 0.90	1.83 ± 0.66	1.50 ± 0.66	*	*
Ash	1.12 ± 0.09	1.05 ± 0.10	1.20 ± 0.07	1.20 ± 0.04	ns	ns
Protein	21.95 ± 1.15	21.25 ± 2.26	22.67 ± 0.97	22.75 ± 0.91	ns	ns
M. triceps brachii						
Moisture	71.19 ± 2.18	71.73 ± 0.37	75.32 ± 0.06	74.52 ± 0.25	ns	ns
Fat	6.16 ± 0.47	6.66 ± 1.99	1.85 ± 0.33	1.88 ± 0.62	*	*
Ash	1.17 ± 0.08	1.15 ± 0.06	1.19 ± 0.02	1.18 ± 0.08	ns	ns
Protein	21.46 ± 1.10	20.45 ± 2.30	21.61 ± 0.36	22.38 ± 0.81	ns	ns

[1] G – Gender; [2] B – Breed.
ns – non significant; * – $p<0.05$.

Pale, soft and exudative (PSE) pork was not an issue in the current trial as the pH measured in the muscles 45 minutes *post mortem* were all <6.0 (results not shown). The ultimate pH of all genotypes was 5.5-5.7, which is normally observed for pork (Table 3).

Table 3. Technological quality of meat from two pig breeds

	Mangalitsa		Landrace		Significance	
	Male	Female	Male	Female	G[1]	B[2]
M. longissimus thoracis et lumborum						
WHC[3], cm^2	12.81 ± 2.40	13.92 ± 1.99	12.86 ± 1.24	11.89 ± 2.54	ns	ns
pH	5.50 ± 0.02	5.51 ± 0.03	5.47 ± 0.03	5.53 ± 0.05	ns	ns
SF[4], kg	5.66 ± 1.09	5.87 ± 1.15	6.40 ± 0.68	6.31 ± 0.87	ns	*
CL[5], %	33.73 ± 3.55	31.55 ± 2.75	28.23 ± 1.71	27.91 ± 2.66	ns	*
M. gluteus medius						
WHC, cm^2	11.08 ± 1.99	11.76 ± 1.51	12.21 ± 1.31	11.50 ± 0.96	ns	ns
pH	5.59 ± 0.10	5.58 ± 0.03	5.61 ± 0.07	5.63 ± 0.08	ns	ns
SF, kg	8.69 ± 1.11	9.40 ± 1.92	7.74 ± 1.82	7.04 ± 1.25	ns	*
CL, %	39.02 ± 2.87	35.35 ± 3.33	27.39 ± 1.82	26.33 ± 1.90	ns	*
M. triceps brachii						
WHC, cm^2	16.76 ± 1.24	17.04 ± 2.07	12.24 ± 1.71	14.28 ± 1.39	ns	*
pH	5.51 ± 0.07	5.49 ± 0.09	5.62 ± 0.02	5.62 ± 0.05	ns	ns
SF, kg	7.75 ± 1.05	7.64 ± 1.16	6.23 ± 0.84	7.36 ± 2.25	ns	ns
CL, %	35.65 ± 2.09	37.51 ± 2.20	29.41 ± 2.35	31.59 ± 3.32	ns	*

[1] G – Gender; [2] B – Breed.
[3] WHC – Water holding capacity (cm^2); [4] SH – Shear force (kg); [5] CL – Cooking weight loss (%).
ns – non significant; * – $p<0.05$.

According to *Ouali et al. (1990)* meat tenderness is affected by the origin and age of animals, their gender, breed, environmental conditions associated with the pre-slaughter stress, the slaughter itself as well as the time of meat ageing. An objective measure of tenderness is the force required to shear a piece of meat with low shear values being desirable. In this trial, breed had a significant effect on shear force (SF) values of cooked meat samples (Table 3). Mangalitsa fatteners had lower SF values for *longissimus*, but higher SF values for *gluteus* muscle, compared with Landrace. Meat shear force values were approximately the same between groups for *triceps brachii* muscle.

Cooking led to a systematic and significant loss of matter and the cooking yields differed depending on the muscle and cooking process *(Gerber et al., 2009)*.

In this trial, cooking weight loss was greater in muscles from Mangalitsa and statistically significant differences ($p<0.05$) in this parameter were found for all analysed meat samples (Table 3).

Water holding capacity (WHC) of muscles were not influenced by gender or pig breed, except for *m. triceps brachii*, where it is determined that Landrace had better WHC compared with Mangalitsa (Table 3).

Conclusion

Compared to the Swedish Landrace, it was found out that the meat from the Mangalitsa had considerably higher intramuscular fat content in all analysed meat samples. As aspected, female fatteners had a significantly lower fat content when compared with male. Moisture content was statistically significantly higher in *gluteus* muscle of Landrace compared with Mangalitsa pigs. As for technological quality of meat, after cooking, meat from Mangalitsa had greater cooking weight loss compared to Landrace and also lower shear force values for *longissimus*, but higher shear force values for *gluteus* muscle. Gender had no statistically significant effect on technological meat quality.

From the obtained results it could be concluded that the autohthonous pig breeds, such as Mangalitsa, had favourable meat quality traits, which are very desirable for production of traditional meat products of high quality.

Acknowledgement

This paper is a result of the research within the project TR31081, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Uticaj pola i rase na fizičko-hemijske karakteristike mesa svinja

N. Stanišić, M. Petrović, Č. Radović, M. Gogić, N. Parunović, S. Stajić, M. Petričević

Rezime

Ovaj ogled je imao za cilj da ispita razlike u fizičko-hemijskim karakteristikama mišića između muških i ženskih tovljenika rase mangulica i švedski landras. Istraživanje je sprovedeno na tri mišića: *longissimus thoracis et*

lumborum, *gluteus medius* i *triceps brachii*. U poređenju sa švedskim landrasom, meso mangulica je imalo znatno veći udeo intramuskularne masti ($p<0,05$). Najveći sadržaj intramuskularne masti utvrđen je kod muških tovljenika rase mangulica u *gluteus medius*-u (6,81%), a najniži ko ženskih tovljenika rase švedski landras u *longissimus*-u (1,12%). Što se tiče tehnološkog kvaliteta, meso mangulice je imalo veći gubitak mase tokom kuvanja u odnosu na meso landrasa i niže vrednosti sile sečenja (SF) za *longissimus*, ali veće vrednosti sile sečenja za *gluteus* mišić. Pol nije imao statistički značajan uticaj na parametre tehnološkog kvaliteta mesa. Na osnovu dobijenih rezultata može se zaključiti da meso mangulice ima veoma dobre parametre kvaliteta, a koji su poželjni pri proizvodnji tradicionalnih proizvoda od mesa.

References

ANTEQUERA T., LÓPEZ-BOTE C.J., CÓRDOBA J.J., GARCÍA C., ASENSIO M.A., VENTANAS J., GARCÍA-REGUEIRO J.A., DÍAZ I. (1992): Lipid oxidative changes in the processing of Iberian pig hams. Food Chemistry, 45, 105-110.

AOAC (1990): Official methods of analysis. Washington, DC: Association of Official Analytical Chemists.

BEJERHOLM C., BARTON-GADE P.A. (1986): Effect of intramuscular fat level on eating quality of pig meat. Danish Meat Research Institute manuscript no. 720E: 1-5.

ESTÉVEZ M., MORCUENDE D., CAVALOPEZ R. (2003): Physico-chemical characteristicsof *M. Longissimus dorsi* from three lines of free-range reared Iberian pigs slaughtered at 90 kg live-weight and commercial pigs: a comparative study. Meat Sci., 64, 499-506.

FRANCI O., BOZZI R., PUGLIESE C., ACCIAIOLI A., CAMPODONI G., GANDINI G. (2005): Performance of Cinta Senese and their crosses with Large White. 1 Muscle and subcutaneous fat characteristics. Meat Sci., 69, 545-550.

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

GRAU R., HAMM R., BAUMANN A. (1953): Über das Wasserbindungsvermögen des toten Säugetiermuskels. I. Mitteilung. Der Einfluß des pH Wertes auf die Wasserbindung von zerkleinertem Rindermuskel. Biochem. Z., 325, 1-11.

LÓPEZ M.O., DE LA HOZ L., CAMBERO M I., GALLARDO E., REGLERO G., ORDÓÑEZ J.A. (1992): Volatile compounds of dry hams from Iberian pigs. Meat Science, 31, 267-277.

OUALI A. (1990): Meat tenderisation: possible causes and mechanisms. A review. Journal of Muscle Foods, 1, 129-165.
PARUNOVIĆ N., PETROVIĆ M., MATEKALO-SVERAK V., RADOVIĆ Č., STANIŠIĆ N. (2013): Carcass properties, chemical content and fatty acid composition of the musculus longissimus of different pig genotypes. South African Journal of Animal Science, 43, 2, 123-136.
PETROVIĆ M., RADOVIĆ Č., PARUNOVIĆ N., MIJATOVIĆ M., RADOJKOVIĆ D., ALEKSIĆ S., STANIŠIĆ N., POPOVAC M. (2010): Quality traits of carcass sides and meat of Moravka and Mangalitsa pig breeds. Biotechnology in Animal Husbandry, 26, 21-27.
STANIŠIĆ N., LILIĆ S., PETROVIĆ M., ŽIVKOVIĆ D., RADOVIĆ Č., PETRIČEVIĆ M., GOGIĆ M. (2012): Proximate composition and sensory characteristics of Sremska sausage producedin a traditional smoking house. 6th Central European Congress on Food, CEFood2012, Novi Sad, Serbia, 23-26. May, Proceedings, 1319-1324.
STANIŠIĆ N., PETROVIĆ M., PARUNOVIĆ N., LILIĆ S., RADOVIĆ Č., GOGIĆ M., PETRIČEVIĆ M. (2013): Physicochemical properties of meat from three different pig breeds. International 57th Meat Industry Conference, June 10-12., Belgrade, Serbia, Proceedings, 190-193.
VOLODKEVICH N.N. (1938): Apparatus for measurement of chewing resistance or tenderness of foodstuffs. Food Research, 3, 221-225.
ŽIVKOVIĆ D., RADULOVIĆ Z., ALEKSIĆ S., PERUNOVIĆ M., STAJIĆ S., STANIŠIĆ N., RADOVIĆ Č. (2012): Chemical, sensory and microbiological characteristics of Sremska sausage (traditional dry-fermented Serbian sausage) as affected by pig breed. African journal of biotechnology, 11, 16, 3858-3867.

Received 29 November 2013; accepted for publication 20 December 2013

Biotechnology in Animal Husbandry 29 (4), p 659-664 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.07'637.12'62
DOI: 10.2298/ BAH1304659D

THE INFLUENCE OF BLOOD AND MILK SERUM ZINC CONCENTRATION ON MILK SOMATIC CELL COUNT IN DAIRY COWS

I. Davidov, M. Radinović, M. Erdeljan, Z. Kovačević, Ž. Jurakić, M.Ž. Radinović

Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
Corresponding author: ivanadav@polj.uns.ac.rs
Original scientific paper

Abstract: The objective of this study was to evaluate the influence of blood and milk zinc concentration on somatic cell count, milk production and occurrence of subclinical mastitis cases. The study was performed on thirty Holstein cows approximate same body weight, ages 3 to 5 years, with equally milk production. Blood samples were taken after the morning milking from the caudal vein and milk from all four quarters was taken during morning milking. All samples of blood and milk were taken to determined zinc, using inductively coupled plasma mass spectrometry. 37.67% (11/30) cows have blood serum zinc concentration below 7µmol/l, and 63.33% or 19/30 cows have blood serum zinc concentration higher then 13µmol/l. Also 30% (9/30) cows have somatic cell count lower then 400.000/ml which indicate absence of subclinical mastitis, but 70% (21/30) cows have somatic cell count higher then 400.000/ml which indicate subclinical mastitis. According to all results in this research, zinc has very important influence on reducing subclinical mastitis and somatic cell count in milk of dairy cows.

Key wards: zinc, somatic cell count, dairy cows

Introduction

Adequate mineral nutrition may be used as a strategy to optimize immune system function by the reduction of metabolic and oxidative stress and therefore it may have a positive effect on defense mechanisms on mammary gland against mastitis *(Weiss and Wyatt, 2002; Cortinhas et al., 2010)*. Zinc is involved in the keratinization of epithelial tissue. These tissues include the lining of the streak canal of teats which is the port of entry to the udder of mastitis- causing microorganisms. Zinc is also involved in the maintenance of immune status. Zinc deficiency might be a factor in predisposing cows to mastitis and high milk somatic

cell count *(Whitaker et al., 1997; Meglia et al., 2004; Cortinhas et al., 2010)*. High somatic cell count reflects subclinical mastitis, which is reputed to cause a greater loss of milk to the dairy industry than clinical cases. With high somatic cell count in milk attracting financial penalties it is important for farmers and their advisers that such claims are soundly based and correct *(Whitaker et al., 1997)*.

Siciliano-Jones et al. (2008) supplemented dairy cows with organic source of Zn, Mn, Cu and Co, and reported no changes in milk composition and somatic cell count. *Griffiths et al. (2007)* studied Zn, Mn and Cu supplementation as an amino acid complex for grazing dairy cows reported increased milk production, but no differences on somatic cell count and milk composition was observed. In contrast, *Kinal et al. (2007)* observed a reduction in somatic cell count with organic sources of Zn, Mn and Cu supplementation for dairy cows during 305 days of lactation.

The objective of this study was to evaluate the influence of blood and milk zinc concentration on somatic cell count, milk production and occurrence of subclinical mastitis cases.

Materials and Methods

The study was performed on thirty Holstein cows approximate same body weight, ages 3 to 5 years, in the same phase of lactation (120-180 days of lactation), with equally milk production. All cows were stabling with dry straw for bedding and with ad libitum access to potable water, and feed by total mixed ration. The total mixed ration contained maize silage, grass silage, cracked wheat, soyabean meal, rapeseed meal, sugar beet and hay.

Blood samples were taken after the morning milking from the caudal vein by applying the principles of asepsis and antisepsis and minimum distress of animal. Blood samples were taken in vacutainers manufacturers BD Vacutainer Systems, Preanalytical Solutions UK with added anticoagulant K3E in quantities of 0.072 ml. After taking the samples should be gently shaken several times in order to anticoagulant mixes with the sample. After sampling each vacutainer is marked with ID number of cow and left at room temperature. The solution was further diluted with water and zinc was subsequently determined using inductively coupled plasma mass spectrometry (Perkin Elmer Elan 6100 ICPMS, Massachusetts, USA).

Milk from all four quarters was taken during morning milking using testers. After sampling each battle with milk sample is marked with ID number of cow and left at incubation on 37˚C for 24 hours in order to segregate milk serum. The solution was further diluted with water and zinc was subsequently determined using inductively coupled plasma mass spectrometry (Perkin Elmer Elan 6100 ICPMS, Massachusetts, USA). Whole milk samples were taken with milk meter for somatic cell count. When quarter milk samples were taken the teat ends were

disinfected. Milk samples for somatic cell count were analyzed by the Fluor-optoelectronic method (Fossomatic; Foss Electric, Hillerod, Denmark). Daily milk production of tested cows was measured using Waikato devices attached to milking machine.

Results and Discussion

After analyzing samples of blood and milk serums of 30 Holstein cows, shows that concentration of zinc varied and somatic cell count as well. Also analyzing milk production, it is noticed that cows with low blood zinc concentration have high somatic cell count and lower milk production. All this results are showed in table 1.

Table 1. Blood and milk serum concentration of zinc, somatic cell count (SCC) and milk production

No. of cows	Blood Zn conc. µmol/l	Milk Zn conc. µmol/l	SCC x10^3	Milk prod. kg	No. of cows	Blood Zn conc. µmol/l	Milk Zn conc. µmol/l	SCC x10^3	Milk prod. kg
1.	15.07	27.48	410	22	16.	13.23	5.65	470	21
2.	16.41	37.98	390	23.5	17.	18.12	6.84	380	24
3.	2.04	70.46	550	18	18.	18.22	5.08	350	24.5
4.	22.69	49.37	310	28	19.	17.59	3.06	370	23
5.	15.76	31.49	410	23	20.	21.15	7.76	330	26
6.	15.05	25.33	450	24	21.	5.58	5.08	560	19
7.	1.79	24.74	540	20	22.	13.98	4.56	430	22
8.	2.84	76.32	560	19	23.	15.86	7.88	400	22
9.	1.78	57.38	560	17.5	24.	6.64	3.12	470	19,5
10.	16.76	45.66	430	19	25.	4.95	6.84	500	18
11.	16.08	27.08	440	18	26.	5.64	1.02	560	17
12.	1.66	56.31	540	18	27.	18.62	1.75	420	20
13.	20.35	76.78	290	27	28.	6.61	3.62	550	18
14.	17.51	69.58	310	25	29.	16.61	6.04	460	21
15.	1.89	77.38	550	20	30.	13.28	1.78	490	21

Siciliano-Jones et al. (2008) reported that there is no changes in milk composition and somatic cell count after supplemented dairy cows with organic source of Zn, Mn, Cu and Co. *Griffiths et al. (2007)* studied Zn, Mn and Cu supplementation as an amino acid complex for grazing dairy cows, and reported increased milk production, but no differences on somatic cell count. In contrast, *Kinal et al. (2007)* observed a reduction in somatic cell count with organic sources of Zn, Mn and Cu supplementation for dairy cows during 305 days of lactation. Analyzing results in this research shows reduction in somatic cell count and absence of subclinical mastitis in cows with blood serum zinc concentration higher

then 13µmol/l. It is known that physiology level of zinc blood serum concentration are between 7-13µmol/l. 37.67% (11/30) cows have blood serum zinc concentration below 7µmol/l, and 63.33% or 19/30 cows have blood serum zinc concentration higher then 13µmol/l. Average value of blood zinc concentration was 12.12 µmol/l. Also 30% (9/30) cows have somatic cell count lower then 400.000/ml which indicate absence of subclinical mastitis, but 70% (21/30) cows have somatic cell count higher then 400.000/ml which indicate subclinical mastitis.

According to table 1, all cows with subclinical mastitis have milk serum zinc concentration between 1.02-77.38 µmol/ and average value was 26.62 µmol/l. In cows without subclinical mastitis milk serum zinc concentration was between 3.06-76.78 µmol/l and average value was 29.37 µmol/l. There is no data of influence of milk serum zinc concentration on milk somatic cell count and milk production.

In table 2, statistical test correlation show a negative correlation between blood serum zinc concentration and somatic cell count, which indicate that zinc from blood have influence on health and secretion of mammary gland. Blood serum zinc concentration and milk production are in positive correlation, which indicate no influence on secretion of mammary gland. Also milk zinc serum concentration, somatic cell count and milk production are in positive correlation which indicate that zinc from milk have not influence on health and secretion of mammary gland.

Table 2. Test correlation between blood and milk serum zinc concentration, somatic cell count (SCC) and milk production

Blood Zn conc.-SCC	Blood Zn conc.-Milk prod.	Milk Zn conc.-SCC	Milk Zn conc.-Milk prod.
-0.91429	0.78091	0.02311	0.05041

According to results in the table 2, there is no significant influence of zinc milk serum concentration to somatic cell count and milk production, but zinc blood serum concentration had significant influence on somatic cell count and reducing subclinical mastitis.

Conclusion

The results indicate that cows with level of zinc in blood serum higher then 13 µmol/l have lower somatic cell count and better milk production. Cows with lower zinc blood serum concentration then 7 µmol/l have high somatic cell count and high incidence of subclinical mastitis. According to all results in this research, zinc has very important influence on reducing subclinical mastitis and somatic cell count in milk of dairy cows.

Uticaj koncentracije cinka krvnog i mlečnog seruma na broj somatskih ćelija mleka visoko mlečnih krava

I. Davidov, M. Radinović, M. Erdeljan, Z. Kovačević, Ž. Jurakić, M.Ž. Radinović

Rezime

Cilj ovog istraživanja je da se utvrdi uticaj cinka iz krvnog i mlečnog seruma na broj somatskih ćelija u mleku krava, kao i njegov uticaj na produkciju mleka i pojavu subkliničkih mastitisa. Istraživanje je sprovedeno na trideset visoko mlečnih krava Holštajn rase. Sve krave su bile približne težine, starosti između 3 i 5 godina i davale su približno istu količinu mleka. Uzorci mleka su uzimani posle jutarnje muže iz kaudalne vene, dok su uzorci mleka uzimani iz sve četiri četvrti pre jutarnje muže. Svi uzorci seruma mleka i krvi su korišćeni za utvrđivanje koncentracije cinka primenom spektormetrije. 37,67% (11/30) krava je bilo sa koncentracijom cinka ispod 7μmol/l, a 63,33% odnosno 19/30 krava je bilo sa koncentracijom cinka iznad 13 μmol/l. Takođe, 30% (9/30) krava je bilo sa brojem somatskih ćelija u mleku ispod 400.000/ml što ukazuje na odsustvo subklinikog mastitisa, dok je 70% (21/30) bilo sa većim brojem somatskih ćelija u mleku od 400.000/ml što je pokazatelj subklinikog mastitisa. Na osnovu dobijenih rezultata može se zaključiti da cink ima bitnu ulogu u smanjenju broja pojavljivanja subkliničkih mastitisa kao i na smanjenje broja somatskih ćelija u mleku visoko mlečnih krava.

References

CORTINHAS C.S., BOTARO B.G., SUCUPIRA M.C.A., RENNO F.P., SANTOS M.V. (2010): Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper and selenium. Livestock Science, 127, 84-87.

GRIFFITHS L.M., LOEFFLER S.H., SOCHA M.T., TOMLINSON D.J., JOHNSON A.B. (2007): Effects of supplementing complexed zinc, manganese, copper and cobalton lactation and reproductive performance of intensivly grazed lactating lactating dairy cattle on the South Island of New Zealand. Anim. Feed Sci. Technol.,137, 69-83.

KINAL S., KORNIEWICZ D., JAMROZ A., SLUPCZYNSKA M., BODARSKI M., ZIEMINSKI R., OSIEGLOWSKI S., DYMARSKI I. (2007): The effctiveness of zinc, copper and manganese applied in organic forms in diets of high milk yielding cows. J. Food Agric. Environ., 5, 189-193.

MEGLIA G.E., HOLTENIUS K., PETERSSON L., OHAGEN P., PERSSON WALLER K. (2004): Prediction of vitamin A, vitamin E, selenium and zinc status

of periparturient dairy cows using blood sampling durig the mid dry period. Acta Vet. Scand., 45, 119-128.
SICILIANO-JONES J.L., SOCHA M.T., TOMLINSON D.J., DeFRAIN J.M. (2008): Effect of trace mineral source on lactation performance, claw integrity and fertility of dairy cattle. Journal of Dairy Science, 91, 1985-1995.
WEISS W.P., WYATT D.J. (2002): Effects of feeding diets based on silage from corn hybrids that differed in concentration and in vitro digestibility of neutral detergent fiber to dairy cows. Journal of Dairy Science, 85, 3462- 3469.
WHITAKER D.A., EAYRES H.F., AITCHISON K., KELLY J.M. (1997): No effect of a dietary zinc proteinate on clinical mastitis, infection rate, recovery rate and somatic cell count in dairy cows. The Veterinary Journal, 153, 197-204.

Received 20 February 2013; accepted for publication 30 October 2013

Biotechnology in Animal Husbandry 29 (4), p 665-673 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.07:637.12'62
DOI: 10.2298/ BAH1304665K

EFFECT OF POST - MILKING TEAT DIPPING ON HYGIENIC QUALITY OF COW'S MILK

D. Kučević[1], M. Plavšić[1], S. Trivunović[1], M. Radinović[1], D. S. Kučević[2]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, 21000 Novi Sad, Republic of Serbia
[2]Superlab doo, 11070 Belgrade, Republic of Serbia
Corresponding author: denis.kucevic@stocarstvo.edu.rs
Original scientific paper

Abstract: The study aimed to investigate the effects of teat disinfection (dipping treatment) after milking on hygienic quality of row milk. The research was conducted on the farm with 30 Holstein-Friesian dairy cows. Animals were kept in a tied housing system. Milking is done by a bucket milking units using vacuum line. The research was carried out during the period of 2 months from 26.11.2012 till 25.01.2013. Results showed that milk immediately after leaving the udder was minimally contaminated with microorganisms (average of both groups with 8,933 CFU/ml). Experimental group had an average 2.668 (133,000 SCC/ml) and control group 3.524 (about 257,000 SCC/ml) ($p<0.05$). The average value of CFU in experimental group was 5,816 (729,000 CFU/ml) and in the control 5.833 (805,000 CFU/ml), ($p<0.05$). During the study period, the average value of SCC in the experimental group decreased to 2.67 (133.020/ml) and the average value of CFU to 5.82 (729.064/ml), ($p<0.05$). Treatment of teat dipping after milking is justifiable and has positive impact on row milk quality.

Key words: Teats dipping, dairy cow, row milk quality

Introduction

Contamination of milk is mostly caused by the contact of milk with microorganisms from the environment (equipment for milking and cooling, air in the stable, milker's hands and clothing, the surface of the skin on udder and the teats) and it's rarer by passing milk through the *ductus papillaris*. The presence of microorganisms can be cause the inflammation of the mammary gland - mastitis and the most important pathogens are among *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli (Miltenburg et al., 1996).* The presence of mastitis in herds of dairy cows inflicts great economic losses because it reduces milk production and quality, increases the cost of healing and staff as well as the number of forced slaughter *(Winter, 2009).* Inflammation of the mammary

gland is accompanied by changes in the number of somatic cell count (SCC), mainly as an increase in SCC in diseased quarters of udder *(Fregonese and Leaver, 2001)*. By monitoring of SCC changes it is possible to successfully manage the health of the udder. Occurrence of diseases of the mammary gland is usually associated with low level of hygiene during the breeding and milking *(Schreiner and Ruegg, 2003)*. Therefore, it is necessary to conduct regular procedures of cleaning in order to maintain hygiene in the stable as well as procedures for the disinfection before and after milking *(Gedek, 1994)*. This reduces the possibility of contamination and adequate disinfection of teats after milking reduces the incidance of new intramammary infections *(Hristov et al., 1997; 2002)*. The greatest risk of infection of the mammary gland may be expected in the first few hours immediately after milking, becuase teat canal stays open for another 30 minutes.

The application of teat hygiene after milking comprises a sinking of teats (or splashing) in a disinfectant. This procedure replaces remains of milk with a layer of disinfectant, temporarily blocking the entrance into the teat canal and has beneficial impact on the skin of teats. These actions reduce post-secretion contamination of milk *Pavicic et al. (2003b),* decrease the number of udder infections caused by pathogens from the environment *Pankey et al. (1987),* and reduce the occurrence of subclinical mastitis *(Lam et al., 1996)*.

Materials and Methods

The study was conducted on the farm with 30 Holstein-Friesian dairy cows. Animals were kept in tied housing system. The usual milking procedure was performed twice a day using a bucket milking unit with a vacuum line. At the beginning of the experiment, only primary udder hygiene was conducted in the herd, based on washing with water and wiping with disposable cloths. For the purpose of experiment, animals were divided into 2 groups. First was experimental group (A), which continued application of the primary hygiene and after milking teats were immerged (dipping treatment) in a special cup containing active compounds based on Pvp-iodine in accordance with EEC regulation 648/2004. The second control group (B) continued only with the primary teat and udder hygiene. Both groups of cows had approximately the same average milk yield, stage of lactation and hygienic quality of raw milk. Difference between the groups wasn't significant. The research was conducted in the period of 2 months, from 26.11.2012 till 25.01.2013. In the determination of the quality of raw milk the somatic cell count (SCC) and number of bacterial colonies (CFU - colony forming unit) were considered. The samples were taken from each individual animal and transported in sterile bottles of 40 ml.

Before the start of the experiment, one specific milk sample from each cow was taken, directly from the teats (udder) in a sterile bottle and thereafter from the milking bucket unit. The aim was to determine the level of contamination of milk before coming into contact with environment, equipment etc. After that, samples were collected as follows: in the first week two samples from each cow in order to determine the initial milk quality (zero day) and then each week one sample until the end of the experiment.

Milk samples were preserved using Asidiol (preservative) in accordance with ISO 13366-2:2006 and IDF 148-2:2006. Analysis of milk samples was performed on automatic analyzers using method of FTIR and flow cytometry. Data were analysed by using the software Statistics 10 *(stat. Soft. Inc. 2012)*. General variability of observed traits was analysed by using the descriptive statistical analysis. The Student t-test was used to determine the significance of differences between the experimental and control group of cows. For the purpose of proper data handling and comparability, the data were transformed logarithmically as:

1) $SCC = Log_2 (SCC /100000) +3$
2) $CFU = Log_{10} (CFU)$

Results and Discussion

The results of the analysis of specific milk samples (directly from the teats in a sterile bottle) showed that the milk immediately after leaving the udder was minimally contaminated by microorganisms (average of both groups amounted 8,933 CFU/ml) and then after milking, the milk samples collected from the bucket milking unit were contaminated on average with about 900,000 CFU/ml (Figure 1).

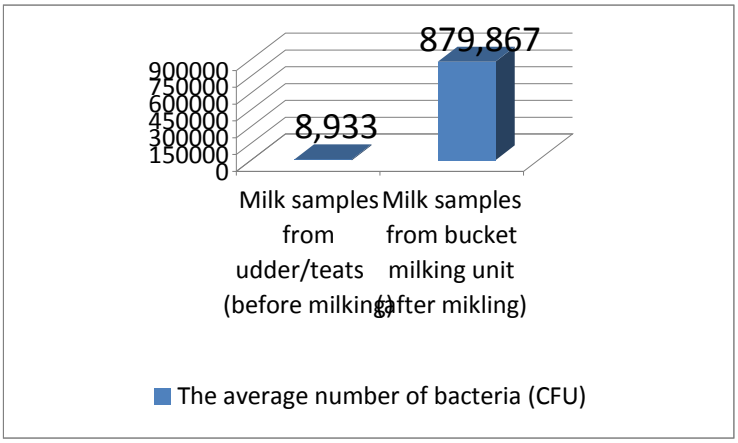


Figure 1. The average number of bacteria (CFU) in milk at start of research

Immediately after leaving the udder, the milk of healthy cows, kept in adequate conditions, is almost sterile and contains the minimum number of microorganisms. To contamination comes mainly during and after milking *(Saran, 1995).* The obtained results indicate the occurrence of contamination after milking (after leaving the udder) due to activity of microorganisms from the environment.

Before the start of the experiment (day zero), the average SCC of 152,400/ml was determined in the experimental group of cows, while the control group had 249,466 SCC/ml. The average number of CFU in the experimental group was 901,946 / ml, whereas in the experimental group it was 950,693/ml. This difference between the groups for both of observed parameters wasn't significant ($p>0.05$).

Table 1. Average values of observed parameters for both groups at the beginning of the experiment (day zero)

Parameters	Cow group	X	SD	SE_x	Min	Max
SCC	Experimental group (A)	152,400.0	221,311.6	57,142.4	33,000	883,000
	Control group (B)	249,466.6	247,865.7	63,998.6	55,000	860,000
Log - SCC	Experimental group (A)	2.878	1.292	0.33	1.40	6.14
	Control group (B)	3.799	1.206	0.31	2.14	6.10
CFU	Experimental group (A)	901,946.6	473,929.1	122,367.9	136,800	1,420,400
	Control group (B)	950,693.3	506,897.6	130,880.4	114,800	1,585,600
Log - CFU	Experimental group (A)	5.853	0.359	0.092	5.14	6.15
	Control group (B)	5.887	0.332	0.085	5.06	6.20

After 2 months of dipping treatment the following results were obtained (Table 2,3).

Table 2. Average values of SCC for both groups at the end of the experiment (t-test)

Parameters	Cow group	X	SD	SE_x	t-value	p-value
Log - SCC	Experimental group (A)	2.668	1.335	0.188	-3.00084	0.003*
	Control group (B)	3.524	1.509	0.213		

Statistically significant value between experimental and control group; *=$p<0.05$

Table 3. Average values of CFU for both groups at the end of the experiment (t-test)

Parameters	Cow group	X	SD	SE_x	t-value	p-value
Log - CFU	Experimental group (A)	5.816	0.209	0.029	-0.35321	0.724 ns
	Control group (B)	5.833	0.257	0.036		

Statistically significant value between experimental and control group; ns= p>0.05

In regard to SCC, the experimental group had on average 2,668 (approximately 133,000 SCC/ml) and control 3.524 (approximately 257,000 SCC/ml). This difference between the groups was significant (p<0,05). The average value of CFU in experimental group was 5.816 (approximately 729,000 CFU/ml) and in control 5.833 (approximately 805,000 CFU/ml), difference statistically wasn't significant (p>0.05).

If only the experimental group of cows is considered, from the beginning of research (day zero) till the end of experiment, the average number of SCC decreased from 2.88 (152,400/ml) to 2.67 (133.020/ml), while the average number of CFU decreased from 5.85 (901.946/ml) to 5.82 (729.064/ml). These differences couldn't be confirmed statistically (p>0,05).

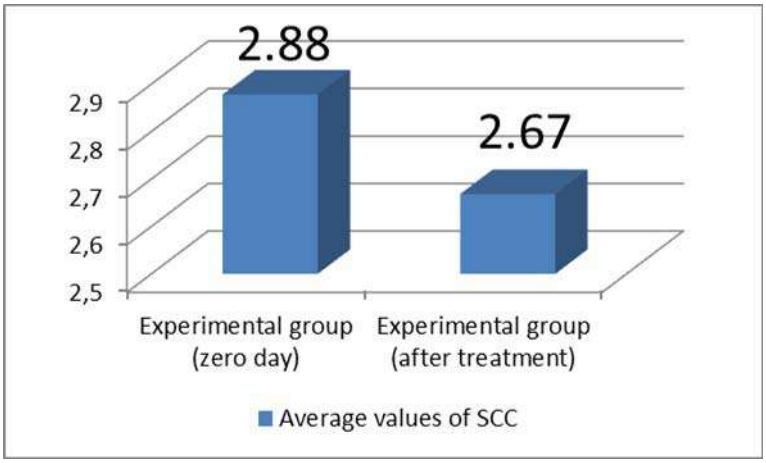


Figure 2. The average number of SCC for experimental group at start and the end of treatment

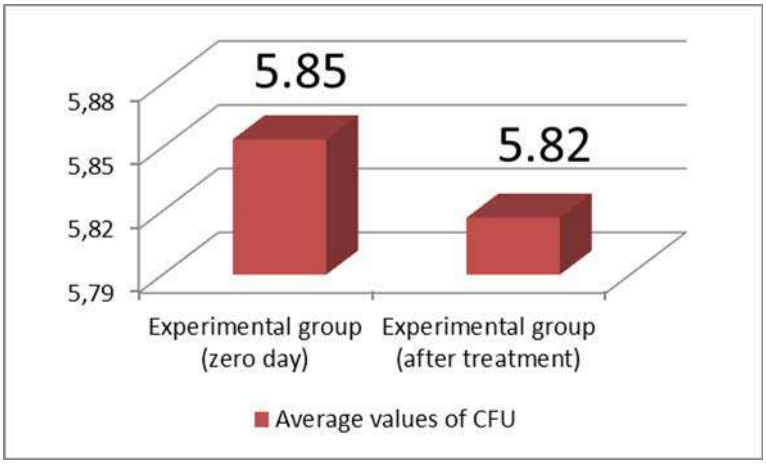


Figure 3. The average number of CFU for Experimental group at start and the end of treatment

Pavicic et al. (2008) have reported that in an experiment with disinfection of teats before and after milking, the average SCC (10.12%) decreased significantly and the total plate count decreased by 24.07%, while in cow group without treatment, the number of SCC increased by 20.97%. Almost identical effect of disinfection before and after milking on reduction of the number of microorganisms is determined by *Petrovic et al. (2006).* The authors point out that introduction of udder disinfection after milking has resulted in reduction in the average number of microorganisms up to 41.6% compared to the group of cows in which only disinfection before milking is applied.

Islam et al. (2009) has found that the average number of microorganisms significantly reduces if at the same time with udder disinfection the disinfection of hands and equipment for a milking is included. Additionally personal hygiene of animals (hygiene of legs, abdomen and back parts of body), significantly influences the number of SCC (decrease) in milk *(Sant'Anna and Paranhos Da Costa, 2010).*

Influence of teat dipping after milking on reduction of the average number of SCC is confirmed by *Bilal et al. (2008),* where the number of SCC in milk after 3 months of treatment was reduced by 53.49% and combined with vaccination against *Staphylococcus aureus* this decrease was more than 62.71%. The positive effect of the teat dipping after milking on reduction the number of SCC in the milk of cows and buffaloes is stated by *Singh and Singh (2002).*

Conclusion

Treatment of teats by dipping was conducted on the farm with 30 Holstein-Friesian dairy cows during the period from 26.11.2012 till 25.01.2013. During the study period, the treatment of teat dipping in the experimental group of cows has led to a reduction in the average number of SCC and CFU compared to the control group. If only the experimental group of cows is considered, from the beginning of research (day zero) till the end of experiment, the average SCC and CFU decreased but difference wasn't statistically significant ($p > 0.05$). Results of research indicate that treatment of teats by dipping after milking has a positive impact on raw milk quality. In order to achieve significant improvement of hygienic quality of raw milk it is necessary to include treatment of dipping before milking (simultaneously with the treatment of dipping after milking), and the treatments must be peformed correctly in accordance with the manufacturer's recommendations. Dipping treatments before and after milking achieve their full effect if procedures related to personal hygiene of employees, hygiene of milking equipment, stables and animals, adequate feeding etc., are conducted regularly and in accordance to the standards and good manufacturing practices.

Efekat tretmana papila posle muže na higijenski kvalitet kravljeg mleka

D. Kučević, M. Plavšić, S. Trivunović, M. Radinović, D. S. Kučević

Rezime

Istraživanje je imalo za cilj da ispita efekte dezinfekcije papila vimena (tretman uranjanja papila) posle muže krava na higijenski kvalitet mleka. Ispitivanje je sprovedeno na gazdinstvu sa 30 mlečnih krava holštajn-frizijske rase u vezanom sistemu držanja. Muža je vršena prenosnom muznom jedinicom sa kantom (muzilicom), uz pomoć vakum voda. Ogled je trajao 2 meseca a sproveden je u periodu od 26.11.2012. do 25.01.2013. Rezultati istraživanja su pokazali da je mleko neposredno nakon napuštanja vimena bilo minimalno kontaminirano mikroorganizmima (u proseku obe grupe sa 8,933 CFU/ml). Eksperimentalna grupa je imala u proseku 2.668 (oko 133,000 SCC/ml) a kontrolna 3.524 (oko 257,000 SCC/ml), ($p<0.05$). Prosečna vrednost CFU u eksperimentalnij grupi je iznosila 5.816 (oko 729,000 CFU/ml) odnosno u kontrolnoj 5.833 (oko 805,000 CFU/ml), ($p>0.05$). Tokom perioda istraživanja u eksperimentalnoj grupi je smanjen prosečan broj SCC na 2.67 (133,020/ml) odnosno prosečan broj CFU na

5.82 (729,064/ml), ($p>0.05$). Tretman uranjanja papila posle muže je opravdan i pozitivno utiče na popravku kvaliteta mleka.

References

BILAL M.A., MUHAMMAD A.A., YOUNAS M., MUHAMMAD G. (2008): Impact of post milking teat dipping and *staphylococcus aureus* vaccination on somatic cell count and serum antibody titre in sahiwal cows. Pak. J. Agri. Sci., 45, 2.

FREGONESI J. A., LEAVER D.J. (2001): Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livest. Prod. Sci., 68, 205-216.

GEDEK W. (1994): Praxisnahe Maßnahmen der Mastitisprophylaxe. Prakt. Tierarzt, 75, 54-56.

HRISTOV S., RELIĆ R., STANKOVIĆ B. (2002): Najznačajniji aspekti dezinfekcije vimena krava. Zbornik radova XIII Savetovanje u zaštiti životne sredine sa međunarodnim učešćem, Kikinda, 75-83.

HRISTOV S., VUČINIĆ M., JOŽEF I. (1997): Dezinfekcija vimena krava pre i posle muže. Zbornik VIII Savetovanja u zaštiti životne sredine sa međunarodnim učešćem, Subotica, 15-21.

ISLAM M.A., ISLAM M.N., KHAN M.A.S., RASHID M.H., OBAIDULLAH S.M. (2009): Effect of different hygienic condition during milking on bacterial count of cows' milk. Bang. J. Anim. Sci., 38, 1-2, 108-114.

LAM T.J.G.M., de JONG M.C.M., SCHUKKEN H.Y., BRAND A. (1996): Mathematical modelling to estimate efficacy of postmilking teat desinfection in split-udder trials of diary cows. J. Dairy Sci., 79, 62-70.

MILTENBURG J.D., LANGE D., CRAUWELS P.A., BONGERS H.J., TIELEN M.M., SCHUKKEN H.Y., ELBERS W.A. (1996): Incidence of clinical mastitis in a random sample of dairy herds in the southern Netherlands. The Veterinary record, 31, 204-207.

PANKEY J.W., WILDMAN E.E., DRECHSLER A.P., HOGAN S.J. (1987): Field trial evaluation of premilking teat disinfection. J. Dairy Sci., 70, 867-872.

PAVIČIĆ Ž., CERGOLJ M., BALENOVIĆ T., ANAMARIA EKERT-KABALIN, VALPOTIĆ H. (2008): Influence of udder sanitation on hygienic quality of cow milk. Vet. Arhiv, 78, 2, 105-112.

PAVIČIĆ Ž., M. VUČEMILO A., TOFANT M., CERGOLJ T., BALENOVIĆ K. MATKOVIĆ (2003b): Značenje primijenjene dezinfekcije u smanjenju onečišćenja mlijeka mikroorganizmima i sprječavanju upala mliječne žlijezde. Zbornik radova, Veterinarski dani, 9.-12. listopada, Šibenik, Hrvaska, 132-142.

PETROVIĆ M., PAVIČIĆ Ž., TOMAŠKOVIĆ A., CERGOLJ M. (2006): Učinak higijene mužnje na mikrobiološku kakvoću mlijeka. Stočarstvo, 60, 403-411.

SANT'ANNA A.C., PARANHOS DA COSTA M.J.R. (2010): The relationship between dairy cow hygiene and somatic cell count in milk. J. Dairy Sci., 94, 3835-3844.
SARAN A. (1995): Disinfection in the dairy parlour. Rev. sci. tech. Off. int. Epiz., 14, 207-224.
SCHREINER D.A., RUEGG L.P. (2003): Relationship between udder and leg hygiene scores and subclinical mastitis. J. Dairy Sci., 86, 3460–3465.
SINGH S., SINGH M. (2002): Post Milking Teat Dip Effect on Somatic Cell Count, Milk Production and Composition in Cows and Buffaloes. Asian-Aust. J. Anim. Sci., 15, 10, 1517-1522.
WINTER P.(2009): Prakischer Leitfaden Mastitis, Vorgehen beim Einzeltier und im Bestan. Parey in MVS Medizinverlage Stuttgart GmbH & Co, KG.

Received 7 August 2013; accepted for publication 10 October 2013

Biotechnology in Animal Husbandry 29 (4), p 675-683 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.38
DOI: 10.2298/ BAH1304675A

POLYMORPHISM IN BMP-15 GENE AND ITS ASSOCIATION WITH LITTER SIZE IN ANGLO-NUBIAN GOAT

S.M. Abdel-Rahman[1], Y.A. Mustafa[2], H.A. Abd Errasool[2], A.A. El-Hanafy[1], A.M. Elmaghraby[1]

[1]Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.
[2]Department of Genetics, Faculty of Agriculture, Alexandria University, Egypt.
Corresponding author: salahmaa@yahoo.com
Original scientific paper

Abstract: For association between BMP-15 (exon2) gene polymorphisms and litter size trait in Anglo-Nubian goat, PCR-SSCP technique was developed. Twenty-five female Anglo-Nubian goats reared under Egyptian conditions were selected according to their litter size. DNA from blood samples of these animals was extracted to amplify 140-bp of the BMP-15 gene affecting litter size production trait in goats. Based on the breeding value, 25 animals were selected from the highest to the lowest litter size productivity. PCR amplification size of the BMP-15 gene (140-bp) was genotyped in all animals. PCR-SSCP analysis of the BMP-15 gene (140-bp) showed three various genotypes BB, BM and MM with frequencies 0.46, 0.43 and 0.11, respectively. The frequencies of the *B* and *M* alleles were 0.68 and 0.32, respectively. The results indicated that the BB genotype was higher in litter size productivity than the other genotypes with significant differences. The result of this study confirmed that BMP-15 gene may be a strong candidate gene for further applications in marker-assisted selection (MAS) for litter size in goats.

Keywords: Anglo-Nubian goat, Litter size, PCR-SSCP, BMP-15 gene polymorphisms

Introduction

Ovulation rate is determined by a complex exchange of endocrine signals between the pituitary gland and the ovary and paracrine and possibly autocrine signals within ovarian follicles involving the oocyte and its adjacent somatic cells (*Dong et al., 1996; Galloway et al., 2000; Eppig, 2001; Galloway et al., 2002*). BMP 15 gene maps to X chromosome and plays an important role in regulating ovulation rate and oocyte quality. The relative importance of BMP15 in early

follicle development is species- specific and appears to be related to differences between mono-polyovulatory species (*Dube et al., 1998; Grapes and Rothschild, 2002; Moore and Shimasaki, 2005; Silva et al., 2004*)**.** Different mutations in BMP 15 gene in women and ewes have been shown to cause defects in folliculogenesis. Five naturally occurring mutations in exon 2 of the sheep BMP15 gene such as FecXG, FecXB, FecXI, FecXH, FecXL leads to infertility in homozygous ewes due to defects in early folliculogenesis (*Chu et al., 2005; McNatty et al., 2005; Bodin et al., 2007*), where as heterozygous ewes have increased ovulation rate and litter size. Ninety-six percent of the world goat populations are owned by small holders in developing countries with rare genetic sources and improvement programs (*Olivier et al., 2005*). So, it is essential to study the genetics and reproduction in goat breeds using modern genetic methods. One of these methods is marker-assisted selection (MAS) which will be useful for increasing and accelerating the rate of genetic improvement on litter size and encourage its uptake it by commercial goat breeders.

Materials and methods

Animals. Twenty-five female Anglo-Nubian goats kept under Egyptian conditions were chosen according to litter size productivity. Blood samples from these animals were collected by Jugular vein puncture into tubes containing an anticoagulant disodium EDTA. The samples were stored at -20°C until needed for DNA isolation.

DNA isolation. Genomic DNA was isolated from whole blood samples using a commercially available kit (GF-1 Blood DNA extraction kit-Vi Vantis). Genomic DNA was separated on agarose gel electrophoresis using 1% agarose (w/v) in 0.5X TBE buffer. To check genomic DNA bands, the gel was photographed using gel documentation system (Syngene, UK).

PCR amplification and genotyping of BMP-15 gene. A 140-bp fragment of exon2 of BMP-15 gene in 25 female goats was amplified by PCR using forward (5'- CACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3') and reverse (5'-GATGCAATACTGCCTGCTTG-3' primers (*Hanrahan et al., 2004*). PCR was performed in a reaction volume of 25 µl using 25 ng of genomic DNA of each sample, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ µl Taq DNA polymerase (Promega, Germany). Thermal cycling (Autorisierter Thermocycler and Mastercycler Gradient) was carried out by initial denaturation at 94°C for 5 min, followed by 35 cycles each at 94°C for 45 sec, annealing temperature at 62°C for 40 sec, polymerization temperature at 72°C for 45 sec and final extension at 72°C for 10 min, then the

samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Single stranded conformational polymorphism (SSCP). Aliquots of 5 µl PCR products were mixed with denaturating solution (98% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue and 10 mM EDTA) and incubated at 98C° for 10 min and then child on ice rapidly. Denaturated DNA was loaded on 10% PAGE gel (10X 10 CM) in 1X TBE buffer and constant voltage 65V for 5 hours. For staining DNA bands and visualizing, the gel was stained with ethidium bromide and photographed by using Gel Documentation system.

Statistical analysis. Data for litter size production in Anglo-Nubian goats was obtained from the farm records to predict breeding value (Table 1). Statistical analysis was performed by SAS version 9.2 (based upon BLUP statistical method) using the following model:

yijk= pi + aj + eijk, where:

pi= Fixed effects (season, year and parity),

aj= Random effect of the ej^{th} does,

eijk= Random error effect.

Table 1. Litter sizes, breeding values (BV) and genotypes of BMP15 gene in experimental animals.

Serial no.	Animal no.	Litter size	BV	Genotype
1	6	2.3	0.2657	BB
2	20	2.5	0.2169	BB
3	22	2.5	0.2153	BM
4	23	1.3	0.2130	BM
5	5	2.0	0.1409	BB
6	14	2.0	0.1153	MM
7	7	1.7	0.1106	BB
8	33	2.0	0.08041	BB
9	32	2.0	0.07218	MM
10	34	2.0	0.03685	BB
11	19	1.0	-0.0097	BB
12	15	1.5	-0.0215	MM
13	11	1.5	-0.0382	BB
14	17	1.5	-0.0545	BB
15	24	1.5	-0.0545	BB
16	26	1.5	-0.0545	BB
17	25	1.0	-0.0716	BB
18	31	1.5	-0.0769	MM
19	12	1.5	-0.112	MM

20	18	1.3	-0.112	MM
21	8	1.3	-0.1245	BB
22	21	1.3	-0.1342	BB
23	9	1.5	-0.1445	BB
24	16	1.3	-0.1925	BB
25	30	2.0	-0.2118	MM

Results and discussion

PCR amplification of the BMP15 (exon2) gene yielded 140-bp in length in all 25 female goats (Figure 1). PCR-SSCP technique was used to identify nucleotide sequence polymorphism by change conformation of alleles within BMP15 (exon2) gene in experimental Anglo-Nubian goat. Characterization and analysis of PCR-SSCP showed two homozygous genotypes: BB genotype in animal numbers 5, 6, 7, 8, 9, 11, 16, 17, 19, 20, 21, 24, 25, 26, 33 and 34 and MM genotype in animal numbers 12, 14, 15, 18, 30, 31 and 32. While, in animal numbers 22 and 23 heterozygous genotype BM was found (Table 1 and Figure 2). The calculated frequencies of BB, BM and MM genotypes were 0.46, 0.43 and 0.11, respectively, and the frequencies of the *B* and *M* alleles were 0.68 and 0.32, respectively (Table 2). The results indicated that the Anglo-Nubian goats with BB genotype had significantly ($p \leq 0.05$) larger size of litter than the goats with other genotypes (BM and MM).

In previous study of BMP15 gene in another goat breed, *Chu et al. (2007)* found two genotypes: AA and AB in Jining Gery goats. Genotype AA was found in low fecundity goat breeds and AB genotype had 1.3 kids more than homozygous AA. In another study on different goat breeds, *Feng et al. (2009)* three genotypes AA, AG and GG in Jining Grey goats were found, while only AA genotype was found in both Liaoning Cashmere and Inner Mongolia Cashmere goats. Boar goat had two genotypes AG and GG, while Angora and Inner Mongolia Cashmere goats had only AA genotype. In a recent study of *Wang et al. (2011)*, three genotypes (AA, BB and AB) were detected in Funiu White goats and their frequency was 0.071, 0.715 and 0.214, respectively. Two genotypes (AB and BB) were detected in Taihang black goats and their frequency was 0.342 and 0.658, respectively. The Funiu white goat with genotype BB had 0.91 or 0.82 kids more than those with AB or AA, respectively. However, these results preliminarily showed that BMP-15 gene is a genetic marker and closely linkage to the litter size trait and consequently, can be used as a marker-assisted selection (MAS) for high litter size productivity in goat.

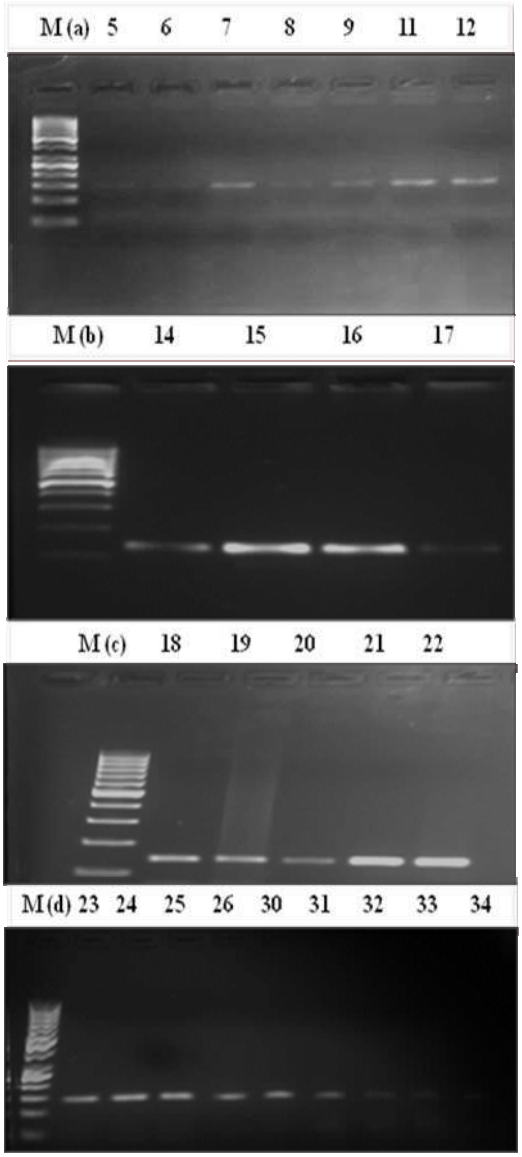


Figure 1. PCR products (140-bp) generated by the BMP15 (exon2) gene primers. Where, lanes: M (a) and M (d) are DNA markers 50-bp, lanes M (b) and M (c) are DNA markers 100-bp and lanes 5-34 are female Anglo-Nubian goats.

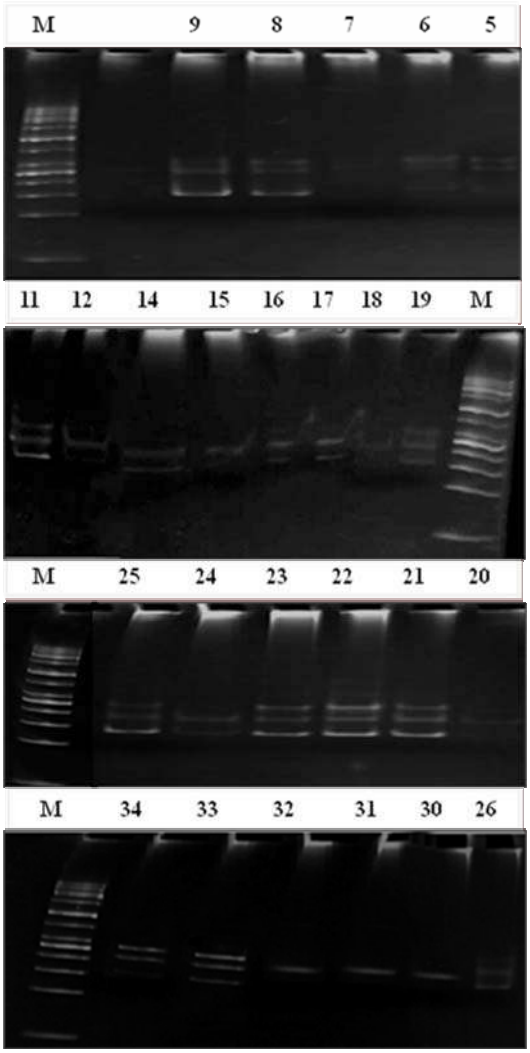


Figure 2: PCR-SSCP analysis of BMP15 gene (140-bp) in 25 animals. Lanes 5, 6, 7, 8, 9, 11, 16, 17, 19, 20, 21, 24, 25, 26, 33 and 34 represent BB genotype, lanes 12, 14, 15, 18, 30, 31 and 32 represent MM genotype, lanes 22 and 23 represent BM genotype and lane M is DNA marker (50-bp).

Table 2. Frequency of genotypes (BB, BM and MM) and alleles (*B* and *M*) in BMP15 locus

Gene	No. of animals	Genotypic frequency			Allelic frequency	
		BB(p^2)	BM(2pq)	MM(q^2)	*B(p)*	*M(q)*
BMP15	25	0.46	0.43	0.11	0.68	0.32

Conclusion

PCR-SSCP technique was developed to associate between BMP-15 (exon2) gene polymorphisms and litter size trait in Anglo-Nubian goat. DNA from blood samples of 25 female Anglo-Nubian goats selected according to their litter size productivity was extracted to amplify 140-bp of the BMP-15 gene. Based on the breeding value, the 25 animals were ordered from the highest to the lowest litter size productivity. PCR-SSCP analysis of the BMP-15 gene (140-bp) showed three different genotypes BB, BM and MM with frequencies 0.46, 0.43 and 0.11, respectively. The results indicated that the BB genotype was higher in litter size productivity than the other genotypes. Consequently, BMP-15 gene can be used as a marker-assisted selection (MAS) to improve litter size production trait in goat.

Acknowledgements

This work was achieved at Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt.

Polimorfizam BMP - 15 gena i njegova povezanost sa veličinom legla anglo - nubijskih koza

S.M. Abdel-Rahman, Y.A. Mustafa, H.A. Abd Errasool, A.A. El-Hanafy, A.M. Elmaghraby

Rezime

Za povezanost BMP-15 (exon 2) polimorfizma gena i osobine veličine legla anglo-nubijskih koza, razvijena je PCR - SSCP tehnika. Dvadeset pet ženskih grla anglo-nubijskih koza gajenih pod egipatskim uslovima odabrana su prema veličini njihovih legala. DNK iz uzoraka krvi ovih životinja je ekstrahovana da bi se amplifikovala 140-bp u BMP-15 genu koji utiče na veličinu legla kao proizvodnu osobinu u koza. Na osnovu priplodne vrednosti, 25 životinja je izabrano od najveće do najmanje veličine legla, odnosno produktivnosti. PCR amplifikacija veličina BMP-15 gena (140-bp) je genotipizirana kod svih grla. PCR-SSCP analiza BMP-15 gena (140-bp) je pokazala tri različita genotipa BB, BM i MM sa frekvencijama 0.46, 0.43 i 0.11. Frekvencije B i M alela su 0.68 i 0.32,. Rezultati su pokazali da je BB genotip bio bolji sa stanovišta produktivnosti veličine legla od drugih genotipova sa značajnim razlikama. Rezultat ovog

istraživanja je potvrdio da BMP-15 gen može biti jak kandidat gen za dalju primenu u marker asistiranoj selekciji (MAS) na veličinu legla u kozarstvu.

References

BODIN L., PASQUALE E.D., FABRE S., BONTOUX M., MONGET P., PERSANI L., MULSANT P. (2007): A novel mutation in the bone morphogentic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinology, 148, 393-400.

Chu M.X., JIAO C.L., HE Y.Q., WANG J.Y., LIU Z.H., CHEN G.H. (2007): Association between PCR-SSCP of the bone morphogentic protein 15 and prolificacy in Jining gery goats. Anim. Biotechnol., 18, 4, 263-274.

DONG J., ALTERTINI D.F., NISHIMORI K., KUMAR T., LU N., MATZUK M.M. (1996): Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature, 383, 531-535.

DUBE J.L., WANG P., ELVIN J., LYONS K.M., CELESTE A.J., MATZUK M.M. (1998): The bone morphogenetic protein 15 gene is x-linked and expressed in oocytes. Mol. Endocrinol., 12, 1809-1817.

EPPIG J.J. (2001): Oocyte control of ovarian follicular development and function in mammals. Reproduction, 122, 829-838.

FENG TAO, ZHAO YOU-ZANG, CHU MING-XING, DI RAN , ZHANG YINGJIE, FANG LI (2009): Bone morphogentic protein 15 as a candidate gene for prolificacy of jining Gery goat.Chin.J. Anim.Vet. Sci., 40, 4, 468-475.

GALLOWAY S.M., MCNATTY K.P., CAMBRIDGE L.M., LAITINEN M.P.E., JUENGEL J.L., JOKIRANTA T.S., MCLAREN R.J., LUIRO K., DODDS K.G., MONTGOMERY G.W., BEATTIE A.E., DAVIS G.H., RIT O. (2000): Mutations in an oocyte-derived growth factor gene (*BMP-15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Gene, 25, 279-283.

GALLOWAY S.M., GREGAN S.M., WILSON T., MCNATTY K.P., JUENGEL J.L., RITVOS O., DAVIS G.H. (2002): Bmp15 mutations and ovarian function. Molecular and Cellular Endocrinology, 191, 15-18.

GRAPES L**.,** ROTHSCHILD M.F**. (***2002***):** BMP15 maps to the X chromosome in swine. Animal Genetics, 33, 165-166.

HANRAHAN J.P., GREGAN S.M., MULSANT P., MULLEN M., DAVIS G.H., POWELL R., GALLOWAY S.M. (2004): Mutations in the Genes for Oocyte-Derived Growth Factors GDF9 and BMP15 Are Associated with Both Increased Ovulation Rate and Sterility in Cambridge and Belclare Sheep (Ovis aries). Biol. Repord., 70, 900-909.

MCNATTY K.P., SMITH P., MOORE L.G., READER K., LUN S., HANRAHAN J.P., GROOME N.P., LAITINEN M., RITVOS O., JUENGEL J.L. (2005):

Oocyteexpressed genes affecting ovulation rate. Mol. Cell. Endocrinol., 234, 57-66.
MOORE R.K., SHIMASAKI S. (2005): Molecular biology and physiologyical role of the oocyte factor, BMP15. Mol. Cell. Endocrinol., 234, 67-73.
OLIVIER J.J., CLOETE S.W.P., SCHOEMAN S.J., MULLER C.J.C. (2005): Performance testing and recording in meat and dairy goats. Small Rumin. Res., 60, 83–93.
SILVA J.R.V., VAN DEN HURK R., VAN TOL A.T.H., ROELEN J.A.B., FIGUEIREDO R.J. (2004). Expression of growth differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), and BMP receptors in the ovaries of goats. Molecular Reproduction and Development, 70, 11–19.
WANG Y., YUANXIAO L., NANA Z., ZHANBIN W., JUNYAN B. (2011): Polymorphism of Exon 2 of bmp15 gene and its relationship with litter size of two Chinese goats. Asian-Aust. J. Anim. Sci., 24, 905-911.

Received 21 October 2013; accepted for publication 20 December 2013

Biotechnology in Animal Husbandry 29 (4), p 685-693 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'632
DOI: 10.2298/ BAH1304685C

EFFECT OF GENOTYPE, SIRE, SEX, GESTATION LENGTH ON BIRTH WEIGHT OF LAMBS

V. Caro Petrović[1], M. P. Petrović[1], Z. Ilić[2], M. M. Petrović[1], B. Milošević[2], D. Ružić Muslić[1], N. Maksimović[1]

[1]Institute for Animal Husbandry, 11080 Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Lesak, Republic of Serbia
Corresponding author: violycaro@yahoo.com
Original scientific paper

Abstract: The birth weight of lambs has an essential role in satisfying sheep production. Also reflected as primary factor later development of young organism. In the study included: gestation length, sire (two sires in each genotype during parenting), sex of lambs at birth, the birth weight of single lambs from well nourished mature ewes of the next genotypes: Miss (1), Wurttemberg (2) and Ile de France (3). The highest birth weight was obtained in genotype 3 with an average birth weight of 4.63 kg in sire 2 and 4.44 kg in sire 1 with a difference of 0.19 kg. Gestation length of genotype 1 ranged from 144 to 153 days; genotype 2, from 148 to 154 days and the genotype 3, from 144 to 151days. The highest average birth weight in genotype 1 with gestation length of 153 days weighed 5.10 kg. Average birth weights of male lambs in all genotypes were higher than the females. There were a very significant effect of genotype, sire, gestation length on birth weight of lambs ($P<0.01$), but sex have no significant effect on birth weight of lambs ($0.065;P>0.05$). The result achieved therefore can be explained that the effect of genotype, sire and gestation length and the interaction between subjects effects such as the next: Sire*Gestation length; Sire*Sex; Gestation length*Sex; Sire*Gestation length*Sex were highly and significantly affected the birth weight of lambs ($P<0.01$; $P<0.05$). The interactions between subjects' effects will be a good indicator to be acquainted more on their influence in a certain trait.

Key words: lamb, birth weight, genotype, sire, sex, gestation length

Introduction

Lambs' birth weight has an important role in achieving a good sheep production (*Petrovic et al., 2011*) and speculated as a primary factor for the later development of young organism (*Petrović, 2007)*. Vitality and mortality of lambs also brought about by the birth weight (*Berhane and Arendonk, 2006; Vantankhah*

and Taleb, 2009). Other studies in animals showed that uterine capacity markedly influences birth weight (*Jenkinson et al., 2007*). The fetal growth and birth weight are regulated by genotype of the fetus, maternal genotype, maternal nutrition and the external environment and in the same way; the birth weight of the individual lambs was significantly related in their mothers' profile (*Oldham et. al., 2011).* Upon primary estimation of breeding values, must specified a model to find which best describe the biological processes wherein this requires study on non-genetic sources of variation that influence the specific measured trait concerned (*Caro Petrović et al.,2013*). Familiarity on such factors affecting variation in birth weight is especially important and conferred the affiliation between birth weight to neonatal and adult health (*Gardner et al., 2007*). Among the influencing factor affecting birth weight of lambs; environmental (year, season, etc.), the keeping and care during the production cycle, diet during stage of pregnancy in observed year and season. Apart from the said environmental factors, the mothers' age, type of birth and sex of lamb, i.e. factors that cannot be called environmental, but a certain biological category (*Petrović et al., 2009).* The weight of the dam at service significantly affected the birth weight of their lambs (*Hussain et al., 2000*). Indeed, the maternal body condition at mating and considering the energy intake prior to conception for at least the 6-8 weeks had significant effect on the birth weight of her lamb. Likewise energy intake of ewe's at late gestation, mostly important in terms of birth weight in sheep (*Buttery et al., 2007*). Wherefore, the birth weight is an easily measured and available substitute for the quantity of fetal growth. Aside from the reported factors which influenced the birth weight, other authors also reported the influence of gestation length on birth (*Osinowo et al., 1994; Vatankhah et al., 2000; Fogarty et al., 2005*) and can be longer for the dam's carrying male lamb (*Vantakhah et al., 2000; Koyuncu et al., 2001; Fofarty et al., 2005*) . There was limited number of research about the effect of sire on lambs' birth weight, the interaction of sire breed × environmental category has significant effect for birth weight (*Osorio-Avalos et al., 2012*) and that this factor should require careful consideration in practical lamb husbandry as affirmed by *Yagoob et al. (2004).*

The aim of this paper is to investigate the influence of some fixed factors on the birth weight variability of lambs.

Materials and Methods

In the study, birth weight records of 180 lambs from 3 genotypes namely: Mis (1), Wurttemberg (2) and Ile de France (3). The ewes were conditioned before mating and fed in accordance with the farm practiced with hay and concentrates. The mating ratio was 30 ewes per sire per genotype (30:1:1) and for every genotype utilized 2 sire. The calculation for gestation length was based on the

successful mating and lambing number of days. The following records were included in the investigation; gestation length, sire (two sires in each genotype during parenting), sex of lambs at birth, the birth weight of single lambs from well nourished mature ewes of a particular genotype. The statistical analysis was performed by using the SPSS software program with the next procedures: General linear model, univariate analysis of variance and between-subjects test and their interactions.

Linear model as presented below:

$$Yijklm = \mu + GTi + SRj + GLk + SXl + \varepsilon ijklm,$$

where: Yijklmnop = birth weight of lth sex, kth gestation length, jth sire, and ith genotype
μ = overall population mean
GTi= effect of genotype (fixed effect –3 classes)
SRj = effect of sire (fixed effect –2 classes)
GLk = effect of gestation length (fixed effect –12 classes)
SXl = effect of sex of lamb (fixed effect –2 classes)
εijklm = residual error

Results and Discussion

In table 1, can be viewed that the highest birth weight was obtained in genotype 3 with an average birth weight of 4.63 kg in sire 2 and 4.44 kg in sire 1 with a difference of 0.19 kg, followed by genotype 1 sire 1 weighed 4.40 kg and sire 2 with 4.39 kg with a difference of 0.01kg while in genotype 2, sire 2 had 4.05 kg and in sire 1 was 3.63 kg for a difference of 0.42 kg.

Table 1. Average birth weight of lambs per genotype and per sire

Genotype	Sire	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.00	1.00	4.40[a]	.073	4.251	4.540
	2.00	4.39[a]	.069	4.257	4.530
2.00	1.00	3.63[a]	.069	3.491	3.763
	2.00	4.05[a]	.067	3.915	4.180
3.00	1.00	4.44[a]	.087	4.270	4.612
	2.00	4.63[a]	.075	4.486	4.782
a. Based on modified population marginal mean.					

The gestation lengths of every genotype observed were in figure 1, wherein the gestation length of genotype 1 ranged from 144 to 153 days; genotype 2, from 148 to 154 days and the genotype 3, from 144 to 151 days. The highest average

birth weight can be viewed in genotype 1 with gestation length of 153 days weighed 5.10 kg; genotype 2 with 3.93 kg with gestation length of 153 days; genotype 3 with gestation length of 149 days weighed 4.80 kg. Among these three genotypes investigated, two of them had similar gestation length that indicated the highest average birth weight within their group.

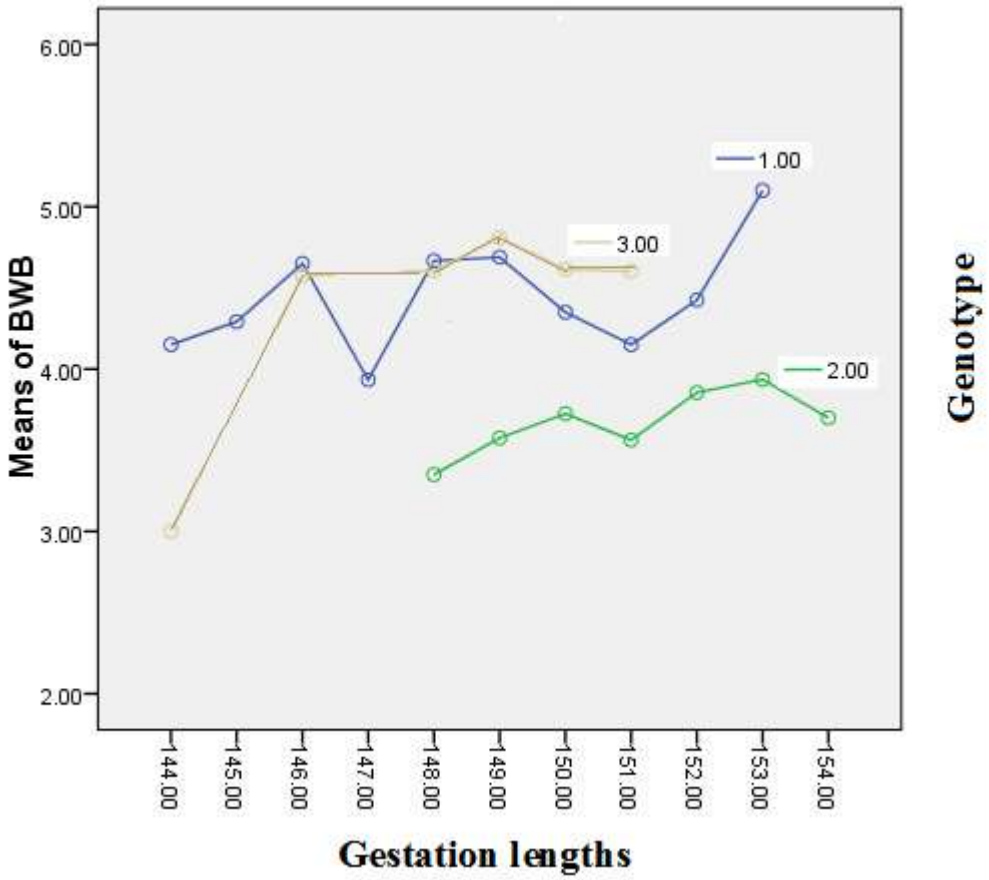


Figure 1. Effect of gestation lengths (days) on body weight at birth (BWB-kg)

As displayed in table 3, the average birth weights of male lambs in all genotypes were higher than the females. Among the weights of male lambs born, the highest birth weight was on genotype 3 followed by genotype 1 and lowest genotype 2. For the lamb born female, the highest average birth weight was genotype 1 followed by genotype 3 and the lowest genotype 2.

Table 2. Average birth weight of lambs per genotype and sex

Dependent Variable: BWB					
Genotype	Sex	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1.00	1.00	4.415[a]	.068	4.281	4.548
	2.00	4.368[a]	.076	4.218	4.518
2.00	1.00	3.797[a]	.081	3.636	3.957
	2.00	3.700[a]	.071	3.560	3.841
3.00	1.00	4.941[a]	.076	4.791	5.092
	2.00	4.176[a]	.085	4.010	4.343

a. Based on modified population marginal mean

Table 3. The test of between –subjects effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	53.987[a]	60	.900	5.435	.000
Intercept	1605.767	1	1605.767	9699.684	.000
Genotype	11.129	2	5.564	33.612	.000
Sire	2.099	1	2.099	12.679	.001
GesL	5.259	10	.526	3.177	.001
Sex	.574	1	.574	3.467	.065
Genotype * Sire	.393	1	.393	2.372	.126
Genotype * GesL	4.969	10	.497	3.001	.002
Genotype * Sex	.064	2	.032	.194	.824
Sire * GesL	3.622	6	.604	3.646	.002
Sire * Sex	1.926	1	1.926	11.631	.001
GesL * Sex	3.407	9	.379	2.286	.021
Genotype * Sire * GesL	1.234	3	.411	2.484	.064
Genotype * Sire * Sex	.550	1	.550	3.320	.071
Genotype * GesL * Sex	.717	5	.143	.866	.506
Sire * GesL * Sex	2.678	4	.669	4.044	.004
Genotype * Sire * GesL * Sex	.077	1	.077	.467	.496
Error	19.700	119	.166		
Total	3364.155	180			
Corrected Total	73.687	179			

a. R Squared = .733 (Adjusted R Squared = .598)

As presented in table 3, the effect of genotype, sire, gestation length have a very significant effect on birth weight of lambs ($P<0.01$). On the said table, have shown that sex have no significant effect on birth weight of lambs (0.065; $P>0.05$). Meanwhile, the interaction between subjects effects such as the next: Sire*Gestation length; Sire*Sex; Gestation length*Sex; Sire*Gestation length*Sex were highly and significantly affected the birth weight of lambs ($P<0.01$; $P<0.05$). In the study of *Iyiola-Tunji et al. (2010)* indicated that sex have no significant effect ($P>0.05$) on birth weight affirmed with ours but their result obtained on

gestation length that had a low and non-significant relationship with birth weight (0.114; P>0.05) and lamb genotype (-0.101; P>0.05) were contradicting on that we obtained. Likewise, the findings of other authors namely: *Yilmaz and Altin (2011); Caro Petrović et al. (2012*); *Ilić et al. (2013)* in their investigation on birth weight affected (P<0.05) by sex of lambs.

Sharma et al., 2011 stated that the large genotype lambs were lighter and smaller when born to small genotype dams; however, the birth weight or body dimensions of small genotype lambs did not differ when born to large genotype dams. The differences in birth weight were reflected in differences in body dimensions between the groups of lambs. Probably, these statements can justify too our findings. *Cloete et al. (1992)* mentioned the significant effect of sire on birth weight. In the investigation of *Olivier et al. (1987*) they found that in variance term between sire were also significant for birth weight, likewise found variation between sires for type traits. *Kincaid (1943)* informed that "the sire could influence only the hereditary characters of the lambs; it can be appeared that in certain rams within each breed transmitted characters to their lambs which influenced birth weight", this remark supported the result attained in the study. Some authors reported that gestation length influenced lamb weight (*Osinowo et al., 1994; Vatankhah et al., 2000; Fogarty et al., 2005)* and can be longer for the dam's carrying male lamb (*Vantakhah et al., 2000; Koyuncu et al.,2001; Fofarty et al., 2005) . Thrift and Durr (1972)* found out in their study that the sex of lamb was not a significant source of variation influencing gestation length but within each sex of lamb, the ewes with heavier lambs at birth had longer gestation length. Furthermore, for the ewes giving birth to single males had 1 day longer gestation length than ewes giving birth to single female lambs, but the difference was not significant. *Babar et al. (2004),* commented that the variation in birth weight reflected the level of management that bound to vary according to the ability of the farm manager and his capability", perhaps these annotation presumably must be considered too as additional support on the results of this study.

Conclusion

The result achieved therefore can be terminated that the effect of genotype, sire and gestation length and the interaction between subjects effects such as the next: Sire*Gestation length; Sire*Sex; Gestation length*Sex; Sire*Gestation length*Sex were highly and significantly affected the birth weight of lambs (P<0.01 ; P<0.05). Precisely, understanding the influencing factors that affects the birth weight will lead changes in breeding and management schemes. Finally, the interactions between subjects' effects will be a good indicator to be acquainted more on their influence in a certain trait. Over and above, the birth weight of every

genotype is useful information for farmers and for the animal breeder in order to be familiarized which fitted in their future needs.

Acknowledgements

This study is part of the projects TR 31053 “Modern biotechnology solutions in the breeding and feeding of cattle sheep and goats for the production of valuable and safety food” and TR 31001 “An environmental approach and implementation of modern biotechnologies as a basis for the improvement of ruminant breeding technology”, and that are financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Uticaj genotipa, oca, pola i trajanja bremenitosti na masu tela jagnjadi pri rođenju

V.Caro Petrović, M. P. Petrović, Z. Ilić, M. M. Petrović, B. Milošević, D. Ružić Muslić, N. Maksimović

Rezime

Masa jagnjadi na rođenju ima bitnu ulogu u proizvodnji ovaca. Takođe se ogleda i kao primarni faktor kasnijeg razvoja mladog organizma. U radu su ispitivani sledeći faktori koji utiči na masu pri rođenju: genotip, otac, trajanje bremenitosti i pol. Istraživanje je obavljeno u populacijama Miss (1), Virtemberg (2) i Il de Frans (3). Najveća porođajna masa je dobijena kod genotipa 3 i to 4,63 kg od oca 2 i 4,44 kg od oca 1, sa razlikom od 0,19 kg. Trajanje bremenitosti genotipa 1 kretalo se u rasponu od 144 do 153 dana, genotipa 2 , od 148 do 154 dana i genotipa 3, od 144 do 151 dan. Najveća prosečna masa od 5,10 kg zabeležena je kod genotipa 1, sa trajanjem gestacije od 153 dana. Prosečne mase pri rođenju kod muških jagnjadi u svim genotipovima bile su veće od ženskih. Utvrđen je veoma značajan efekat genotipa, oca i trajanja bremenitosti na porođajnu masu jagnjadi ($P<0,01$), ali pol nije imao značajan uticaj na masu jagnjadi (0,065 ; $P>0,05$). Rezultati dobijeni u ovim istraživanjima pokazuju da su efekti genotipa, oca, trajanja bremenitosti i interakcije između efekata kao što su : otac * dužina bremenitosti; otac * pol; dužina bremenitosti * pol; otac * dužina bremenitosti * pol, vrlo značajno i značajno uticali na porođajnu masu jagnjadi ($P<0,01$, $P<0,05$) ispitivanih genotipova. Interakcije između efekata su dobar

pokazatelj boljeg upoznavanja njihovog uticaja na masu tela jagnjadi pri rođenju, koja je od ključnog značaja za kasniji razvoj mladog organizma.

References

BABAR M.E., AHMAD Z., NADEEM A., YAQOOB M. (2004): Environmental Factors Affecting Birth Weight in Lohi Sheep. Pakistan Vet. J., 24, 1, 5-8.

BERHAN A., ARENDONK J.V. (2006): Reproductive performance and mortality rate in Menz and Horro sheep following controlled breeding in Ethiopia. Small Rumin. Res. 63, 297-303.

CARO PETROVIĆ V., PETROVIĆ P M., 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, 743-749.

CARO PETROVIC V., PETROVIC P. M., ILIC Z., PETROVIC M. M., TENEVA A., STOJKOVIĆ J., RUZIĆ MUSLIĆ D. Some Non-Genetic Factors Influencing Early Growth Traits of Lambs. Poster presentation at the International Symposium held at Hotel Park Belgrade Serbia on October 2- 4, 2013.

CARO PETROVIĆ V., PETROVIĆ M.P., PETROVIĆ M.M., ILIĆ Z., STOJKOVIĆ J., RUŽIĆ-MUSLIĆ D., MAKSIMOVIC N. (2013): The Linear Relationship between Growth Traits Of Sharplanina Lambs In Extensive Farming Practices Biotechnology in Animal Husbandry 29, 2, 287-297.

GARDNER D.S., BUTTERY P.J., D. Z., SYMONDS M.E. (2007): Factors affecting birth weight in sheep: Maternal Environ. Reprod., 133, 297-307.

HUSSAIN A., BABAR M.E., ALI S., HASAN ZIA-UL (2000): Effect of Body Weight of Ewes on Birth Weight of Lambs on Rambouillet Breed of Sheep, Pakistan Journal of Biological Science 3, 1, 179-180.

ILIĆ Z., JEVTIĆ-VUKMIROVIĆ A., PETROVIĆ P.M., CARO PETROVIĆ V., MILOŠEVIĆ B., SPASIĆ Z., RISTANOVIĆ B. (2013): Effect of Mating Method, Sex and Birth Type On Growth Of Lambs. Biotechnology in Animal Husbandry 29, 2, 277-286.

IYIOLA-TUNJI A.O., AKPA B.I., NWAGU G.N., ADEYINKA I.A., OSUHOR C.U., LAWAL T.T., OJO O.A. (2010): Relationship between Gestation Length and Birth Weight in Nigerian Sheep and Their Crosses. Animal Production, 12, 3, 135-138.

JENKINSON C.M.C., BLAIR H.T., KENYON P.R., HARDING J.E., BLOOMFIELD F.H., BREIER B.H., P. GLUCKMAN D. (2007): Maternal constraints in sheep breeds with diverse birth weight. Proc. N.Z. Soc. Anim. Prod., 67, 187–191.

KINCAID C.M. (1943): Influence of the Sire on the Birth Weight of Lambs .Journal of Animal Science, 2, 152-156.
OLDHAM C.M., THOMPSON A.N., FERGUSON M.B., GORDON D.J., KEARNEY G.A., PAGANONI B.L (2011): The birth weight and survival of Merino lambs can be predicted from the profile of live weight change of their mothers during pregnancy. Animal Production Science, 51, 9, 776-783.
OSINOWO O.A., ABUBAKAR B.Y., TRIMNELL A.R. (1994): Genetic and phenotypic relationships between gestation length, litter size and litter birth weight in Yankasa sheep. Anim. Rep. Sci., 34, 2, 111-118.
OSORIO-AVALOS J., MONTALDO H.H., VALENCIA-POSADAS M., CASTILLO-JUÁREZ H., ULLOA-ARVIZU R. (2012): Breed and breed x environment interaction effects for growth traits and survival rate from birth to weaning in crossbred lambs.Journal Animal Science, 90, 12, 4239-47.
PETROVIĆ P.M., RUŽIĆ- MUSLIĆ D., MAKSIMOVIĆ N., MEMIŠI N. (2009): Effect of Environmental and Paragenetic Factors On Birth Mass Variability of Mis Sheep Populations Biotechnology in Animal Husbandry, 25, 3-4, 213-219.
PETROVIC P.M. (2007): Sustainable sheep breeding (Monograph). Institute for Animal Husbandry Belgrade, p. 256.
PETROVIC P.M., RUZIC MUSLIC D., CARO PETROVIC V., MAKSIMOVIC N. (2011) Influence of environmental factors on birth weight variability of indigenous Serbian breeds of sheep. African Journal of Biotechnology, 10, 4673-4676.
SHARMA R.K., BLAIR H.T., JENKINSON C.M.C., KENYON P.R, COCKREM J.F., PARKINSON T.J. (2011): Uterine environment as a regulator of birth weight and body dimensions of newborn lambs. Journal Animal Science, 90, 1338-1348.
THRIFT F.A., DURR R.H. (1972): Relationship between Gestation Length of Artificially Inseminated Ewes and Number, Weight and Sex of Lambs Born. Journal Animal Science, 34, 3, 441-444.
VATANKHAH M., TALEBI M.A. (2009): Genetic and Non-genetic factors Affecting Mortality in Lori-Bakhtiari Lambs. Asian-Aust. J. Anim. Sci., 4, 459-464.
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.
YAQOOB M., MERRELL B.G., SULTAN I.J. (2004): Comparison of Three Terminal Sire Breeds For Birth Weight of Lambs Kept Under Upland Grassland Conditions In The Northeast Of England. Pakistan Vet. J., 24, 4, 196-198.

Received 28 November 2013; accepted for publication 25 December 2013

Biotechnology in Animal Husbandry 29 (4), p 695-704 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.31'633.2
DOI: 10.2298/ BAH1304695B

YIELD POTENTIAL AND QUALITY OF FORAGE MIXTURES OF ALFALFA WITH COCKSFOOT AND TALL FESCUE DEPENDING ON THE NITROGEN FERTILIZATION

Z. Bijelić[1*], Z. Tomić[1], D. Ružić-Muslić[1], V. Mandić[1], A. Simić[2], S. Vučković[2]

[1] Institute for Animal husbandry, Autoput 16, Belgrade
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, Zemun
*Corresponding author: zonesh@gmail.com
Original scientific paper

Abstract: The study was performed at the Institute for Animal Husbandry in the three year period, in field and laboratory conditions. The experiment involved two mixtures of grasses and legumes: mixture A (alfalfa (cv. K -28), 50% and cocksfoot (cv. K-40), 50%) and mixture B (alfalfa (cv. K -28), 33.3 % , cocksfoot (cv. K -40) , 33.3 %, and tall fescue (cv. K -20) , 33.3 %), and pure stand of alfalfa (M). The experiment was conducted in a randomized block design with four replications. Fertilization was carried out using the split method, in quantities of of 0, 70 and 140 kg N ha $^{-1}$. The aim of this study was to determine the phytocoenological stability of mixture of alfalfa (*Medicago sativa* L.) with cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.), the yield and quality of fodder obtained from the grassland, depending on the mixture composition and nitrogen fertilization. Studied factors had a statistically significant impact on dry matter yield and protein yield. Alfalfa has achieved significantly higher yields compared to its mixtures. Dry matter yield increased with the addition of N fertilizers. Protein yield was highest in pure alfalfa crop, followed by its mixture cocksfoot. Fertilization has also increased protein yield.

Key words: yield, quality, alfalfa, mixtures, N fertilization

Introduction

Grasslands can provide sufficient quantities of inexpensive and high - quality forage for livestock production, for feeding of large and small ruminants. However, this option requires the proper use of grassland areas, whether natural or sown, that is very precise planning and management systems. In Serbia, according to the statistical data from the 2012, there were 590 927 hectares of meadows and 702,887 hectares of pastures (SBS, 2012). Comparing statistics from previous

years, we can conclude that the production area decreased. For these reasons, attention should be directed towards sown grasslands, or mixtures of grasses and legumes. There are numerous benefits of grass - legume mixtures that lead to thir use, such as: nitrogen fixating ability of legumes where grass can use adopted nitrogen (*Tekeli and Ates, 2005*), the high productivity of mixtures during the entire growing season (*Nešić at al. 2007*) as well as high nutritional value as compared to pure grass crops (*Berdahl et al., 2001*). Cattle can be fed green food because the possibility of bloat is reduced and the performance is better compared to cattle fed monocrops (*Sanderson et al. , 1999*) .

When planning a grass-legume surfaces, it is very important to choose the adequate species for the mixture, which should be primarily based on the adaptation of species to growing conditions, purpose of the grassland, consistency, yield and its seasonal distribution and nutritional value (*Tekeli and Ates, 2005*). The exmination/study of the botanical composition of grass - legume mixtures led to the conclusion that cocksfoot was the most compatible species suitable for growing in mixtures with very competitive legumes, and in some cases its competitiveness exceeds the competitiveness of legumes forcing them out of the mixture, (*Casler and Walgenbach, 1990*). In addition to cocksfoot, tall fescue is also suitable grass species for growing mixed with clover (*Hannaway et al., 1999*) . Grass- legume mixtures are energy high-quality forage with a well-balanced contents of protein, carbohydrates, minerals and vitamins.

The aim of this study was to determine the phytocoenological stability of the mixture of alfalfa (*Medicago sativa* L.) with cocksfoot (*Dactylis glomerata L.*) and tall fescue (*Festuca arundinacea* Schreb.), the yield and quality of fodder obtained from the grasslands, depending on the mixture composition and nitrogen fertilization.

Material and methods

In order to solve the given task, tests were carried out at the Institute for Animal Husbandry in three year period, in two phases: in field and laboratory conditions. The study included two mixtures of grasses and legumes : A mixture (alfalfa (cv. K -28), 50% and cocksfoot (cv. K-40), 50% and mixture B (alfalfa (cv. K -28), 33.3 % cocksfoot (cv. K-40), 33.3 %, and tall fescue (cv. K -20, 33.3 %), and pure alfalfa crop (M). The experiment was conducted in a randomized block design with four replications, the size of the experimental plots of 10 m^2. Sowing experiment was performed in the 2003. Meadow fertilization was carried out using the split method, at the beginning of the vegetation and after first cutting in the total weight of 0, 70 and 140 kg N ha^{-1}. Botanical composition (% grasses, legumes, weeds) of samples has been done in green, sampling 1m^2 by separating and measuring the species. The chemical composition of the feed was analyzed in

the laboratory of the Institute for Animal Husbandry, using standard laboratory analyses. Chemical analyzes included the content of crude protein (CP), crude fiber (CF) and mineral substances. The content of metabolizable energy (ME) was calculated by the formula :

ME(MJ/kg DM) = 14,06-0,0137gCF+0,00483gCP-0,0098gCA (*Schenkel,* 1998, quoated by *Petkova*, 2006)

The data obtained in the botanical analysis, dry matter yield and chemical quality of dry mass were analysed by ANOVA two-factorial analysis of the variance for studied factors, the type of mixture and N fertilization, and significance of differences was tested using LSD test (Stat.Soft., STATISTICA 8, 2001).

The land/soil on which the grassland was established was low carbonate chernozem, of favourable water, air, thermal regime and a very good particle-grain structure (pH = 7.3, N = 1975 ppm, P_2O_5 = 9.0 mg $100g^{-1}$, K_2O = 16 mg $100g^{-1}$).

Average temperatures for vegetation period according to investigation years were 12.6, 13.24 and 14.09 ^{0}C, and total precipitation 486.1, 543.2 and 532.3 mm which was 72.34%, 77.5% and 69.05% of total annual precipitation.

Results and discussion

Botanical analysis of samples of green mass from grasslands A and B showed that participation in these mixtures of legumes, grasses and weeds for years quite varied, but the studied factors did not express their effect in any of the years studied. (Table 1).

Table 1. The percentage of species in the mixture (%) depending on the mixture and N fertilization for three year study period

Mixture	I exp. year			II exp. year			III exp. year		
	L	G	F	L	G	F	L	G	F
Mixture A	52.10	46.33	1.57	56.76	41.80	1.44	67.58	30.43	1.99
Mixture B	52.00	46.25	1.75	59.44	39.92	0.64	66.33	32.24	1.43
Level of signific.	ns	ns	ns	ns	ns	**	ns	ns	ns
N fertilization									
0	60.87	37.96	1.17	68.11	31.16	0.73	71.42	26.75	1.83
70	50.38	47.96	1.66	56.44	42.48	1.08	68.05	30.41	1.54
140	44.90	52.96	2.14	49.77	48.93	1.30	61.38	36.85	1.77
Level of signific.	ns	ns	ns	ns	ns	ns	ns	ns	ns

L-legumes; G-grass; F-forbs

In the mixture A the percentage of alfalfa increased from 52.10 to 67.58%, as well as the percentage of weeds, while the share of cocksfoot decreased from 46.3 to 30.43%. In the three-component mixture B also share of alfalfa increased each

year from 52.0 to 66.3% and the share of grass reduced from 46.25 to 32.24%. In the mixture A the percentage of both species is similar to the sowing level with small variations of ≈ 10 % depending on the year, which according to *Casler et al. (1990)* is a sign of good competitive abilities for cocksfoot grown in mixture with alfalfa. In the grass mixture B, share of grasses was lower than it was at the time of sowing, which means that although tall fescue has good capacity for growing in mixture with alfalfa (*Hannaway et al., 1999*) nevertheless it has lower competitiveness than cocksfoot in the given environmental conditions. Increasing the share of alfalfa in the mixture could be explained by a greater adjustment ability of alfalfa to grow on existing soil conditions in relation to cocksfoot and tall fescue, which can be seen from the study of dry matter yield of alfalfa, cocksfoot and tall fescue on chernozem by *Nešić et al. (2005)* and *Tomić et al. (2006).*

Although fertilization had no statistically significant effect slight increase in share of grass and weeds was noticeable, and decrease in share of legumes which is consistent with the research by *Stoeva and Vateva* (2010).

Total yield, quality and seasonal distribution of forage are of great importance for livestock production. The total dry matter yield and quality of forage grass are shown in Table 2. The most important aspect of forage quality is the content and availability of nutrients for animals. Many factors affect the quality of forage such as: stage of maturity of crops at the time of cutting, the ratio of grasses and legumes, the presence of weeds, diseases, etc. Crude protein is one of the most important parameters of food quality. Crude protein content determines the biological value of food.

The yields of both mixtures varied considerably by years of study. Both studied factors had statistically significant impacts on the achieved yields of dry matter in all three years.

Table 2. Total dry matter yield (t ha^{-1}) of alfalfa and its mixtures with cocksfoot and tall fescue, monitored in regard to N fertilization in three year investigation period

Treatments	DM yield t ha^{-1}			
	I exp. year	II exp. year	III exp.	Average
Mixture				
Alfalfa	10.34^{a}	9.97^{a}	10.36^{a}	10,51^{a}
Alfalfa +cocksfoot	10.74^{a}	9.13^{b}	8.88^{b}	9,77^{b}
Alfalfa +cocksfoot +tall fescue	9.70^{b}	8.87^{b}	8.87^{b}	9,53^{b}
Level of significance of mixtures	**	**	**	**
Fertilization with N (kgN ha^{-1})				
0	9.92^{b}	9.09^{b}	8.88^{b}	9,10^{b}
70	10.67^{a}	9.40^{ab}	9.76^{a}	9,80^{a}
140	10.21^{ab}	9.48^{a}	9.49^{ab}	9,64^{ab}
Level of significance of N fertilization	*	*	*	**

In the first experimental year, the two-component mixture A had the highest yield of 10.74 t ha^{-1}, which was not significantly different from the yield of pure alfalfa crop, whereas mixture B achieved significantly lower yield of 9.70 t ha^{-1} compared to monoculture and two-component mixture A. *Vassilev (2004)* presented in his research that alfalfa mixtures with cocksfoot achieved higher dry matter yield of 15.6-16.8 t ha^{-1} compared to mixtures with tall fescue 15.1-15.6 t ha^{-1}. In the next two years, alfalfa has achieved a significantly higher yield compared to its mixture. The results were consistent with the results of *Halling and Wallgren (1996)* and *Kunelius et al. (2005),* but slightly higher than the results obtained by *Vassilev and Chakarov (1999),* and *Loges et al. (2000*), whose studies reported yields of 6.86 and 7.53 t ha^{-1}. Average for the three-year test period of research alfalfa achieved significantly higher yields of dry matter in relation to its mixtures with grasses, while fertilization with 70 kg N ha^{-1} proved to be the most effective.

In all three investigated years, fertilization significantly increased the yield, but the differences between the quantities of 70 and 140 kg N ha^{-1} were not significant so that the increased investment in higher amounts of nitrogen fertilization were not economically justified.

Table 3. Quality, protein yield and content of metabolic energy of alfalfa and its mixtures in all three investigation years

Treatments	CP	CF	ASH	YP	ME
	gkg^{-1}			t ha^{-1}	Mcal
I exp. year					
Alfalfa	168.6	309.9	84.5	1.7^{a}	9.80
Alfalfa +cocksfoot	163.2	310.3	83.6	1.6^{a}	9.78
Alfalfa +cocksfoot +tall fescue	157.8	322.8	83.4	1.4^{b}	9.58
Level of significance	ns	ns	ns	**	ns
II exp. year					
Alfalfa	185.1^{a}	270.8	96.2	1.9^{a}	10.30
Alfalfa +cocksfoot	170.9^{ab}	285.4	89.8	1.5^{b}	10.09
Alfalfa +cocksfoot +tall fescue	158.3^{b}	279.7	93.2	1.4^{c}	10.08
Level of significance	**	ns	ns	**	ns
III exp. year					
Alfalfa	181.6	290.8	86.9	1.9^{a}	10.10
Alfalfa +cocksfoot	174.4	300.9	86.7	1.5^{b}	9.93
Alfalfa +cocksfoot +tall fescue	180.3	297.4	80.9	1.6^{b}	10.06
Level of significance	ns	ns	ns	**	ns

With the increase of share of legumes in the mixture the crude protein content also increases, and the crude fibre content decreases. The tested mixtures were not significantly different in regard to the content of crude protein ranging in mixture A from 163.2 to 174.4 g kg^{-1} DM, depending on the investigation year, in mixture

of B from 157.8-180.3 g kg $^{-1}$ DM. Pure crop of alfalfa had a higher crude protein content than alfalfa mixtures. The content of crude fibre, ash and metabolic energy showed no significant differences between monocultures and mixtures. Protein yield was highly dependent on the type of mixture. In all three years alfalfa achieved significantly higher protein yield compared to mixture B, and the value of the protein yield in mixture A for the first experimental year was not statistically significantly different from the yield of pure crop. A mixture of alfalfa and cocksfoot achieved significantly higher protein yield compared to mixture of alfalfa, cocksfoot and tall fescue in the first two years of testing, while in the final year of examination yields were not statistically different. Similar to our results *Chakarov and Vassilev (1992)* obtained the higher values of protein yield (1.15 t ha $^{-1}$) for mixtures of alfalfa and cocksfoot compared to the mixture of alfalfa, cocksfoot and tall fescue (1.04 t ha $^{-1}$).

According to the NRC (2001) the daily needs of small and large ruminants (sheep and cattle) for the specific content of crude protein in animal feeds range from 9.1 to 15.0% DM for sheep and 7.4 to 16.6% DM for cattle. Comparing the results of analysis of crude protein in forage of alfalfa mixtures with cocksfoot and tall fescue, with data from the NRC (2001), we recognize the very good quality of forage of examined mixtures that can fully meet the daily requirements of sheep and cattle.

Table 4. Quality and yield of protein and content of metabolizable energy of alfalfa and its mixtures depending on nitrogen fertilization in all three years

Treatments	CP	CF	ASH	YP	ME
	gkg^{-1}			t ha^{-1}	Mcal
I exp. year					
0	157.1	318.9	84.5	1.48^{b}	9.62
70	163.8	311.4	82.0	1.65^{a}	9.78
140	168.7	312.7	85.0	1.62^{a}	9.75
Level of significance	ns	ns	ns	*	ns
II exp. year					
0	165.3	277.2	91.0	1.49^{b}	10.17
70	172.2	279.6	95.1	1.59^{a}	10.13
140	176.8	279.1	93.0	1.66^{a}	10.18
Level of significance	ns	ns	ns	*	ns
III exp. year					
0	173.8	296.1	84.2	1.52^{b}	10.02
70	178.8	299.7	85.2	1.73^{a}	9.98
140	183.7	293.3	84.9	1.77^{a}	10.10
Level of significance	ns	ns	ns	*	ns

CP-crude proteins; CC-crude fiber; YP-protein yield; ME-metabolizable energy

N fertilization had no statistically significant effect on the crude protein content, although the results show that the content slightly increased with the addition of N. In the control treatment CP content ranged from 157.1-173.8 g kg^{-1} DM, and treatment with 140 kg N ha^{-1} from 168.7-183.7 g kg^{-1} DM. Like in the case of mixtures, fertilization had no significant effect on the content of crude fiber, ash and metabolic energy. Contrary to our study, in the research carried out by *Samuil et al. (2012)* mixture and fertilization had a significant impact on the quality of forage. According to them, increasing the participation of legumes in the mixture and N fertilization increased the content of total nitrogen and decreased the crude fiber content. Protein yield was dependent on the N fertilization at the level of of $P \leq 0.05$. Adding of N resulted in an increase of protein yield. Variations in the amount of protein yield between the amount of nitrogen 70 and 140 kg N ha^{-1} were not significant, so in this case, greater quantity of nitrogen, 140 kg N ha^{-1} was not justified.

Conclusion

Alfalfa achieved significantly higher yields of DM compared to its mixtures, the mixture of alfalfa and cocksfoot had higher yields than mixtures with tall fescue. Yields increased with addition of N fertilizers, but the difference in yield between the medium and the highest dose of nitrogen were not statistically significant, and consequently economically justified. Protein yield was the highest in pure alfalfa crop, followed by the mixture of alfalfa and cocksfoot, while fertilization increased the yield of protein, but also high nitrogen rate of 140 kg N ha^{-1} was not justified because it did not not significantly increase the yield of protein in relation to the fertilization dose of 70 kg N ha^{-1}.

Mixture of alfalfa and cocksfoot are giving satisfactory DM yield and fodder quality,and could be recommended in practice. In order to achieve satisfactory yields, crops should be fertilized with N mineral fertilizers but with smaller amounts of nitrogen (70 kg N ha^{-1}), because only this is economically justified considering the difference in the yields compared to the larger amount of nitrogen (140 kg N ha^{-1}).

Acknowledgement

This research is part of the Project EVB: TR-31053 financial supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Prinos i kvalitet krmnih smeša lucerke sa ježevicom i visokim vijukom u zavisnosti od djubrenja azotom

Z. Bijelić, Z. Tomić, D. Ružić-Muslić, V. Mandić, A. Simić, S. Vučković

Rezime

Ispitivanja su radjena u Institutu za stočarstvo u trogodišnjem periodu ispitivanja u poljskim i laboratorijskim uslovima. U eksperiment su uključene dve smeše trava i leguminoza: Smeša A (lucerka (K-28), 50% i ježevica (cv. K-40), 50%) i Smeša B (lucerka (K-28), 33,3%, ježevica (cv. K-40), 33,3% i visoki vijuk (cv.K-20), 33,3%), kao i čist usev lucerke (M). Ogled je izveden po metodu slučajnog blok sistema u četiri ponavljanja, Đubrenje travnjaka je obavljeno split metodom, količinama od 0, 70 i 140 kgN ha^{-1}. Cilj ovih istraživanja je da odredi fitocenološku postojanost smeša lucerke (*Medicago sativa* L.) sa ježevicom (*Dactylis glomerata* L.) i visokim vijukom (*Festuca arundinacea* Schreb.), prinos i kvalitet dobijene stočne hrane sa travnjaka u zavisnosti od sastava smeše i djubrenja azotnim djubrivima. Ispitivani faktori imali su statistički značajnog uticaja na prinos suve materije i prinos proteina. Lucerka je ostvarila značajno veće prinose u odnosu na njene smeše. Prinosi suve materijeSM su se povećavali dodatkom N mineralnih djubriva. Prinos proteina bio je najveći u čistom usevu lucerke, a zatim u smeši lucerke i ježevice. Djubrenje je takođe povećalo prinos proteina.

References

BERDAHL, J. D., KARU, J. F., HENDRICKSON, J. R. (2001): Dry matter yield of cool-seson Grass Monocultures and grass-alfalfa binary mixtures. Agronomy Journal, 93, 463-467.

CASLER, D. M., WALGENBACH, R. P. (1990): Ground cover potential of forage grass cultivars mixed with alfalfa at divergent locations. Crop Science, 30, 825-831.

CHAKAROV, R., VASSILEV, E. (1992): Growing of lucerne forage in mixtures with grasses. Eucarpia Erba Medica, X-a Conferenze Internacionale, Lodi , 73-77.

HALLING, A.M., WALLGREN, B. (1996): Effect of harvest system and nitrogen rate on the performance of lucerne or red clover in mixtures with grass. EGF 96.

HANNAWAY, D., S. FRANSEN, J. CROPPER, M. TEEL, M. CHANEY, T. GRIGGS, R. HALSE, J. HART, P. CHEEKE, D. HANSEN, R. KLINGER, AND W. LANE (1999). Tall Fescue. PNW504 Oregon State University Extension Service, Corvallis, OR.

KUNELIUS, T. H., DURR, H. G., MCRAE, B. K., FILLMORE, A. S. (2005): Performance of timothy-based grass/legume mixtures in cold winter region. Journal of Agronomy and Crop Science, 192, 159-167.
LOGES, R., KASKE, A., INGWERSE, K., FRIEDHELM, T. (2000): Yield, forage quality, residue nitrogen and nitrogen fixation of different forage legumes. The World Grows Organic, Proceeding of the 13th International IFOAM Scientific Conference, Basel, 83.
NEŠIĆ Z., TOMIĆ Z., ŽUJOVIĆ M., KRNJAJA V. (2005): Production characteristics of domestic Alfalfa (*Medicago sativa* L.) cultivars in agro ecological conditions of Srem district. Biotechnology in Animal Husbandry, vol. 21, iss. 5-6-2, pp. 169-174
NEŠIĆ, Z., TOMIĆ, Z. VUČKOVIĆ S., ŽUJOVIĆ M. (2006): Yield and botanical composition of pure alfalfa and alfalfa-orchard grass mixtures at different levels of nitrogen. Sustainable Grassland Productivity, Proceeding of the 21st General Meeting of European Grassland Federation, Vol. 11, 273-275.
NRC (2001). National Research Council, (2001): Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington,
D. C. RZS (2012): Republički zavod za statistiku, http://webrzs.stat.gov.rs/WebSite/public/ReportView.aspx
SAMUIL, C., VINTU, V., SIRBU, C., SURMEI, G. M. (2012): Behaviour of fodder mixtures with alfalfa in north-eastern Romania. Romanian Agricultural Research, No. 29, 227-235.
SANDERSON, A., ELWINGER, M., GERALD, F. (1999): Grass species and cultivar effect on establishment of grass-white clover mixture. Agronomy Journal, 91, 889-897.
SCHENKEL H.(1998): Methods for determination of energetic feed value – Scientific base and practical experience, Arch. Tierernahrung, 51, 2/3, 155 – 165 in PETKOVA, M. (2006): Energetic and protein values of new bulgarian feedstuffs. Biotechnology in Animal Husbandry 22 (1-2), 133-138. STOEVA, K. AND VATEVA, V. (2010): Effect of organo-mineral fertilization on growth and development of perennial grass mixture, cultivated in Strandzha region. Agricultural science and technology, vol. 2, No 4, 211 – 214.
TEKELI, S.A., ATES, E. (2005): Yield potential and mineral composition of white clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinaceae* Schreb.) mixtures. Journal of Central European Agriculture, 6, 1, 27-34.
TOMIĆ, Z., NEŠIĆ, Z., KRNJAJA, V. , ŽUJOVIĆ, M. , VORKAPIĆ, M. (2006): Testiranje novih sorti višegodišnjih trava u godini setve. Biotechnology in Animal Husbandry 22 (5-6), 81 - 88
VASSILEV, E., CHAKAROV, R. (1999): Methods for growing and using management of alfalfa. Influence of the alternative utilization for seed and forage production on the alfalfa forage productivity in the first regrowth. Pastagens e Foragens, 20, 113-123.

VASSILEV, E. (2004): Forage productivity of some Bulgarian lucerne in mixtures with grasses. Proceeding of the 20th European Grassland Federation, Land use systems in Grassland Dominated Regions. Grassland Science in Europe, 9, 401-403.

Received 11 November 2013; accepted for publication 20 December 2013

Biotechnology in Animal Husbandry 29 (4), p 705-714 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.32
DOI: 10.2298/ BAH1304705N

GENOTYPIC AND ECOLOGICAL EFFECTS ON LEAFINESS OF RED CLOVER (*Trifolium pratense L.*)

G. Naydenova

Experimental Station of Soybean, Pavlikeni 5200, Bulgaria
Corresponding author: gmvg@abv.bg
Original scientific paper

Abstract: The objective of this experiment was to: 1) examine the degree of influence of hereditary factors and conditions of growth (region of cultivation, season of growth and type of cultivation) on the phenotypic variance of leafiness in breeding populations of red clover; 2) quantify heritability and experimental variances in order to assess the opportunities for red clover breeding for improved leafiness. Five genotypes of red clover (four synthetic breeding populations and variety Sofia 52) were screened in the study. Among the factors studied as environmental variables, the season of growth had the strongest effect on the leafiness of red clover. In second vegetation of the life cycle of plants there was a significant additive genetic variance ($h_{ns}^2 = 0.31$) of the trait of leaf proportion in the fresh forage and the recurrent phenotypic selection for this criterion could be used in the breeding for leafiness in this species. It can be concluded from the results in this experiment that a higher degree of heritable genetic variation of leafiness must be searched for in connection with the variation of additional characteristics – thickness of stems, rates of formation and growth of stems, growth features related to regrowth (secondary growth) and age of sward (plants) and probably to persistence of genotypes.

Key words: red clover, leafiness, breeding, heritability

Introduction

Legumes are the major element of grassland ecosystems, to which the function to increasing forage quality is assigned. Leafiness is a particularly important morphological trait in forage legumes. Proportion of leaves in the forage positively correlates with main forage quality parameters – carbohydrates, protein and vitamins content and protein digestibility (*Brink and Fairbrother, 1992; Katic et al., 2005; Chourkova, 2011*).

Red clover leaves are an important ingredient of quality forage (*Vasiljevic et al., 2009*). Until the budding stage the protein content in plant is directly related to its content in the leaves and consequently to the degree of leafiness. The rate of

decline in digestibility was lower in red clover leaves than stems *(Sanderson and Wedin, 1989*). Leaves in red clover are the morphological fraction, related also to another specific quality character of this species, namely content and activity of the polyphenol oxidase enzyme, which protects proteins and glycerol-based lipid in the rumen (*Sullivan et al., 2004; Michael et al., 2009; Parveen et al., 2010*). It was found that specific polyphenol oxidase activity was higher in red clover leaves compared to stems, while leaf age had no significant effect.

Although the parameters of proportion of leaves in the forage or leaf/stem ratio are main agronomic traits in variety testing of red clover (*Mihovski and Yancheva, 1998*), they are seldom used as an independent or main breeding criterion. On the one hand, the morphological types with great leafiness have usually lower productivity and persistence (*Goranova, 2002*). On the other hand, when using this species for haymaking, a part of leaf mass is lost to one extent or another in the process of harvesting and this makes the breeding improvement for this trait meaningless. However, in the aspect of using red clover for grazing, the breeding for great leafiness proves to be an important breeding aim and can be considered as breeding for productivity, since great leafiness of stems provides higher palatability and intake potential of forage (*Poli, 1998*). Ensiling is another main mode of using red clover, which also necessitates breeding for green forage quality, and consequently for leafiness.

Red clover is a polymorphic species. Proportion of leaves in total plant DM yield is primarily a function of morphological and biological type (*Goranova, 2002*), life cycle (*Brink and Fairbrother, 1992*) or level of ploidy (*Leto et al., 2004*), but is strongly influenced also by ecological conditions – temperatures and rainfall during the growth period (*Leto et al., 2004, Drobná, 2009*), intensity of sunshine, photoperiod, shading (*Rhykerd et al., 1959; Bowley et al., 1987; Taylor and Quesenberry, 1996*), diseases and insect pests.

The objective of this experiment was to: (1) examine the degree of influence of hereditary factors and conditions of growth on the phenotypic variance of leafiness in breeding populations of red clover; (2) quantify heritability and experimental variances in order to assess the opportunities for red clover breeding for improved leafiness.

Material and methods

Five genotypes of red clover, including four complex hybrid (synthetic) breeding populations, developed in one of our previous breeding works and variety Sofia 52, which was used as a standard variety, were screened in the study. The populations are a result of recurrent phenotypic breeding for specific adaptation to fore-mountain and mountain conditions of cultivation. In addition to productivity for haymaking use, they have been also consolidated according to different

additional breeding criteria, namely: the material used for development of population A is late-flowering, for population B – with great leafiness and even seasonal distribution of yield, for S – with good persistence, for D – with high seed productivity.

The study was conducted under swards conditions at two locations (mountain and lowland region) during the following two growth cycles: 2005-2007 and 2008-2010, respectively (Table 1). In the first growth cycle the populations were cultivated alone and in binary mixtures with meadow timothy (*Phleum pretense L.*) variety Troyan, and during the second one – only as a continuous crop. The seeds of the experimental populations of red clover originated from two successive generations (Syn 1 and Syn 2).

Table 1. Geographic, soil and climatic data for the regions and the periods of the investigation.

	Mountain region	**Lowland region**
Trial period	**2005-2007**	**2008-2010**
Location	**latitude – 42 º 88' N** longitude – 24º 72' E **altitude - 384m**	**latitude – 43 º 24' N** longitude – 25 º 32' E **altitude - 144m**
Soil characteristics	**soil type – planosols. district** soil pH - 5.4 in N_2O	**soil type – leached chernozem** soil pH - 7.1 in N_2O
Climatic data	rainfall amount during the growing season (April- October) average for the trial period - 1132 mm **mean annual temperature – 10.2ºS**	rainfall amount during the growing season (April- October) average for the trial period - 347 mm mean annual temperature – 12.8 ºS

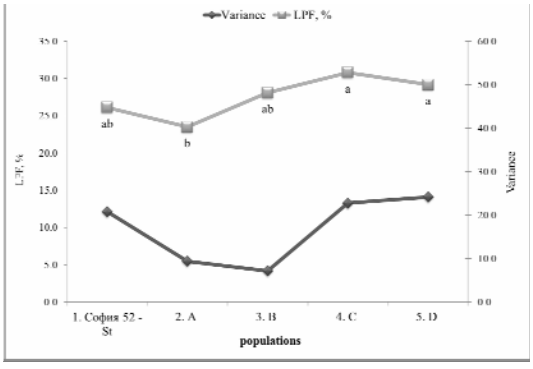
The experiments were carried out as randomised complete block design with 5 replications. The harvest plot area was 1 m^2. The sowing was conducted in early April for both growth cycles, at a sowing rate of 22 kg ha^{-1}. The leafiness was determined through the portion of leaf fraction in the mowed fresh biomass at haymaking ripeness of plants - at the stage of budding-early flowering. Through representative average samples of 1.5 kg in weight for each variant (or 0.3 kg from each replication) a morphological analysis of freshly cut biomass was performed. Weight percentage of leaves, stems and inflorescences in the fresh mass was determined. The number of obtained and hence analyzed cuts according to years was the following: 2006 – 3, 2007 – 1, 2009 – 2 and 2010 – 2. The populations were also characterized according to the following parameters: height of stems (HS, cm), variance of stem height (s^2), thickness of stems (TS, mm) and number of branches per stem (BS, nm). Biometric measurements were done on 30 stems from every genotype, 6 from each replication.

Data were analyzed with analysis of variance procedures and means comparison made with Least Significant Difference at 5% level of probability (LSD at $P<0.05$). The statistic program STATGRAPHICS PLUS was used. The studied sources of variation included two regions of cultivation (mountain or lowland region), growth season or cut (spring growth or summer regrowth) and

two types of cultivation (alone or in mixtures). They were assessed in a factorial combination with the genotype factor. The setting of the trial did not allow an analysis of influence of the factor of "age of sward" (or age of plants), so the variance of leafiness was examined in separate dispersion complexes for second and third vegetation (for a two- or three-year sward, respectively). Degree of influence of the studied factors in the dispersion of leafiness was determined through correlation relationship (η^2). The coefficient of heritability, which was calculated as a share of the genetic variance from the observed phenotypic variance ($H^2_{bs}= \sigma_g^2/\sigma^2_{ph}$), was used as an indicator of existence of genotypic differences in the studied trait in the studied group of populations. The additive inheritance of leafiness was assessed through the coefficient of heritability in a narrow sense, which was calculated through regression (b) and correlation (r) of Syn 2 as compared to Syn 1 generation of populations ($h^2_{ns}=b/2r_{xy}$).

Results and Discussion

It was found that there was a significant effect of the conditions of growth (regions of cultivation - season of growth - type of cultivation combination), as well as of the genotype on the proportion of leaf mass in the forage during second vegetation, when the crop reached full development (Table 2). The highest values of leaf proportion in the forage were observed for populations S and D (Figure 1). In third vegetation, the studied factors were not a significant source of variability of leafiness. Then the breeding populations were inferior to the standard variety in leafiness with non-significant differences (Figure 2).



The means followed by same letters did not differ significantly, P=0.05

Figure 1. Leaf proportion in forage (LPF) mean for second vegetation, %

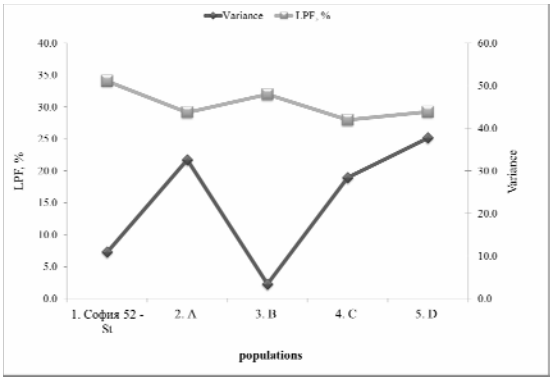


Figure 2. Leaf proportion in forage (LPF) mean for third vegetation, %

Table 2. Analysis of variance, significance and degree of factorial influences by leaf proportion in forage in red clover populations.

	Source of variation	df	MS	η^2
Second vegetation	E [a)]	5	35.9 *	0.29
	G	4	47.8 *	0.31
Third vegetation	E	1	5.7 NS	0.03
	G	4	47.8 NS	0.36
Second vegetation/ primary spring growth	R	1	7.41 NS	0.05
	G	4	32.90*	0.87
Second vegetation/ summer regrowth	R	1	72.04 P<0.10	0.29
	G	4	28.82 NS	0.47
Third vegetation/ primary spring growth	R	1	13.60 NS	0.03
	G	4	46.16 NS	0.46
Mountain region/ second vegetation	GS	1	11.03 NS	0.09
	G	4	21.91 P<0.10	0.73
Lowland region/ second vegetation	GS	1	82.6*	0.28
	G	4	47.5*	0.65
Lowland region/ third vegetation	GS	1	1.00 NS	0.01
	G	4	19.46 NS	0.51
Second vegetation/ primary spring growth	TC	1	0.48 NS	0.01
	G	4	26.61 NS	0.76
Second vegetation/ summer regrowth	TC	1	0.40 NS	0.01
	G	4	2.83 NS	0.14
Third vegetation/ primary spring growth	TC	1	0.65 NS	0.02
	G	4	60.87 NS	0.67

[a)] Environment (E) is a regions of cultivation (R) - growth season or cut (GS) - type of cultivation (TC) combination.
* Significant at the 0.05 probability levels

Region of cultivation and season of growth influenced the morphology of red clover by affecting the physiological processes of growth and development. Low soil moisture, increased temperatures and high sunshine intensity (characteristic of lowland conditions, as compared to mountain ones, or of the conditions of summer regrowth in comparison with the conditions of primary spring growth) limited the growth and as a consequence, they increased the portion of leaf fraction (*Leto et al., 2004; Drobná, 2009*). In this study at the same season of growth and equal age of sward (plants), the region of cultivation had a non-significant effect on the phenotypic variance of leafiness, in spite of significant differences in the values of growth ecological factors between mountain and lowland regions of study. Only in the summer regrowth during second vegetation there was a significant tendency ($P<0.10$, Table 2) to greater leafiness under the considerably drier conditions of the lowland region, by 4.7% on average for all populations. This result could be considered as an indicator of adaptive potential of the studied breeding populations.

Seasonal differences in ecological factors were greater in the lowland region, as compared to the mountain one. As a result, in lowland conditions the

season of growth was a significant source of variability of the observed morphological trait ($P<0.05$, Table 2). In the summer cut of second vegetation, the portion of leaf mass, on average for all populations, was 5.7% greater than that in the spring regrowth, and in some genotypes (Sofia 52 and D) this increase reached to 9.1%.

Growth rate and morphological development of red clover were influenced by the processes of competition and synergism in mixed cultivation (*Pineiro and Harris, 1978; Guretzky et al., 2004*). According to the results, in mountain conditions the cultivation in mixture with meadow (common) timothy did not influence the leafiness of red clover (Table 2). In addition, there was no significant genotype reaction through leafiness to mixed cultivation. Timothy, as a component of the mixture, had slight regrowth, and in summer conditions it did not compete red clover practically.

The significant genotypic variance of leafiness observed in second vegetation was related to region of cultivation and season of growth ($P<0.05$, Table 2). Populations S and D reacted to dry conditions to the greatest extent through a great portion of the leaf fraction. According to our results, this was related not only to suppressed vegetative growth (smaller height of generative stems), but it was also a result of the variation of other characters, related to leaf proportion in the fresh forage – namely, morphology of stems, as well as rate of their formation and growth. The population D, at growth in dry conditions, formed considerably thinner and branched stems (Table 3), which increased the portion of leaf fraction. Growth of population S in the lowland region was characterized by uneven and continuous formation of generative stems (dispersion in the variation sequences for height of stems under mowing was the highest in this population, Table 3), which resulted also in high values of leaf proportion in the forage. This population has been bred for persistence and has heterogeneous biotype composition. The uneven rates of growth of stems were probably related also to greater biotype segregation in Syn 2 generation, which was cultivated in the lowland region. In population V, bred through recurrent phenotypic selection for the criterion of great leaf proportion in forage, there was a significantly lower variance of the trait according to regions, cuts and years (Figure 1, 2).

Table 3. Means of populations for height of stems (HS, cm), variance of stem height (s^2), thickness of stems (TS, mm) and number of branches per stem (BS, nm) at lowland region.

Populations	Second vegetation/ primary spring growth		Second vegetation/ summer regrowth		Third vegetation/ primary spring growth		Second vegetation/ primary spring growth	Second vegetation/ summer regrowth	Third vegetation/ primary spring growth	Second vegetation/ primary spring growth	Second vegetation/ summer regrowth	Third vegetation / primary spring growth
	HS cm	s^2	HS cm	s^2	HS cm	s^2	TS, mm			BS, nm		
Sofia52 St	58.0	84.4	40.0	68.1	64.1	34.3	4.7	4.8	4.8	2.3	3.7	2.6
A	45.9	63.8	40.8	70.7	68.9	138.6	4.7	4.4	4.3	2.8	3.2	3.3
B	51.4	39.5	31.4	69.6	62.6	72.7	4.4	4.4	4.5	2.2	3.4	3.0
C	53.6	84.3	33.6	118.6	56.5	491.6	4.2	3.7	4.0	2.0	2.8	2.9
D	58.9	58.2	44.7	52.3	65.1	80.3	4.4	3.2	3.8	1.7	4.2	3.2
Mean	53.6		38.1		63.4		4.5	4.1	4.3	2.2	3.5	3.0
$LSD_{0,05}$	2.7				5.1		0.4	0.6	0.5	0.4	0.8	0.7

As far as the rates of growth and development are a function of life cycle, the leafiness, being related to them, was influenced by the factor of age of sward to a great extent. In third vegetation there was a tendency to growth with finer and unequally tall generative stems, with more branches, due to natural thinning of swards (Table 3). This resulted in increased leaf proportion in the fresh forage, which had an average value of 37.0% for all populations with a respective value of 27.5% in second vegetation (Table 4). The setting of the trial did not allow an assessment of significance of this difference, but the age of sward (plants) was probably a factor having a considerable effect on the studied trait.

The observed significant genotypic influence in the total dispersion of leafiness exceeded the effects of the conditions of growth ($\eta^2_G > \eta^2_E$, Table 2). Broad sense heritability, as an indicator of interaction between leaf proportion in forage and genotype had a value of medium significance in second vegetation ($H_{bs}^2 = 0.33$, Table 4), and a low value – in the third one ($H_{bs}^2 = 0.17$). In addition, in second vegetation 31% of variation of leafiness was influenced by additive genetic causes ($h_{ns}^2 = 0.31$). In third vegetation there was no additive genetic variance ($h_{ns}^2 = 0.03$). According to the hereditary assessment of the additive effects, the trait had

good response to breeding, but in the second year of life the plants. Also, the recurrent phenotypic selection for the criterion of leaf proportion in forage could be used with a good result with regard to phenotypic stability of the trait, which was confirmed by the results of population B bred for leafiness by the method specified.

Table 4. Variance components, broad sense (H^2_{bs}) and narrow sense (h^2_{ns}) heritability estimates for leaf proportion in fresh forage.

	Second vegetation	Third vegetation
Mean	27.5	37.0
Range	19.9-38.7	26.6-45.6
CV_{ph}	17%	14%
σ_e^2	12.1	26,8
σ_g^2	6.0	5.3
σ_{ph}^2	18.1	32.1
H_{bs}^2	0.33	0.17
h_{ns}^2	0.31	0.03

Conclusions

Among the factors studied as environmental variables, the season of growth had the strongest effect on the leafiness of red clover. In second vegetation of the life cycle of plants there was a significant additive genetic variance (h_{ns}^2 = 0.31) of the trait of leaf proportion in the fresh forage and the recurrent phenotypic selection for this criterion could be used in the breeding for leafiness in this species. It can be concluded from the results in this experiment that a higher degree of heritable genetic variation of leafiness must be searched for in connection with the variation of additional characteristics – thickness of stems, rates of formation and growth of stems, growth features related to regrowth (secondary growth) and age of sward (plants) and probably to persistence of genotypes.

Acknowledgment

Research was financed by the Agricultural Academy, Bulgaria, project P 109.

Uticaj genotipa i ekoloških faktora na olistalost crvene deteline (*Trifolium pratense* L.)

G. Naydenova

Rezime

Cilj ovog eksperimenta bio je da se: 1) ispita stepen uticaja naslednih faktora i uslova rasta (region gajenja , sezone rasta i vrste uzgoja) na fenotipske varijanse olistalosti priplodnih populacija crvene deteline, 2) kvantifikuju naslednost i eksperimentalne varijanse u cilju procene mogućnosti za uzgoj crvene deteline za poboljšanu olistalost. Pet genotipova crvene deteline (četiri sintetičke populacije i sorta za priplod Sofia 52) su prikazani u studiji. Među faktorima koji su ispitivani kao promenljive iz životne sredine, sezona rasta je imala najjači uticaj na olistalost crvene deteline. U drugoj vegetaciji životnog ciklusa biljaka postoji značajna aditivna genetička varijansa (hns2=0.31) na osobinu udeo lista u svežoj krmi i rekurentna fenotipska selekcija za ovaj kriterijum može da se koristi u oplemenjivanju na olistalost ove vrste. Na osnovu rezultata u ovom eksperimentu može se zaključiti da viši stepen nasledne genetske varijacije olistalosti se mora tražiti u vezi sa variranjem dodatnih karakteristika - debljina stabljike, stopa formiranja i rasta stabljike, karakteristike rasta u vezi sa ponovnim porastom (sekundarni rast) i starost biljaka i verovatno sa perzistencijom genotipova .

References

BOWLEY S., TAYLOR N., DOUGHERTY C. (1984): Physiology and morphology of red clover. Advances in Agronomy, 37, 317-347.

BRINK G., FAIRBROTHER T. (1992): Forage quality and morphological components of diverse clovers during primary spring growth. Crop Science, 32, 4, 1043-1048.

CHOURKOVA B. (2011): Biochemical traits and correlation associations between chemical composition and some parameters yield in birds' foot trefoil. Banat's Journal of Biotechnology II(3), 25-28.

DROBNÁ J. (2009): Yield and forage quality of Romanian red clover (*Trifolium pratense L.*) varieties studied in Slovakia. Not. Bot. Hort. Agrobot. Cluj, 37, 1, 204-208.

GORANOVA, G., 2002. Study of populations and varieties of red clover with a view to breeding and seed production. PhD Thesis, Agricultural Academy, Bulgaria.

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.

KATIC S., MILIC D., VASILJEVIC S. (2005): Variability of dry matter yield and quality of lucerne genotypes depending on geographic origin. EGF, Grassland science in Europe, 10, 537-540.

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.
LETO J., KNEŽEVIĆ M., BOŠNJAK K., MAĆEŠIĆ D., ŠTAFA Z., KOZUMPLIK V. (2004): Yield and forage quality of red clover (*Trifolium pratense L.*) cultivars in the lowland and the mountain regions. Plant Soil Environ., 50, 9, 391–396.
MIHOVSKI TS., YANCHEVA H. (1998): Comparative evaluation of red clover in the conditions of Central Northern Bulgaria. Journal of Mountain Agriculture on the Balkans, 1(3-4), 299-302.
PARVEEN I., THREADGILL M., MOORBY J., WINTERS A., (2010): Oxidative phenols in forage crops containing polyphenol oxidase enzymes. J Agric Food Chem., 58, 3, 1371-1382, doi: 10.1021/jf9024294.
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.
POLI, C. (1998): Effects of physical and biochemical characteristics of contrasting legume swards on selective behaviour of grazing cattle. PhD Thesis, College of Sciences, Massey University, Palmerston North.
RHYKERD C., LANGSTON R., MOTT G. (1959): Influence of light on the foliar growth of alfalfa, red clover, and birds' foot trefoil. Agronomy Journal, 51, 4, 199-201.
SANDERSON, M.., WEDIN, W. (1989): Phenological stage and herbage quality relationships in temperate grasses and legumes. Agronomy journal, 81, 864-869.
SULLIVAN M., HATFIELD R., THOMA S., SAMAC D. (2004): Cloning and characterization of red clover polyphenol oxidase cDNAs and expression of active protein in Escherichia coli and transgenic alfalfa. Plant physiology, 136, 2, 3234-3244.
TAYLOR, N. AND QUESENBERRY, K. (1996): Red clover science, Kluwer Academy Publishers.
VASILJEVIĆ S., MILIĆ D., MIKIĆ A. (2009): Chemical attributes and quality improvement of forage legumes. Biotechnology in Animal Husbandry, 25, 5-6, 493-504.

Received 24 July 2013; accepted for publication 31 October 2013

Biotechnology in Animal Husbandry 29 (4), p 715-725 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.15
DOI: 10.2298/ BAH1304715T

EFFECTS OF AGROECOLOGICAL CONDITIONS AND HYBRID COMBINATIONS ON MAIZE SEED GERMINATION

M. Tabaković[1*], Đ. Glamočlija[2], S. Jovanović[1], V. Popović[3], D. Simić[4], S. Anđelković[5]

[1]Maize Research Institute, Slobodana Bajica 1, Zemun Polje, Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, Zemun, Serbia
[3]Institute of Field and Vegetable Crops, Maxim Gorky 30, Novi Sad, Serbia
[4]Institute of PKB Agroeconomic, Padinska Skela, Belgrade, Serbia
[5]Delta Agrar, Milentija Popovica 7 b, 11070 Belgrade, Serbia
*Corresponding author: E-mail address: mtabakovic@mrizp.rs
Original scientific paper

Abstract: Germination energy and seed germination of four maize combinations cultivated under different growing conditions were observed. Analysis of hybrid seed of four commercial combinations derived at the Maize Research Institute, Zemun Polje, grown in three locations, were done on the working sample of 4 x 100 seeds under laboratory conditions. The experimental data was processed for the mean and total variability ($\overline{X}$ and C.V.) for both seed traits and for each test variant. The total average values of germination energy and germination in the period of investigation amounted to 94.5% and 94.8%, respectively. The coefficient of variation for germination energy was 0.67%, while for germination it was 0.39%. In the five-year study, the hybrid combination ZP704 had the highest values of germination energy (95.9%) and total germination (96.3%).Test results indicate that coefficients of variations for both observed traits over years were low, which points out to homogeneity of the material. Significant effects of factors (hybrid and location) and their interaction on observed seed properties were established by the analysis of variance. Gained results indicate that all four hybrids had high level of expression of traits under changeable agro-ecological conditions, as well as, a great effect of factors (hybrid and location) on germination energy and seed germination.

Key words: maize, hybrid seed, fraction, location, germination energy, germination

Introduction

A necessary and appropriate number and arrangement of plants in the hybrid maize commercial crop can be primarily provided by sowing seeds of good physiological-physical and mechanical properties.

From the scientific, research and technological point of view, germination is a physiological trait of hybrid maize seed that depends on embryo viability and environmental conditions under which this process occurs. Seed germination is a complex, biochemical, physiological and morphological process and is a first critical moment in the formation of a new plant. According to *Ujević and Kovačević (1972),* the germination process proceeds in three stages, starting with the enzyme activation, continues through degradation of reserve materials and translocation and mobilisation of soluble substances as well as reactions that provide the growth. Studies carried out by *McDonald et al., (1994*) show that the most active water uptake by maize seeds occurs during the first six hours of seed imbibition and that it is more pronounced in the embryo than in the endosperm. Physical and mechanical seed traits depend on agro-ecological conditions during the seed formation and maturation. Physiological traits of maize hybrid seed also depend on agro-ecological conditions (*Fenner and Thompson, 2005*). According to studies performed by *Shien and McDonald, (1982)* the seed size and shape of two maize inbred lines did not affect see quality. Based on results presented by *Chasso (2000)* and *Maric at al. (2013)* temperatures of both, surface layers of the soil and air, are the main physiological stress factor of the early growth of the maize plant in the no-tillage growing system. *Stone et al. (1999)* have established that the soil temperature directly affected meristem tissues of roots and shoots.

All traits of hybrid seeds of maize also depend on a genotypic combination and an interaction of the combination with agro-ecological conditions during the growing season of seed crops. *Lee et al. (2002)* have determined significant differences in tolerance to low temperatures related to a genotype, and these differences affected the growth dynamics of roots and above ground plant organs. Genetic traits of a hybrid combination, as stated by *Sikder et al. (2009)*, have a great effect on seed germination.

The issue that arises in commercial hybrid maize growing practices is how to harmonise traits of seeds originating from different combinations and produced under various agro-ecological conditions. Success in resolving this issue will be reflected in the optimal utilisation of heterosis effects that are involved in a hybrid combination or a genotypic constitution of hybrid maize seed.

The research programme encompassed experimental studies of traits of hybrid seeds of the F1 generation, mathematical-statistical processing, evaluation and the analysis of obtained data.

Materials and Methods

The seed of hybrid combinations ZP 341, ZP 434, ZP 684 and ZP 704 produced in the following three locations in Bačka: surroundings of Subotica (northern Bačka - B 1), surroundings of Novi Sad (southern Bačka - B 2) and surroundings of Sombor (western Bačka - B 3) was used as a material in the present study. Trials in all locations were carried out on chernozem type of soil, which, by its physical and mechanical properties, met requirements of maize production.

Standard cropping practices for seed maize production were applied in all three locations. The establishment of macro-trials for each hybrid combination was done according to standards prescribed by the Regulation on control of the seed production, the content and the method of keeping records on production of seedlings of agricultural crops and the form on the report on the production of mycelia of edible and medicinal fungi (*Official Gazette of RS, issue 60/2006*). The distribution of precipitation and heat was observed over pheno-phases during the growing season of seed maize. Data on meteorological conditions were recorded in hydrometeorological stations closest to trial fields. Weather conditions significantly varied over years and locations during the five-year period of investigation.

Working samples of 1000 g, obtained from natural seed material of 25-30 kg, were used to perform the analysis of germination energy and seed germination, by methods of Regulation on Seed Testing Quality of Agricultural Crops (Official Gazette 47/87) and (ISTA, 1996) in the following manner: working sample of 4x100 seeds was used under the standard temperature and between paper (t=20/30^{o}C, under 16/8 h light/dark conditions), germination energy - 4 days, germination seed - 7 days.

Gained experimental data were processed by the appropriate mathematical-statistical methods using the statistical software package STATISTICA 10.0 for Windows.

Each of obtained parameters was processed by the statistical analysis using descriptive statistics for each parameter at the annual level (2007-2011). Differences among analysed maize hybrids cultivated in three locations of Bačka were observed by the analysis of variance for the factorial trial set up according to the randomised block design and by the LSD test for the probability levels 5% and 1% (*Maletić, 2005*). Levene's test of homogeneity of variance was used with the aim to reach unbiased conclusions about effects of observed factors on maize seed traits, as well as the possibility to apply parametric tests (ANOVA and LSD-test).

Agroecological conditions. Data on average temperatures and precipitation sums for the vegetation season in the 2007- 2011 period were processed for each trial location.

According to recorded data on average temperatures, the warmest year was 2010 in the location of B1, with the highest average temperature of 19.5^{o}C (Table 1).

The precipitation sums were uniform for the first four years of investigation, ranging from 320 to 470 mm (Table 2). In 2011, the precipitation sum was significantly higher (above 700 mm), with the highest value of 751 mm in the location of B2.

Water requirements of crops depend on the developmental stage, hence the optimal water regime is determined not only by the total precipitation but also by its distribution. The higher water requirements (100-150 mm) are in the summer months (June-July). The highest five-year precipitation average was recorded in April, while the maximum precipitation sum (210 mm) was recorded in the location of B3 in 2011. The maximum amount of precipitation of 92 mm was recorded in the location of B1 in 2007.

Table 1 Average air temperatures in locations of Bačka, Serbia, 2007-2011

Y	2007			2008			2009			2010			2011		
M	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3
IV	14	12	12	14	13	13	13	13	12	14	14	14	11	12	12
V	17	16	16	18	18	18	18	18	18	19	18	18	17	17	16
VI	20	19	20	23	22	22	22	21	21	20	19	19	20	20	20
VII	25	23	23	25	23	23	22	21	21	24	22	23	21	23	22
VII	20	19	19	23	22	22	23	22	21	24	23	22	21	21	21
IX	19	18	17	14	14	14	16	15	15	20	19	18	16	16	15
X	14	13	12	10	10	10	13	13	12	13	11	11	11	9	8
A	18	17	18	18	17	18	18	18	18	19	18	18	17	17	17

Y- Years, M- Month, A- Average, B 1- northern Bačka, B 2- southern Bačka, B 3- western Bačka

Table 2 Monthly precipitation sums in locations of Bačka, Serbia, 2007-2011

Y	2007			2008			2009			2010			2011		
M	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3
V	92	66	88	1	0	1	23	22	41	27	4	14	90	64	54
V	50	70	51	171	99	69	42	15	21	78	50	53	160	114	93
VI	135	104	147	39	71	87	124	116	113	104	127	109	174	172	210
VII	12	61	18	24	39	44	60	42	48	74	58	29	53	99	107
VII	98	125	115	53	80	57	25	14	21	35	19	50	47	169	49
IX	13	24	8	52	79	96	77	94	64	38	13	2	122	68	129
X	23	18	17	88	101	101	38	18	22	53	82	71	62	67	59
S	422	467	444	428	469	453	388	351	330	409	353	327	707	751	701

Y- Years, M- Month, S-Summ, B 1- northern Bačka, B 2- southern Bačka, B 3- western Bačka

Results and Discussions

Laboratory tests, as well as results obtained in the field, confirm that germination and germination energy of maize seeds are higher at higher temperatures (*Sabovljević et al,. 2011; Rosić, 1959; Stikić, 2005; Pinnel, 1949*). According to *Chassot (2000)*, the mean daily air temperature necessary for successful maize cultivation should be 8-10oC and 10-12^{o}C in the stage of germination and the stage of emergence, respectively. Temperature conditions were uniform over locations, while the precipitation distribution was satisfactory over locations and years. Maize tolerance to extreme conditions is genetically predetermined, limiting values for the photosynthetic activity amount to 15oC in the majority of genotypes (*Lee et al., 2002*).

The five-year average seed germination and germination energy of all observed hybrids was above 90% (Table 3). The total average values of germination energy and germination in the period of investigation amounted to 94.5% and 94.8%, respectively (Table 3). The coefficient of variation for germination energy and germination was 0.67% and 0.39%, respectively. In the five-year study, the hybrid combination ZP704 had the highest values of germination energy (95.9%) and total germination (96.3%). This hybrid was followed by the hybrid ZP684 with germination energy of 94.8% and germination of 95.4%. The hybrid ZP434 ranked third and last considering germination energy and germination, respectively. Furthermore, the hybrid ZP341 had the lowest germination energy, while it was just behind ZP684 considering germination. Based on the evaluation of locations, the mean germination energy and. germination for all hybrids in the observed period were determined in the location B3 and B2, respectively.

The coefficient of variation was low (1% < x< 7%) pointing out to material homogeneity and the insignificant effect of locations.

Table 3 Means and variations in traits germination energy and seed germination

Year	Location	Energy				Mean	Germination			Mean	
		ZP341	ZP434	ZP684	ZP704	ek	ZP341	ZP434	ZP684	ZP704	uk
2007	B 1	97.8	93.8	93.4	94.8	94.9	97.8	93.8	93.4	96.2	95.3
	B 2	96.8	95.8	93.8	95.8	95.5	98.8	95.8	93.8	95.8	96.0
	B 3	96.2	98.2	94.6	95.4	96.1	96.2	98.2	94.6	95.4	96.1
	Average	96.9	95.9	93.9	95.3	95.5	97.6	95.9	93.9	95.8	95.8
	CV	1.18	2.32	2.83	4.70	0.72	1.18	2.32	2.83	4.70	0.74
2008	B 1	82.5	95.6	94.0	98.2	92.5	94.0	95.6	97.0	98.2	96.2
	B 2	98.0	90.1	94.0	98.8	95.2	98.0	90.1	95.0	98.8	95.4
	B 3	92.6	95.2	96.0	98.8	95.6	93.2	95.2	96.0	98.8	95.8
	Average	91.0	93.6	94.6	98.6	94.43	95.0	93.6	96.0	98.6	95.8
	CV	6.19	2.92	2.45	1.19	5.69	5.57	2.31	2.01	1.19	0.32
2009	B 1	93.1	97.2	94.4	89.6	93.5	93.9	97.2	94.4	89.8	93.8
	B 2	82.6	98.2	95.0	97.8	93.4	95.8	98.2	95.0	97.8	96.7
	B 3	96.4	96.0	94.4	92.8	94.9	83.4	96.0	96.2	95.0	92.6
	Average	90.7	97.1	94.6	93.4	93.93	91.0	97.1	95.2	94.2	94.4
	CV	6.59	2.16	1.90	4.72	1.41	5.08	1.85	1.71	3.38	8.89
2010	B 1	91.2	91.4	95.4	96.6	93.6	92.5	91.4	96.2	98.0	94.5
	B 2	92.2	92.0	97.8	98.2	95.0	92.2	92.0	98.6	98.2	95.2
	B 3	96.4	91.6	96.4	93.8	94.5	96.6	91.6	96.4	94.2	94.7
	Average	93.2	91.6	96.5	96.2	94.4	93.7	91.6	97.0	96.8	94.8
	CV	3.01	2.84	2.16	2.78	1.01	2.67	2.22	1.73	2.17	0.26
2011	B 1	95.8	90.8	95.2	96.2	94.5	95.8	90.8	95.2	96.2	94.5
	B 2	96.0	93.0	93.6	96.0	94.6	96.0	93.0	93.6	96.0	94.6
	B 3	96.0	92.0	94.8	95.2	94.5	96.0	92.0	95.8	95.8	94.9
	Average	95.9	91.9	94.5	95.8	94.5	95.9	91.9	94.8	96.0	94.7
	CV	1.34	2.69	1.59	1.62	0.01	1.34	2.48	1.76	1.66	0.09
2007 – 2011	B 1	92.1	93.8	94.5	95.1	93.9	94.8	93.8	95.2	95.7	94.3
	B 2	93.8	93.8	94.5	97.3	94.8	96.2	93.8	95.2	97.3	95.2
	B 3	94.6	94.6	95.2	95.2	94.9	93.1	94.6	95.8	95.8	94.9
	Average	93.6	94.1	94.8	95.9	94.5	94.7	94.1	95.4	96.3	94.8
	CV	3.26	0.43	0.33	3.09	0.67	4.82	0.43	0.24	1.61	0.39

B 1- northern Bačka, B 2- southern Bačka, B 3- western Bačka

In order to apply tests and parametric tests, homogeneity of variance of selected samples was checked by Levene's test (Table 4). Obtained results indicate that samples were not uniform for both properties ($F=4.1093$, $p=0.00$; $F=4.3618$, $p=0.00$) in 2007 and for germination energy ($F=3.3463$, $p=0.0001$) in 2008 and therefore in the process of testing these results the level of significance has to more rigorous, i.e. 1%.

Table 4 Levene`s test

Yers	Test	Energy	Germination
2007	F	4.1093	4.3618
	p-level	0.0000	0.0000
2008	F	3.3463	1.6485
	p-level	0.0001	0.0643
2009	F	2.8835	2.8330
	p-level	0.0005	0.0006
2010	F	2.8417	2.8440
	p-level	0.052	0.053
2011	F	1.5481	2.9763
	p-level	0.052	0.0589

Effects of factors on germination energy and seed germination were determined by the two-factorial analysis of variance (Table 5). The difference in seed germination energy as well as in seed germination was significant over all hybrids in all five years of production. The location, as a second factor, had no statistical significance for neither of observed properties in 2007 (F=1.09, F= 1.89) nor for just germination energy in 2009 (F=1.73). The effect of the interaction of the two factors was observed in the five-year production. The values of F test in all tests point out that the hybrid combination x location interaction is of great significance for differences occurred in seed germination energy and seed germination.

Table 5 Statistical significance of differences in germination energy and seed germination (F and LSD test)

Yers	Test	Energy			Germination		
		H	L	I	H	L	I
2007	F-test	6.68**	1.09	2.47**	8.59**	1.89	3.09
	LSD 0.01	1.456	1.629	3.256	0.976	1.091	2.182
	LSD 0.05	1.917	2.146	4.286	1.185	1.236	2.873
2008	F-test	43.38**	16.01**	26.49**	46.29**	12.17**	27.08**
	LSD 0.01	0.876	0.980	1.960	0.784	0.876	1.756
	LSD 0.05	1.154	1.290	2.580	1.032	1.154	2.308
2009	F-test	23.67**	1.73	5.43**	46.59**	17.31**	17.49**
	LSD 0.01	1.735	1.940	3.881	0.876	0.980	1.960
	LSD 0.05	2.284	2.554	5.108	1.154	1.290	2.580
2010	F-test	16.9**	6.98**	4.69**	23.67**	6.38**	8.07**
	LSD 0.01	1.095	1.224	2.448	0.803	0.898	1.796
	LSD 0.05	1.441	1.611	3.222	1.057	1.182	2.365
2011	F-test	18.68**	5.77**	3.97**	11.39**	5.27**	4.09**
	LSD 0.01	0.784	0.877	1.753	0.784	0.876	1.753
	LSD 0.05	1.032	1.541	2.308	1.032	1.154	2.308

H-hybrid, L-location, I-interaction

Effects of factors on germination energy and seed germination were determined by the two-factorial analysis of variance (Table 5). Observed factors did not have only statistically significant effect on germination energy and seed germination, but also had a very high partial eta-squared value. To which extent will certain factors affect expressing of particular traits depends on the duration of their action (*Rakić et al., 2012*). Therefore, both factors, as well as their interaction, had a great effect on changes in germination energy and seed germination. It has been determined that many factors affected the process of maize seed germination, individually but also in the interactions, but gained results referred to seed as a whole (*Sabovljević et al., 2011*). Stress agroecological conditions, such as drought, are some of factors affecting morphological and physiological traits of seed (Camacho et al., 1994). According to data obtained by Walles and Bressman (cit. *Glamočlija, 2012*), the amount of precipitation necessary to achieve the grain yield of 6.35 t ha-1 is 480-600 mm. Germination and emergence are stages which cannot start without water, hence the minimum soil moisture necessary for seed germination amounts to 305 g/kg, (*Hunter and Erickson, 1952*). According to (*Mayer and Poljakoff-Mayber, 1982*) the amount of absorbed water depends on colloid properties.

In 2007 and 2009, agro-ecological conditions did not statistically significantly affect these traits. The highest partial eta-squared values for germination energy of both factors ($\eta 2$= 0.6196, $\eta 2$=0.4447) were recorded in 2008. Furthermore, the interactions of factors in 2007 significantly contributed to the differences in germination energy of observed hybrids ($\eta 2$=0.79992). The highest partial effect of the hybrid combinations as well as of the interaction of factors on the differences in seed germination was recorded in 2008 ($\eta 2$=0.6343 and $\eta 2$=0.8024, respectively), while in 2009, the location had the highest partial effect ($\eta 2$=0 4639).

Conclusion

Based on studies about effects of agro-ecological conditions and hybrid combinations on germination energy and total germination the following can be concluded that effects of observed factors on the trait expression of maize hybrid seed differ in their extent and modes - they can be immediate and direct, more or less indirect, they can occur through greater or smaller interactions and in a modified mode. Means and variations in traits of maize hybrid seed are of complex nature and are a resultant of effects of all stated factors and all stated ways of their actions. Results obtained by the analysis of variance, F-test values and probabilities of these values indicate that the effect of certain factors on the expression of observed seed traits is not equal for all hybrid combinations. Results obtained by the analysis of effects of factors on the trait expression of maize hybrid seeds show

the major significance of a genotypic or a hybrid combination of these seeds, i.e. of plant traits, as well as of flowering congruence of parental inbreeds in the seed crop.

Acknowledgement

This work is part of project number TR-31006, financed by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Uticaj agroekoloških uslova i hibridne kombinacije na klijavost semena kukuruza

M. Tabaković, Đ. Glamočlija, S. Jovanović, V. Popović, S. Anđelović

Rezime

U ovim istraživanja proučavane su energija klijanja i klijavost semena kukuruza četiri genotipske kombinacije u različitim agroekološkim uslovima uspevanja. Analize hirbidnog semena četiri komercijalne kombinacije Instituta za kukuruz Zemun Polje, proizvedene na tri lokaliteta, urađene su u laboratorijskim uslovima na radnom uzorku 4 x 100 semena. Eksperimentalni podaci obrađeni su na srednju vrednost i ukupnu varijabilnost ($\overline{X}$ i C.V.) za obe osobine semena i svaku varijantu istraživanja.

Ukupna prosečna vrednost energije klijanja u posmatranom periodu bila je 94.5%, a klijavost 94.8%. Koeficijent varijacije za energiju iznosio je 0.67%, a za klijavost 0.39%. Hibridna kombinacija ZP704 u petogodišnjim istraživanjima imala je najviši nivo energije klijanja (95.9%) i ukupne klijavosti (96.3%). Rezultati testova ukazuju da koeficijenti varijacije ispitivanih karakteristika kod obe osobine su mali i ukazuju na homogenost materijala. Dvofaktorijalnom analizom varijanse utvrđen je značajan uticaj faktora (hibrid i lokacija), kao i njihova interakcija na ispitivane osobine semena.

Dobijeni rezultati pokazali su da sva četiri hibrida imaju visok nivo ispoljavanja ispitivanih osobina u promenljivim agroekološkim uslovima, kao i visok uticaj faktora (hibrid i lokalitet) na energiju i klijavost semena.

References

BEKAVAC G., PURAR B., JOCKOVIC DJ. (2008): Relationships between line per se and testcross performance for agronomic traits in two broad-based populations of maize (Article), EUPHYTICA, 162, 3, 363-369

CAMACHO, R.G. AND CARABALLO D.F. (1994): Evaluation of morphological characteristics in Venezuelan maize (*Zea mays* L.) genotypes under drought stress. Science of Agriculture (Piracicaba, Braz.). 51 (3), 453-458

CHASSOT A. (2000): Early growth of roots and shoots of maize as affected by tillage-induced changes in soil physical properties. Zurich. Diss. ETH No. 13907

FENNER, M. AND THOMPSON M. (2005): The ecology of seeds. Cambridge University, UK.

GLAMOCLIJA, Dj. (2012): Especially farming, grain legumes and grains (corn), Faculty of Agriculture, Belgrade, 122-176.

HUNTER, J.R. AND ERICKSON A.E. (1952): Relation of seed germination to soil moisture tension. Agronomy Journal 44, 107-109.

ISTA 1996. ISTA handbook on seed sampling. International Seed Testing association. Zurich, Switzerland.

LEE, E.A., STAEBLER M.A., TOLLENAAR M. (2002): Genetic variation and physiological discrimination for cold tolerance in maize (*Zea Mays* L.) during an early autotrophic phase of development. Crop Sci. 42, 1919–1929

MALETIĆ R., (2005): Statistics. Faculty of Agriculture, Belgrade,

MARIC V., GLAMOCLIJA DJ., POPOVIC V., DJUKANOVIC L. (2013): Maize hybrids from different maturity groups over sowing densities. Institute of PKB Agroekonomik, Padinska Skela, Belgrade, 19, 1-2, 117-124

MAYER A.M. AND A. POLJKOFF-MAYBER (1982): The germination of Seeds. Third edition, Pergamon Press, Great Britain

McDONALD M.B., SULLIVAN J., LAVER M.J. (1994): The pathway of water uptake in maize seeds. Seed Science and Technology, 22, 79-90.

PINNEL E. L. (1949): Genetic and environmental factors affecting seed corn germination at low temperature; J. Am. Loc. Agron., 41, 562-568.

RAKIĆ S., JANKOVIĆ S., KRIVOKAPIĆ M., JOVANOVIĆ R., IKANOVIĆ J. (2012): Grain quality and status of Oats (Avena sativa L.) during storage. Biotechnology in Animal Husbandry 28 (4), 863-871.

ROSIC K. (1959): The influence of certain fractions of the seeds develop, the growth and yield of maize. Hybrid corn Yugoslavia, 9, 3-17.

SABOVLJEVIĆ R., SIMIĆ D., MILOSAVLJEVIĆ S., ACIMOVIĆ B., GORANOVIĆ DJ., SELAKOVIĆ D. (2011): Effect calibration properties of hybrid seed corn, Institute of PKB Agroekonomik, Belgrade, vol. 17. No. 1-2, 81-100.

SHIEN W.J. AND McDONALD B.M. (1982): The influence of seed size, shape and treatment on inbred seed corn quality. Seed Sci Techno., 10, 307-313.
SIKDER S., HASAN M.A. AND HOSSAIN M.S. (2009): Germination Characteristics and Mobilization of Seed Reserves in Maize Varieties as Influenced by Temperature Regimes. Journal of Agriculture and Rural Development, No 7 (1&2), 51-56
STIKIĆ, R. (2005): Physiology of growth and development of plants, Faculty of Agriculture, University of Belgrade.
STOJAKOVIC, M., JOCKOVIC Dj., BEKAVAC G., PURAR, B. NASTASIC A. (1999): Variability and interrelationships between traits of two maize populations. Acta Agronomica Hungarica, Vol.47, (4), 383-390.
STONE, P. J., SORENSEN I.B, JAMIESON P.D. (1999): Effect of soil temperature on phenology, canopy development, biomass and yield of maize in a cool temperature climate. Field Crop. Res. 63, 169–178.
UJEVIĆ, A.I., KOVACEVIĆ J. (1972), Seed Testing. Department of seeds, Zagreb.
ZIVANOVIC Lj., IKANOVIC J., POPOVIC V., KAJGANA M., RAKIC S., MILUTINOVIC M. (2012): The effect of nitrogen fertilization on yield of maize. „Agrosym Jahorina“, 215-219.
*OFFICIAL GAZETTE OF RS, issue 60/2006: Regulation on control of the seed production, the content and the method of keeping records on production of seedlings of agricultural crops and the form on the report on the production of mycelia of edible and medicinal fungi.
* OFFICIAL GAZETTE OF RS, issue 47/87: Regulation on the quality of seeds of agricultural plants

Received 27 May 2013; accepted for publication 25 September 2013

Biotechnology in Animal Husbandry 29 (4), p 727-740 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'636.592
DOI: 10.2298/ BAH1304727W

REVIEW OF THE PHYSIOLOGICAL AND PATHOLOGICAL WELFARE INDICATORS APPLIED IN TURKEYS (*MELEAGRIS GALLOPAVO*)

T.T.N. Watanabe, S. Lolli, L. Ferrari, V. Ferrante

Università di Milano, Department of Veterinary Science and Public Health, Via Celoria 10, Milan 20133, Italy
Corresponding author: valentina.ferrante@unimi.it
Communication

Abstract: Concern on animal welfare has become an important issue in Europe for a decade now. In commercial poultry husbandry, there are many trials to standardize the production in order to reduce the economic loss caused by poor welfare at marketing age. As it known, factors such as density, group size, space availability, maturation, lighting, feeding, and transportation can have effects on welfare of turkeys. However, to ensure a better quality of live for the birds as well as the industry as good performance, reducing the mortality and condemnations it is important to have another point of view as different kind of indicators. This paper reviews the available scientific literature related to the turkeys' welfare according to the main relevant physiopathology indicators by taking into a count whether they are feasible or not for being used. We addressed foot pad dermatitis and breast skin lesions as being the most relevant indicators so far. They may be relevant to improve the welfare assessment indicators of turkeys. However, measurements of corticosterone, enzyme activities, cytokines, and hematological profile seemed to be flourishing indicators to be applied more often. In this way associating the indicators that were previously studied to these new ones, it is assumed that animal, producer, industry and consumer may have a suitable bond between them (poultry chain) according to their different interests.

Keywords: welfare, pathological and physiological indicator, turkey

Introduction

For many years, commercial turkey husbandry has been practiced in many countries. In order to increase its profitability, and meet the consumers' demands, research is conducted and regulations are put in place. In poultry chain production, welfare concerns were already pointed out (*Voris et al., 1998; Bessei, 2006; Department for Environment Food and Rural Affairs, 2009; Welfare Quality®,*

2009; Beaumont et al., 2010); nonetheless, causes of downgrades and condemnations are still the industries' main worry (*Petracci et al., 2006; Shepherd and Fairchild, 2010*).

Studies have been developed regarding better management factors influencing domestic turkey flocks, such as diet (*Hocking et al., 1999; Mirabito et al., 2003*), light (*Hart et al., 1999; Sherwin et al., 1999a; Moinard et al., 2001*), animal stocking density and group size (*Martrenchar et al., 1999; Buchwalder and Huber-Eicher, 2004, 2005a*), environmental enrichment (*Sherwin et al., 1999b; Martrenchar et al., 2001)*, and transportation (*Wichman et al., 2010*). Thus, turkey behavior could be modified by variations in such factors (*Hughes and Grigor, 1996; Sherwin and Kelland, 1998; Buchwalder and Huber-Eicher, 2003*) while welfare issues could be accomplished (*Martrenchar, 1999*). The effect of social and rearing conditions on the behavior of turkeys has recently been reviewed by *Marchewka et al. (2013).*

The solution to what is still being considered as a problematic welfare issue will be found by dual-way cooperation between consumers, farms and the complex animal production-slaughterhouse industries (*Waiblinger et al., 2006; Humane Farm Animal Care, 2008*).

In broiler chickens, among the welfare issues, high susceptibility to metabolic disorders and low locomotor activity were considered the most important (*Bessei, 2006*). It is inferred that similar issues are seen in turkeys.

Using well-defined indicators to assess welfare is extremely important in order to enhance their welfare evaluation. In this case, changes in behavior, haematological profile, plasma hormones concentration, immune measures and enzymatic activities could be mentioned (*Duncan, 1981; Hocking et al., 1999*).

The purpose of this review was to analyze, based on the existing literature, the physiological and pathological indicators that could be associated with welfare in turkey production. Therefore, improvement of the birds' health status, carcass quality, management, economic issues, and providing satisfactory welfare, would lead to better conditions for turkey production.

Pathological Indicators

Foot pad Dermatitis

Foot pad dermatitis (FPD) is defined as a necrotic lesion and inflammation of the foot pad. The main concern is that it could be the route of entry for other microorganisms potentially leading to the appearance of new diseases (*Shepherd and Fairchild, 2010*).

Lesions related to FPD are important not only for food safety and product downgrading, but also for animal welfare; therefore, decreasing them has been considered one of the main goal for the poultry industries (*Shepherd and Fairchild,*

2010). In addition, FPD was classified as the key welfare indicator in the United Kingdom in turkey production (*Clark et al., 2002*), when considering the criteria of welfare assessments in Europe and the United States (*National Chicken Council, 2010; Shepherd and Fairchild, 2010*).

Severe FPD in commercial turkey flocks are common lesions, whereas, they are unlikely in broilers (*Clark et al., 2002*). In addition, poor litter moisture seemed to be a good example of management deficiencies and the most likely cause of the FPD in turkeys; consequently, decreasing animal welfare (*Mayne et al., 2004; Shepherd and Fairchild, 2010*).

These kinds of lesions in female turkeys were more severe than males during the fattening period according to *Krautwald-Junghanns et al. (2011),* even though Clark et al. (2002) reported higher frequency in males. It could be associated with the differences in animal density in different countries, (e.g. in Germany, the turkey's density for males is approximately 2.8 birds/m^2, while for females is approximately 5.1 birds/m^2). Nevertheless, the severity of these pathological lesions is associated with other flock management factors such as litter moisture. Moreover, *Grosse Liesner (2007)* stated that genetic predisposition is also an important element of FPD.

Mayne et al. (2007b) demonstrated that FPD could be most likely associated with a rapid inflammatory response, rather than an allergic response to an environmental stimulus in the litter.

Wu and Hocking (2011) conducted an experiment with female growing turkeys in order to observe the effects of litter and animal age on FPD. The most important conclusion was that high moisture litter is the main cause of FPD, which affected the severity and the prevalence of these lesions (*Mayne et al., 2007a; Youssef et al., 2011*). Accordingly, short exposure to wet litter (4h/day; *Abd El-Wahab et al., 2012*; 8h/day; *Youssef et al., 2010*) could be enough for the development and marked increase in severity of FPD.

Thus, to reduce FPD in the flock, good litter management is essential to maintain the level of moisture under the 30% (*Wu and Hocking, 2011*); this result may be reached by using floor heating, or even providing soft litter such as lignocelluloses rather than wood shavings (*Abd El-Wahab et al., 2011*). Furthermore, there is no significant difference in litter pH between different bird groups, suggesting no relation between organic material and FPD, such as bird's droppings and bedding material (*Wu and Hocking, 2011*). On the other hand, according to *Abd El-Wahab et al. (2013)*, when the electrolyte in the diet changes, mainly the sodium/ potassium rate, indirectly interferes with the litter quality due to the increase of water intake and the increases of the excreta moisture, causing the occurrence of the 'wet litter conditions'.

Breast Skin Lesions

Breast skin lesions such as breast buttons, blisters, and purulent bursitis observed in conventional intensive turkey farming are still considered as the most frequent problems and as a result, they are one of the main reason of economic loss (*Krautwald-Junghanns et al., 2009*).

Mitterer-Istyagin et al. (2011) found the prevalence of these alterations in turkey tom than in turkey hen flocks. Although the origin is not clear and probably multifactorial, it was possible to infer that the differences between fattening period regarding body weight and the laying time may be the cause. They also conclude that sex-related influences and age status were important reasons for these results, but could also be determinate by breeding, litter moisture, and management quality.

Muscular and Skeletal Lesions

As occurs in broilers, turkeys also have skeletal abnormalities associated with higher body weight (*Wyers et al., 1991*). In this case, tibial dyschondroplasia (TD) has been described as the cause of enormous economic losses and also an animal welfare problem (*Pines et al., 2005; Tatara et al., 2006*). According to *Hocking et al. (2002),* there is no predisposition for this disease related to diet with higher or lower calcium: phosphorus ratios. Furthermore, the turkeys affected by TD have not been observed to have any locomotion problems as occurs in broilers (*Simsa et al., 2007*). Therefore, the welfare issue of TD could in this case be primarily related to location on the body where osteomyelitis may develop (*Wyers et al., 1991*). Additionally, *Tatara et al. (2006)* discovered the beneficial effects of orally administrating ornithine alpha-ketoglutarate in turkeys with skeletal disorders, which resulted in increased amino acid synthesis. It was shown that the quality of the bone is improved by higher bone mineral density of trabecular and cortical bone as well as the maximum elastic and ultimate strengths were increased.

Hocking et al. (2005) evaluated the efficacy and optimum doses of non-steroidal anti-inflammatory drugs in domestic fowl suffering from articular pain. This model could be useful when applied to different avian species such as turkeys, due to the lack of studies presently available.

In order to demonstrate the efficiency of treatments using analgesics such as synthetic opioids (butorphanol) and anti-inflammatory steroids (betamethasone) in adult turkeys with degenerative join disorders, studies were carried out with regarding locomotion, spontaneous and sexual activities (*Duncan et al., 1991; Buchwalder and Huber-Eicher, 2005b*). It was demonstrated that in all the treatment groups, an increase in the natural function activities was observed.

Physiological Indicators

Corticosteroid Evaluation

It is known that stress events which could be defined as a trigger or stressor that causes a stress response, such as catching and transport of live turkeys (*Marchewka et al., 2013*), may affect animal behavior, decrease the immune system when fighting disease and change population performance (*Korte, 2001; Shini et al., 2010*).

Glucocorticoid hormones are synthesized and released through activation of the hypothalamic-pituitary-adrenal axis complex. Conventionally, it is possible to evaluate glucocorticoid levels in the blood; however the components only remain in circulation for a short period of time (minutes). Nonetheless, another way of measuring them is by analyzing animal feathers, which has been used in order to determine more chronic stress experience indicators (activity durations of the hormone is days-to-weeks) (*Bortolotti et al., 2009*). In addition, corticosterone (CORT) is the main avian glucocorticoid that could be quantified by feather analysis (*Botolotti et al., 2008*).

According to *Botolotti et al. (2009),* CORT is a stable hormone in feathers that could be used individually or in the flock, which gives the information about the time when the stress event happened and how the bird responded. It is a non-invasive and feasible method (*Botolotti et al., 2008*). Furthermore, this analysis used samples that were collected over many years and stored by taping the calamus to a sheet of paper in a binder kept at room temperature. Therefore, it is a good way to track stress, which is one of the most important factors that influences animal welfare. However, *Lattin et al. (2011)* reported that caution is necessary in the interpretation of CORT results extracted from feathers, due to the effect of the sample and effectiveness of the antibodies used in the feather assay.

Hocking et al. (1999) demonstrated that food restriction in commercially turkeys' production causes increase of plasma CORT during the rearing period, even if the traditional birds at 4 and 8 weeks of age have a relative high levels of this hormone.

Corticosterone metabolites' levels in faeces-urine also could be evaluated, however, many limitations have been discussed, for instance, artifacts caused by sample age, storage and transportation, diet, captivity and biological status (*Millspaugh and Washburn, 2004; Tempel and Gutiérrez, 2004; Möstl et al., 2005; Cabezas et al., 2007; Hayward et al., 2010*).

Stoyanchev et al. (2007) gathered accurate data about natural humoral immunity in turkeys, which had muscular dystrophy, reared under conditions of poor welfare and stress. In general, lysozyme concentrations (LC) were higher in sick animals than in healthier ones due to the body attempt to go through the

disease. Nevertheless, under stress challenging the blood serum LC were decreased due to the presence of cortisol.

Furthermore, *Franciosini et al., (2011)* showed LC was lower in broiler turkeys reared in the backyard group (pen measuring 6 m long X 4 m wide that were subdivided into 2 connected spaces, one of which opened) when compared with industrial (13,500 birds with natural light and ventilation) and experimental flocks (optimized light, ventilation, temperature and density according to *Anonymous, 2000*). These results suggest that stress situations possibly caused by predators could be a reason for those findings as well as weather conditions for instance. Moreover, there are difference between different rearing systems and natural immune parameters.

Cytokines belong in many ways of the immune response path and are important in leucocytes development, and their roles. Interleukins (IL) are a type of cytokines that are produced by leucocytes and influence other leucocytes (Snyder, 2007).

Shini et al. (2010) carried out an experimental study in chickens regarding the effects on the expressions of the proinflammatory cytokines (e.g. IL-1β, IL-6) and chemokines (CC) (e.g. CCLi1, CCLi2) of leucocytes and heterophils under CORT oral administration. During a chronic treatment of CORT (1 week), there were a down regulation of cytokines and CC which suggest that there was suppression in the immune response. However, expositing birds to acute stress (until 24h) can cause the increase of immune system. They cited as well that IL and CC could be important markers in order to assess the influence of stress factors in their immune system.

Wu et al. (2007, 2008) reported that IL-1β and IL-8 proteins in chicken, duck, goose, turkey and pigeon have significantly structural and functional homology, which could be used as adjuvant in vaccine for all these species. It is considered as an important tool regarding to modulating the immune system.

Hematological Profile

According to *Maxwell (1993),* the increase of heterophil to lymphocytes ratio (HLR) and basophils are well-known variables indicating stress, such as heat and incorrect transportation. However, there was no evidence of the HLR changes by food restriction in turkeys (*Hocking et al., 1999)*, neither with the crate height during short-term confinement (*Wichman et al., 2010*).

HLR has been used for many years as the method to evaluate the stress, such as, in birds. When the immune system releases CORT, which elevates its blood concentration, afterwards, there is an increase of nonlymphocytes leukocytes (heterophil) and decrease of lymphoid leucocytes (lymphocytes), thus the HLR changing occurs (*Shini et al., 2010*). However, understanding of these mechanisms according to the molecular point of view should be more detailed. It was observed

that during an exogenous application of CORT in 7-wk-old chicken experiment, the HLR markedly increased during 1h, 3h, and 24h post administration, which can help the innate immune response (*Shini et al., 2010*).

Huff et al. (2005) compared the effects of 2 different stressors (such as transport and dexamethasone treatments) on the measure of HLR and resistance to *Escherichia coli*, from 3 different turkeys' genetic lines, (according to their rate of growth). Their data supported the concept of lighter and slower growing line birds are more resistance to stress. In addition, HLR were increased in both stressors that were used.

Proportion of basophiles seemed to have no changes into its values during food-restrict event (*Hocking et al., 1999*).

Activities of Plasma Enzymes

Creatine kinase (CK), aspartate aminotransferase (ASAT), alkaline phosphatise (APL) and lactate are some examples for physiological measures of animal welfare which could be considered as turkey welfare indicators.

Wichman et al. (2010) showed that there was no difference between the size of crate and the activity of CK or ASAT; however, frequently changes in turkey behavior were noticed. Moreover, lactate levels were significant lower with male birds in the 55cm crates than in 40cm.

Hocking et al. (1999) observed that the activity of lactate dehydrogenase (LDH) in turkeys from 12 to 24 weeks of age was higher in male turkeys that were fed *ad libitum*. In addition, the performance of ASAT was similar to one found in LDH. However, APL was inversely of the LDH.

Conclusion

In summary, it may be concluded from this review that foot pad dermatitis (FPD), breast skin alterations, corticosterone measure analyses, immune measures (e.g. cytokines and chemokines), hematological profile, and enzyme activities (e.g. creatine kinase, aspartate aminotransferase, alkaline phosphatase and lactate) are suitable indicators of the welfare in the turkey rearing methods and in agreement with the bird protection; hence, the fittest welfare protocol using theses indicator should be built and applied. On the contrary, it is important to notice the real feasibility of those indicators. The aims should be well-understanding of their usability in order to select the proper indicators for the assessment of the turkey welfare.

The proper welfare assessment cannot be done considering solely each factor as singular; it should be evaluated based on the factors that are also involved, such as the rearing methods for turkeys, management, and breeding.

The FPD and breast blisters are for now considered the most practical welfare indicators in turkeys regarding the feasibility for collecting this data scoring. Furthermore, it has a great importance if consider how practical, no time consuming and reliable welfare indicator(s) could be applied on the complex farm-slaughterhouse. Therefore, the real welfare picture could be defined.

Further studies are needed in order to obtain the useful tools in order to figure out which is the best way to deal with these challenges: intensive animal production chain versus poultry protections concerns. Perhaps, the main scope is the equilibrium between the research, farms, industries and consumers.

Acknowledgements

The authors are grateful to Leigh Murray for providing comments and English revision on the manuscript.

Pregled fizioloških i patološkim indikatora dobrobiti ćurki (*Meleagris gallopavo*)

T.T.N. Watanabe, S. Lolli, L. Ferari, V Ferrante

Rezime

Pitanje dobrobiti životinja je preko jedne decenije jedno od važnijih pitanja u Evropi. U komercijalnom živinarstvu, bilo je mnogo pokušaja da se standardizuje proizvodnja u cilju smanjenja ekonomskih gubitaka izazvanih lošom dobrobiti u uzrastu kada se živina plasira na tržište. Kao što je poznato, faktori kao što su gustina naseljenosti, veličina grupe, dostupnost prostora, sazrevanje, osvetljenje, ishrana i prevoz, mogu imati uticaj na dobrobit ćurki. Međutim, da bi se obezbedio bolji kvalitet života za ptice, kao i za industriju dobre performanse, smanjenje smrtnosti i gubitaka, važno je imati još jednu tačku gledišta kao različite vrste indikatora. Ovaj rad razmatra raspoloživu naučnu literaturu koja se odnosi na dobrobit ćurki "u skladu sa glavnim relevantnim fizičko-patološkim pokazateljima uzimajući u obzir da li su oni izvodljivi ili ne. U radu je pažnja usmerena na dermatitis nogu i lezije kože grudi kao najrelevantnije indikatori do sada. Oni mogu biti od značaja za poboljšanje indikatora ocenjivanja dobrobiti ćurki. Međutim, merenja kortikosterona, aktivnosti enzima, citokina i hematološki profil su indikatori za koje se čini da će se u budućnosti češće primenjivati. Na ovaj način, povezivanjem indikatora koji su ranije ispitivani sa novim, pretpostavlja se da će postojati odgovarajuću vezu između životinja, odgajivača, industrije i potrošača (lanac proizvodnje živine) u skladu sa njihovim različitim interesima.

References

ABD EL-WAHAB A., BEINEKE A., BEYERBACH M., VISSCHER C.F., KAMPHUES J. (2011): Effects of floor heating and quality on the development and severity of foot pad dermatitis in young turkeys. Avian Dis., 55, 429-434.
ABD EL-WAHAB A., VISSCHER C.F., BEINEKE A., BEYERBACH M., KAMPHUES J. (2012): Experimental studies on the effects of different litter moisture contents and exposure time to wet litter on development and severity of foot pad dermatitis in young fattening turkeys. Arch. Geflugelkd., 76, 55-62.
ABD EL-WAHAB A., VISSCHER C.F., BEINEKE A., BEYERBACH M., KAMPHUES J. (2013): Effects of high electrolyte contents in the diet and using floor heating on development and severity of foot pad dermatitis in young turkeys. J. Anim. Physiol. Anim. Nutr., 97, 39-47.
ANONYMOUS. (2000): Manual British United Turkeys. Warren Hall, Chester, UK.
BEAUMONT C., LEBIHAN-DUVAL E., MIGNON-GRASTEAU S., LETERRIER C. (2010): The European experience in poultry welfare - A decade ahead. Poult. Sci., 89, 825-831.
BESSEI W. (2006): Welfare of broilers: a review. World's Poult. Sci. J., 62, 455-466.
BORTOLOTTI G.R., MARCHANT T.A., BLAS J., CABEZAS S. (2009): Tracking stress: localisation, deposition and stability of corticosterone in feathers. J. Exp. Biol., 212, 1477-1482.
BORTOLOTTI G.R., MARCHANT T., BLAS J., GERMAN T. (2008): Corticosterone in feathers in a long-term, integrated measure of avian stress physiology. Funct. Ecol., 22, 494-500.
BUCHWALDER T., HUBER-EICHER B.V. (2003): A brief report on aggressive interactions within and between groups of domestic turkeys (*Meleagris gallopavo*). Appl. Anim. Behav. Sci., 84, 75-80.
BUCHWALDER T., HUBER-EICHER B. (2004): Effect of increased floor space on aggressive behaviour in male turkeys (*Meleagris gallopavo*). Appl. Anim. Behav. Sci., 89, 207-214.
BUCHWALDER T., HUBER-EICHER B. (2005a): Effect of group size on aggressive reactions to an introduced conspecific in groups of domestic turkeys (*Meleagris gallopavo*). Appl. Anim. Behav. Sci., 93, 251-258.
BUCHWALDER T., HUBER-EICHER B. (2005b): Effect of the analgesic butorphanol on activity behaviour in turkeys (*Meleagris gallopavo*). Res. Vet. Sci., 79, 239-244.
CABEZAS S., BLAS J., MARCHANT T.A., MORENO S. (2007): Physiological stress levels predict survival probabilities in wild rabbits. Horm. Behav., 51, 313-320.

CLARK S., HANSEN G., MCLEAN P., BOND P.J.R., WAKEMAN W., MEADOWS R., BUDA S. (2002): Pododermatitis in turkeys. Avian Dis., 46, 1038-1044.

DEPARTMENT FOR ENVIRONMENT FOOD AND RURAL AFFAIRS, LONDON, UK. (2009): Codes of Recommendations for the Welfare of Livestock: Turkeys.
http://archive.defra.gov.uk/foodfarm/farmanimal/welfare/onfarm/othersps/turkcode.htm. Accessed Sep. 2012.

DUNCAN I.J.H. (1981): Animal rights-animal welfare: a scientist's assessment. Poult. Sci., 60, 429-499.

DUNCAN, I.J.H., BEATTY E.R., HOCKING P.M., DUFF S.R.I. (1991): Assessment of pain associated with degenerative hip disorders in adult male turkeys. Res. Vet. Sci., 50, 200-203.

FRANCIOSINI M.P., BIETTA A., MOSCATI L., BATTISTACCI L., PELA M.,. TACCONI G, DAVIDSON I., PROIETTI P.CASAGRANDE. (2011): Influence of different rearing systems on natural immune parameters in broilers turkeys. Poult. Sci., 90, 1462-1466.

GROSSE LIESNER, B.B. (2007): Comparative investigations on the performance and the appearance (incidence and manner) of primarily noninfectious health problems in male fattening turkeys of five different strains. Doctoral Diss. University of Veterinary Medicine, Hannover, Germany.

HART N.S., PARTRIDGE J.C., CUTHILL I.C. (1999): Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey (*Meleagris gallopavo*). Vision Res., 39, 3321-3328.

HAYWARD L.S., BOOTH R.K., WASSER S.K. (2010): Eliminating the artificial effect of sample mass on avian fecal hormone metabolite concentration. Gen. Com. Endocr., 169,117-122.

HUMANE FARM ANIMAL CARE. (2008): Animal Care Standards for Turkeys. http://www.certifiedhumane.org/uploads/pdf/Standards/English/Std08.Turkeys.3J.pdf Accessed Sep. 2012.

HOCKING P.M., MAXWELL M.H., MITCHELL M.A. (1999): Welfare of food restricted male and female turkeys. Br. Poult. Sci., 40, 19-29.

HOCKING P.M., ROBERTSON G.W., GENTLE M.J. (2005): Effects of non-steroidal anti-inflammatory drugs on pain-related behaviour in a model of articular pain in the domestic fowl. Res. Vet. Sci., 78, 69-75.

HOCKING P.M., WILSON S., DICK L., DUNN L.N., ROBERTSON G.W., NIXEY C. (2002): Role of dietary calcium and available phosphorus in the aetiology of tibial dyschondroplasia in growing turkeys. Br. Poult. Sci., 43, 432-441.

HUFF G.R., HUFF W.E., BALOG J.M., RATH N.C., ANTHONY N.B., NESTOR K.E. (2005): Stress response differences and disease susceptibility reflected by

heterophil to lymphocyte ratio in turkeys selected for increased body weight. Poult. Sci., 84, 709-717.
HUGHES B.O., GRIGOR P.N. (1996): Behavioural time-budgets and beak related behaviour in floor-housed turkeys. Anim. Welf., 5, 189-198.
KORTE S.M. (2001): Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav. Rev., 25(2), 117-1142.
KRAUTWALD-JUNGHANNS M.E., ELLERICH R., BOHME J., CRAMER K., DELLAVOLPE A., MITTERER-ISTYAGIN H., LUDEWIG M., FEHLHABER K., SCHUSTER E., BERK J., ALDEHOFF D., FULHORST D., KRUSE W., DRESSEL A., NOACK U., BARTELS T. (2009): Examination of rearing standards and health status in turkeys in Germany. Berl. Munch. Tierarztl. Wochenschr., 122, 271-283.
KRAUTWALD-JUNGHANNS M.E., ELLERICH R., MITTERER-ISTYAGIN H., LUDEWIG M., FEHLHABER K., SCHUSTER E., BERK J., PETERMANN S., BARTELS T. (2011): Examination on the prevalence of footpad lesions and breast skin lesions in British United Turkeys Big 6 fattening turkeys in Germany. Part I: Prevalence of footpad lesions. Poult. Sci., 90, 555-560.
LATTIN C.R., REED J.M., DESROCHERS D.W., ROMERO L.M. (2011): Elevated corticosterone in feathers correlates with corticosterone-induced decreased feather quality: a validation study. J. Avian Biol., 42, 247-252.
MARCHEWKA J., WATANABE T.T.N., FERRANTE V., ESTEVEZ I. (2013): Review of the social and environmental factors affecting the behavior and welfare of turkeys (*Meleagris gallopavo*). Poult. Sci., 92, 1467:1473.
MARTRENCHAR A. (1999): Animal welfare and intensive production of turkey broilers. World's Poult. Sci. J., 55, 143-152.
MARTRENCHAR A., HUONNIC D., COTTE J.P., BOILLETOT E., MORISSE J.P. (1999): Influence of stocking density on behavioural, health and productivity traits of turkeys in large flocks. Br. Poult. Sci., 40, 323-331.
MARTRENCHAR A., HUONNIC D., COTTE J.P. (2001): Influence of environmental enrichment on injurious peacking and perching behaviour in young turkeys. Br. Poult. Sci., 42, 161-170.
MAXWELL M. H. (1993): Avian leucocyte responses to stress. World's Poult. Sci. J., 49 34-43.
MAYNE R.K., ELSE R.W., HOCKING P.M. (2004): Histopathological changes that occur in the turkey footpad as a result of wet and dry litter substrates. Br. Poult. Sci., 45 (Suppl. 1), 41-42.
MAYNE R.K., ELSE R.W., HOCKING P.M. (2007a): High litter moisture alone is sufficient to cause footpad dermatitis in growing turkeys. Br. Poult. Sci., 48, 538-545.
MAYNE R.K., POWELL F., ELSE R.W., KAISER P., HOCKING P.M. (2007b): Foot pad dermatitis in growing turkeys is associated with cytokine and cellular

changes indicative of an inflammatory immune response. Avian Pathol., 36(6), 453-459.
MILLSPAUGH J.J., WASHBURN B.E. (2004): Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol., 138, 189-199.
MIRABITO L., ANDRE L., BOUVAREL I. (2003): Effect of providing 'whole wheat' in the diet on pecking behaviour in turkeys. Spring meeting of the WPSA French branch meeting abstracts. p. 776-778. (Abstr.)
MITTERER-ISTYAGIN H., LUDEWIG M., BARTELS T., KRAUTWALD-JUNGHANNS M.-E., ELLERICH R., SCHUSTER E., BERK J., PETERMANN S., FEHLHABER K. (2011): Examinations on the prevalence of footpad lesions and breast skin lesions in B.U.T. 6 fattening turkeys in Germany. Part II: Prevalence of breast skin lesions (breast buttons and breast blisters). Poult. Sci., 90, 775-780.
MOINARD C., LEWIS P.D., PERRY G.C., SHERWIN C.M. (2001): The effects of light intensity and light source on injuries due to pecking of male domestic turkeys (*Meleagris gallopavo*). Anim. Welf., 10, 131-139.
MÖSTL E., RETTENBACHER S., PALME R. (2005): Measurement of corticosterone metabolities in bird's droppings: An analytical approach. Ann. N.Y. Acad. Sci., 1046, 17-34.
NATIONAL CHICKEN COUNCIL. (2010): National Chicken Council Animal Welfare Guidelines and Audit Checklist. http://www.nationalchickencouncil.org/wp-content/uploads/2012/01/NCC-Animal-Welfare-Guidelines-2010-Revision-BROILERS.pdf Accessed Sep. 2012.
PETRACCI M., BIANCHI M., CAVANI C., GASPARI P., LAVAZZA A. (2006): Preslaughter mortality in broiler chicken, turkeys, and spent hens under commercial slaughtering. Poult. Sci., 85, 1660-1664.
PINES M., HASDAI A., MONSONEGO-ORNAN E. (2005): Tibial dyschondroplasia-tools, new insights and future prospects. World's Poult. Sci. J., 61, 285-297.
SHEPHERD E.M., FAIRCHILD B.D. (2010): Footpad dermatitis in poultry. Poult Sci., 89, 2043-2051.
SHERWIN C.M., KELLAND A. (1998): Time-budgets, comfort behaviours and injurious pecking of turkeys housed in pairs. Br. Poult. Sci., 39, 325-332.
SHERWIN C.M., LEWIS P.D., PERRY G.C. (1999a): Effects of environmental enrichment, fluorescent and intermittent lighting on injurious pecking amongst male turkey poults. Br. Poult. Sci., 40, 592-598.
SHERWIN C.M., LEWIS P.D., PERRY G.C. (1999b): The effects of environmental enrichment and intermittent lighting on the behaviour and welfare of male domestic turkeys. Appl. Anim. Behav. Sci., 62, 319-333.

SHINI S., HUFF G.R., SHINI A., KAISER P. (2010): Understanding stress-induced immunosuppression: Exploration of cytokine and chemokine gene profiles in chicken peripheral leukocytes. Poult. Sci., 89, 841-851.
SIMSA S., HASDAI A., DAN H., ORNAN E.M. (2007): Induction of tibial dyschondroplasia in turkeys by tetramethylthiuram disulfide (Thiram). Poult. Sci., 86, 1766-1771.
SNYDER P.W. (2007): Diseases of immunity. Pages 193-251 in Pathologic Basis of Veterinary Disease. M. D. McGavin and J.F. Zachary, eds. 4th ed. Mosby Elsevier, St Louis.
STOYANCHEV K., SOTIROV L., STOYANCHEV T., LALEV M., YARKOV D., YOTOVA I. (2007): Study on the level of natural humoral immunity in turkey-broilers with muscular dystrophy, reared under conditions of either animal welfare or stress. Rev. Med. Vet., 158 (6), 314-319.
TATARA M.R., ŚLIWA E., KRUPSKI W., BRODZKI A., PASTERNAK K. (2006): Ornithine alpha-ketoglutarate increases mineralization and mechanical properties of tibia in turkeys. Bone, 39, 100-105.
TEMPEL D.J., GUTIÉRREZ R.J. (2004): Factors related to fecal corticosterone levels in California Spotted Owls: Implications for assessing chronic stress. Conserv. Biol., 18 (2), 538-547.
VORIS J.C., MACMARTIN D., BRADLEY F. (1998): Animal Care Series: Turkey Care Practices. Stull C, ed. 2nd ed. University of California Cooperative Extension Service, Davis, CA.
WAIBLINGER S., BOIVIN X., PEDERSEN V., TOSI M.V., JANCZAK A.M., VISSER E.K., JONES R.B. (2006): Assessing the human-animal relationship in farmed species: A critical review. Appl. Anim. Behav. Sci., 101, 185-242.
WELFARE QUALITY (2009): Welfare Quality assessment protocol for poultry (broilers, laying hens). Welfare Quality Consortium, Lelystad, The Netherlands.
WICHMAN A., NORRING M., PASTELL M., ALGERS B., PÖSÖ R., VALROS A., SALONIEMI H., HÄNNINEN L. (2010): Effect of crate height during short-term confinement on the welfare and behaviour of turkeys. Appl. Anim. Behav. Sci., 126, 134-139.
WU Y.F., LIU H.J., CHIOU S.H., LEE L.H. (2007): Sequence and phylogenetic analysis of interleukin (IL)-1β-encoding genes of five avian species and structural and functional homology among these IL-1β proteins. Vet. Immunol. Immunopathol., 116, 37-46.
WU Y.F., SHIEN J.H., YIN H.H., CHIOUW S.H., LEE L.H. (2008): Structural and functional homology among chicken, duck, goose, turkey and pigeon interleukin-8 proteins. Vet. Immunol. Immunop., 125, 205-215.
WU K., HOCKING P.M. (2011): Turkeys are equally susceptible to foot pad dermatitis from 1 to 10 weeks of age and foot pad scores were minimized when litter moisture was less than 30%. Poult. Sci., 90, 1170-1178.

WYERS M., CHEREL Y., PLASSIART G. (1991): Late clinical expression of lameness related to associated osteomyelitis and tibial dyschondroplasia in male breeding turkeys. Avian Dis., 35, 408-414.
YOUSSEF I.M.I., BEINEKE A., ROHN K., KAMPHUES J. (2011): Effects of litter quality (moisture, ammonia, uric acid) on development and severity of foot pad dermatitis in growing turkeys. Avian Dis., 55, 51-58.

Received 10 December 2013; accepted for publication 25 December 2013

Biotechnology in Animal Husbandry 29 (4), p 741-749 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 619
DOI: 10.2298/ BAH1304741V

IMPORTANCE AND APPLICATION OF MARKETING IN SMALL ANIMAL PRACTICE

V. Vidić, S.Savić, B.Vidić, Ž.Grgić

Scientific Veterinary Institute "Novi Sad", Novi Sad, Republic of Serbia
Corresponding autor: vaso@niv.ns.ac.rs
Communication

Abstract: The main goals of marketing are the following: firstly, to attract new customers by emphasizing the potential value of the product or service offered and secondly, to keep the existing customers by keeping them satisfied with the product or service by constantly producing superior value and exceeding their ***expectations.*** Contrary to the U.S.A., where greatest achievements were accomplished in the field of marketing theory and application and its implementation into veterinary practice, as well as in appropriate perception of importance of marketing idea and philosophy, this issue has not been appropriately addressed in our country. The main goal of marketing in veterinary practice is not only to sell a product or service, but also to create mutual profitable long-term relations with clients. Disregarding personal reasons for practicing veterinary medicine, particularly in small-animal practice, the veterinarian should approach such practice as any other business activity that should provide profits. In that respect, it is of key importance to adopt marketing approach and management concept. The four basic marketing instruments are product, price, promotion and place that make so-called *marketing mix*, which is applicable also in the veterinary practice. In veterinary medicine, the product pertains to spent items (e.g. drugs, food) or veterinary services. By determining the price, the basis is the cost price and added profit margin, depending on the product or service offered. The promotion encompasses communication with the owners, presenting them products and services offered, describing the features of the clinic or infirmary, etc. Contrary to other business branches, position of veterinary unit is not so important, yet it must provide good communication with animal owner. The overall experience of the customer, starting from entering the clinic / infirmary until leaving it, must be professional, pleasant and effective. Only accomplishing this goal will enable competitiveness or justify even a higher price for a superior service.

Key words: marketing, marketing mix, veterinary services

Introduction

Marketing as a term has developed from an English word "market". Most of the people would say that marketing is advertizing or selling, but marketing is made out of quite a lot of different procedures then the two named ones. There is no consent about the unique definition of marketing, not even in the literature. Definition of American Marketing Association states that marketing is an activity and set of processes for creation, communication, distribution and trade of products and services, which have a value for the buyers, clients, partners and society as a whole (23).

Pride and Ferell (*Pride and Ferell, 2010*) state that marketing is a process of creation, distribution, promotion and determination of product and service prices, what facilitates satisfying the client, develops and maintains satisfactory relation with all interest groups in the changing environment. Clients expect to benefit in exchange for the price that they pay in the transaction, while a company expects to profit from a price charged (*Pride and Ferell, 2010*). The aim of marketing, according to Kotler, is to build mutually profitable long lasting relations with the clients, and not only to sell the product or a service. This requires from a marketing managers to know their clients good enough so that they can make an appropriate offer of products and services, which meet their demands (*Kotlter, 2003*). Kotler defines marketing as a professional function that identifies unfulfilled wishes and needs, defines and measures their size and potential profitability, determines which target markets an organization can serve the best, decides about certain products and services which will be out in the market and invites everybody in the organization to be pointing to satisfying the client (*Kotlter, 2003*). This definition includes all aspects of marketing and determines it in the best way.

Two basic objectives of marketing are: attracting new customers by highlighting the potential value of the product or service offered and keeping the existing clients by constantly meeting and exceeding their expectations with a product or service (*Moore and Pareek, 2010*). In modern business, the second demand demonstrated greater significance compared to the first one. The research has shown that attracting new clients can be more expensive then retaining the existing ones. Moreover, exceeding the expectations of the client is the key point of this strategy. It is no longer enough "just" to satisfy the clients, they have to be delighted, because that is the only sound way how to get to the loyal customer, what is certainly the most valuable asset of every company.

Although marketing as a scientific and teaching discipline has emerged recently, i.e. in 1905 at the University of Pennsylvania, the history of marketing is much longer in the practice. Kotler quotes an opinion of Drucker that the concept of marketing was accepted for the first time in 17th century in Japan, when family

Mitsu opened something that could be named a first department store (*Kotler, 1988*). The evolution of marketing encompasses three phases (*Pride and Ferell, 2010*):

1) Product Orientation: in the second half of XIX century, the industrial revolution was at its full speed and the demand was way over the offer, so the aim was only to produce as much as possible and satisfy that demand.

2) Sales Orientation: from 1920 until the beginning of 1950, the offer of products has been growing while the demand was reduced, which was primarily due to the Great Depression. Because of this, the efforts for selling were growing and the most important marketing activities were personal sales, advertizing and distribution.

3) Marketing Orientation: it has been noticed that the promotion itself cannot do much, wishes and demands of customers had to be revealed and such products must be produced. Up to that time, the products were produced before thinking what would be done with them, which was not a good way of working. A customer is not an opponent, it is necessary to keep him by fulfilling his wishes and working on long-term relations. All employees at the company have to think in a good marketing way, regardless the job they are doing, since satisfied customer is the most important goal for all of them.

The history of marketing in veterinary practice is not very long (*Međugorac, 2009; Vidić, 2012a*). The development of marketing in veterinary practice was significantly supported by the articles published in the journal Veterinary Economics and numerous books published mostly in the U.S. (*Ackermann, 2007*). These articles and books address all issues relevant for veterinary practice, from the moment of taking decision to establish a practice and the best way to do it, everyday activities in the practice and adjusting to variable working environment to making decision to sell the practice. In Serbia, marketing in veterinary practice has been rarely discussed issue among professionals (*Vidić, 2012b*). Since recently, veterinary practice has been considered a business requiring high quality of service since strong competition imposes the necessity of using marketing tools in order to achieve competitive advantage, survive and grow in the market (*Boboš et al., 2013,Vidić et al.2007, Vidić et al.2011b*).

Marketing Mix in veterinary practice

Marketing mix is a combination of instruments used by the organization or a company in accomplishing its goals. Marketing mix is one of the main concepts in marketing and the main instruments are product, price, place and promotion (*Vasiljev, 2005*). Marketing mix is often referred to as a “strategy 4P” because of the first letters of English words for the four instruments: product, price, place, promotion (*Tadić, 2011*). Each of these marketing mix instruments is a variable

manipulated by managers in order to achieve company's goals. A synergy effect produced by combining these instruments in the right way is also very important. Of course, this strategy can be used only if the operating costs are lower than the effects. The company aims to find out a combination of instruments to provide maximum difference between the effect and cost.

In the book "Business basic for veterinarians", Ackerman states that the instruments of marketing mix can be successfully applied in veterinary practice; however, with taking into consideration the specificities of this practice (*Ackerman, 2002*). In veterinary practice, a "product" can be an expendable product such as feed for pets or medicines, or veterinary service. It is very important to regard the product as a value delivered to the client and his/her pet and not as a physical entity or service. In managing the veterinary practice and deciding which product should be included in their offer, the most important basic question veterinarians should answer is: *What is the aim of veterinary practice?* Possible answers could be low costs, to be an innovator and offer a hi-tech service, or be in the middle of those two concepts with a "client friendly" strategy and a medium price. After defining this target, the management of the practice can easily decide which products and services should be offered.

When speaking about pricing strategies, a basic rule is that all calculations should rely on the cost price. There are four strategies used in veterinary practice (*Ackermann, 2007*):

1) Competitive prices – used for products that are widely available in the market and can be bought, like vaccines. It is highly important that these prices are kept at the same level with competition in order not to lose the clients.
2) Value-based pricing –implicates prices of products and services, which are exclusive first-class services associated with high level of veterinarian expertise required. A highly qualified personnel employed at the veterinary clinic is a prerequisite for setting high prices and making extra profit.
3) Cost-based pricing – this is the mostly used method as it is simple to calculate. A selling price is formed when selling margin is added to the cost price. Development of internet has offered to clients an easy access to the wide range of products thus reducing the retail possibilities at veterinary clinics. In this respect, veterinary clinic should only purchase products that require professional assistance or advice of a veterinarian. Furthermore, the clients have impression to receive better product with professional service from a veterinary clinic then from the internet.
4) Variable cost pricing - this strategy is highly effective in covering all expenses related to product or service offered. If the clinic is capable of increasing the range of products and services without increasing the labor or space needed, this method is very effective.

Promotion encompasses communication with animals' owners using personal or non-personal techniques about the range of products and services offered, as well as about the characteristics of your veterinary practice (Savić et.al.2010). The main objective of the promotion is to increase awareness of the quality of your veterinary practice among clients. When it comes to veterinary medicine, public relations is the most important way of promotion– appearance on TV or radio, publishing articles, organizing "open-door days" etc. Moreover, online advertising (web site, log), advertisements in professional literature, yellow pages and various public programs are also of particular importance. Although all aforementioned actions are very effective, the research has shown that the majority of clients choose a certain practice mainly based on the reference and experience of other animal owners. Thus, the best way of promotion of a veterinary practice is to provide always the best possible service (*Molhoek and Edenburg, 2009*).

The location of a veterinary practice or clinic is important and that gives the first impression to the client about the practice (Stowe, 1999). Location is not as important as in some other business branches but it would surely help if the practice was on a good spot with an easy and fast access and a big enough parking space. The entire practice space should be clean, light and odor-free, since it indirectly informs the client about the high quality service that he / she will receive for their money. The ***waiting room*** is where the ***first impressions*** of the clients are formed, which than determines their expectations regarding the surgery room. Things like professional magazines, photos of the clients with their pets on the walls, flowers etc. are very effective. Furthermore, taking consideration of clients' needs while waiting is very important, thus it is necessary to provide the waiting room with phone, toilet, toys for kids, coffee on the house, etc. All this increases the perception of client's importance and enables the creation of a long lasting relation with the client (*Silk, 2006*).

Client relationship management

Regardless of the reasons for practicing veterinary medicine (e.g. love for animals or other) it should be considered a business. Clients are the most important aspect of every business and it is necessary to focus on client relationships and to meet their needs (*Međugorac, 2009*). Establishing a client-oriented practice requires a well-organized teamwork, where all team members work collaboratively sharing the same philosophy. Client-oriented attitude pertains not only to veterinarians but also to the personnel working at the reception desk. Every member of the team should continuously strive to delight the client by offering the most remarkable service. Consistency is the most crucial part of the interaction with the client. It is accomplished through creating and applying appropriate workplace protocols and procedures. Such protocols precisely define everyone's particular

duties and responsibilities within the company. The first impression of the client is very important, 65% of the picture about the practice comes from the interaction with the personnel and even 68% of clients leave the practice because of the indifferent relation of the employees (*Ackermann, 2007*). The first step towards successful serving of the client is to get to know him/her as well as possible, learn and meet the wishes and needs of the client and his pet. Therefore, the main question is how to get to know the client? Firstly, it is necessary to identify the groups of clients, i.e. service consumers. This strategy is called market segmentation. Veterinary medicine services imply many kinds of different clients. One of the ways to get to know clients better is to give them a questionnaire about: who they are, how close they live, their incomes, which animals they have, etc. Furthermore, it is necessary to analyze which clients bring most money to the practice and pay them some extra attention. According to Pareto's law, 80 % of the practice income is achieved from 20 % of the clients. These clients do not come to particular veterinary practice because of the prices but rather because of good service quality so the quality must be maintained at the highest level. Meeting the needs of such clients (making appointments, house visits, feed and medicine delivery, etc) whenever it is possible is highly recommendable. Of course, it does not mean that other clients are not important, but these deserve some kind of a bonus for their loyalty. Segmentation strategy enables offering specialized veterinary services to specific clients when justified. This primarily relates to growing number of clients having exotic animals as pets (*Ackermann, 2002*).

Dr. Lowell Ackerman speaks of six "C" management relations with the client in veterinary practice (*Ackerman, 2007*):

1) Consistency: clients want a unique attitude about the way of treatment, not different opinions from different veterinarians at the clinic
2) Compassion: clients are attached to their animals and they expect that the veterinarians show love and compassion for the animals
3) Client service: clients like to be treated as important for the veterinary organization or they would leave, which is very expensive - it is more expensive to attract a new client then to keep an old one, and old clients are usually more profitable then the new ones
4) Convenience: in modern society, people have less and less time and so it is necessary to meet the clients' demands, and they will reward that. It is necessary to make it easy for the clients.
5) Competence: clients cannot estimate the expertise of a veterinarian because the lack of professional knowledge. It is necessary to use every opportunity to suggest one's level of expertise (degree, license, rewards…).
6) Cost: no client ever wants to overpay the service, no matter how professional it is. They would rather go to the competition. It is very important to take care about the pricing policy and consider all the aspects.

Conclusion

As in any other business areas, marketing in veterinary practice is getting increasingly important in relation to the other activities within the company. This is a result of considering veterinary practice a business as any other branch, sharing the same goals and problems in realization of these goals. In the light of current globalization, growing competition and expansion of innovative technologies associated with massive availability of information, a fight for every customer is the main task and the prerequisite for the survival of each particular veterinary practice. Understanding that the client is its most valuable asset and a reason for the existence of veterinary practice should be the milestone of their working policy, and clients should be treated accordingly. Clients experience in communication with veterinary practice should be simple, professional, pleasant and effective. If a veterinary company fulfills all the aforementioned conditions, it is on a good way of creating mutually profitable and long lasting relationship with its clients that ensures its survival in the market.

Acknowledgment

Paper is financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia, project No. III- 43007.

Značaj i primena marketinga u veterinarskoj maloj praksi

V. Vidić, S.Savić, B.Vidić, Ž.Grgić

Rezime

Marketing ima dva cilja. Prvi je, da privuče nove klijente naglašavajući potencijalnu vrednost proizvioda ili usluge koji se nude. Drugi, zadržati postojeće klijente tako što će oni biti permanentno zadovoljni uslugom ili proizvodom, tako što ćemo prevazilaziti njihova očekivanja i uvek im pružati više.

Za razliku od SAD gde je najviše urađeno kako u teoriji tako i praksi na implementaciji marketinga u veterinarskoj delatnosti i razvoju svesti kod veterinara pre svega o prednostima prihvatanja marketing filozofije, u Srbiji se ova tema nije značajnije izučavala. Cilj marketinga u veterinarskoj delatnosti je da izgradi uzajamno profitabilne dugoročne odnose sa klijentima, a ne samo da proda određeni proizvod ili uslugu. Bez obzira na lične razloge

pojedinaca da se odluče za bavljenje veterinarskom delatnošću, a pre svega poslovima male prakse, potrebno je ovu delatnost posmatraju kao i svaki drugi posao, koja treba da obezbedi profit. Da bi se ovo postiglo, neophodno je usvojiti marketinški način razmišljanja i upravljati marketingom.

Temeljni instrumenati marketinga su proizvod, cena, promocija i mesto koji čine takozvani marketing miks koji se može primeniti i na veterinarsku praksu. U veterini proizvod se odnosi ili na potrošne stavke, kao što su lekovi ili hrana, ili na usluge veterinara. Kod utvrđivanja cene, polazna osnova mora biti cena koštanja, na koju se dodaje marža koja zavisi od vrste proizvoda i usluge koja se nudi. Promocija predstavlja komunikaciju sa vlasnicima životinja o proizvodima i uslugama koje se nude i o karakteristikama klinike ili ambulante. Za razliku od drugih delatnosti, lokacije veterinarske organizacije nema toliku važnost, ali svakako mora biti u funkciji dobrog poslovanja sa vlasnikom životinja. Celokupno iskustvo klijenta od ulaska u kliniku ili ambulantu do izlaska mora biti profesionalno, ugodno i efikasno. Samo ako se ovo ostvari moguće je biti konkurentan ili čak naplatiti više za superiornu uslugu.

References

ACKERMAN J.L. (2002): Business Basic for Veterinarians, ASJA, Lincoln, New York.

ACKERMAN J.L. (2007): Blackwell's Five Minute Veterinary Practice Management Consult, Blackwell Publishing, USA.

BOBOŠ S., RADINOVIĆ M., VIDIĆ B., PAJIĆ M., VIDIĆ V., GALFI A. (2013): Mastitis therapy - direct and indirect costs. Biotechnology in Animal Husbandry, ISSN 1450-9156, 29, 2, 269-275.

KOTLER P. (2003): Marketing insights from A to Z: 80 Concepts every manager needs to know. John Waley & Sons, New Jersey.

KOTLER P. (1988): Upravljanje marketingom 1. Zagreb, Informator.

KOTLER P. (2008): Upravljanje marketingom. Zagreb.

MEĐUGORAC S. (2009): Marketing veterinarskih usluga, Magistarski rad, Ekonomski fakultet, Zagreb.

MOLHOEK A.W.I, EDENBURG N. (2009): The effectiveness of marketing concepts in veterinary practices, Tijdsch.voor Diergk., 134, 1, 4-10.

MORE K., POREEK N. (2010): Marketing: the basics, Niketh Pareek, Routledge, New York.

PRIDE M.W., FERELL O.C. (2010): Marketing, South-Western cengage learning, Mason, USA.

SAVIĆ S., VIDIĆ B., LAZIĆ S., LAKO B., POTKONJAK A., LEPŠANOVIĆ Z. (2010): *Borrelia burgdorferi* in ticks and dogs in the province of Vojvodina, Serbia.Parasite, ISSN 1252-607X, 17,357-361.
SILK J.A. (2006): What is Marketing? Harvard Business school Publishing Corporation.
STOWE J.D. (1999): Brand loyalty and veterinary practice marketing. Can.Vet.J.,40, 816-81 .
TADIĆ M. (2011): Marketing veterinarskih usluga. Vet. Stanica, 45, 5, 471- 480.
VASILJEV S. (2005): Marketing, četvrto izdanje, Prometej, Novi Sad.
VIDIĆ B, SAVIĆ-JEVĐENIĆ S., GRGIĆ Ž., BUGARSKI D., MALJKOVIĆ M. (2007): Infectious abortion in sheep. Biotechnology in animal husbandry. - ISSN 1450-9156. -. 23, 5-6, 383-389.
VIDIĆ B., SAVIĆ S., PRICA N. (2011b): Paratuberculosis in cattle - the outspread, diagnostics and control. New Perspectives and Callenges of Sustainable Livestock Production.Book 1, 3rd International Congress, Belgrade, 5-7 October 2011, Beograd, Institute for Animal Husbandry, 335-344.
VIDIĆ V. (2012a): Marketing usluga savremene veterinarske prakse. Arhiv veterinarske medicine, ISSN 1820-9955, 5, 2,.67-76,.
VIDIĆ V. (2012b): Marketing in veterinary. Proceedings, Second International epizootiology Symposium XIV Serbian epizootiology days, April 18-21, 2012, Belgrade, Sekcija za zoonoze, ISBN 978-86-83115-20-4, 271-277.
VIDIĆ B., SAVIĆ S., VIDIĆ V., JOVIČIN M., PRICA N. (2013): Economic impact of paratuberculosis on milk production. Biotechnology in Animal Husbandry, ISSN 1450-9156, 29, 2, 183-191.
www.marketing.com

Received 11 September 2013; accepted for publication 20 December 2013

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. Škrbić, 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 Dušana 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. Škrbić, 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 živine, zahtevi potrošača. Živinarstvo, 8-9, 49-58.

PAVLOVSKI Z., MAŠIĆ B. (1994): Odnos potrošača prema živinskim proizvodima. Živinarstvo, 7-9, 77-82.

PETROVIĆ D.M., GUTIĆ M., BOGOSAVLJEVIĆ-BOŠKOVIĆ 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 Mašić, 1994)*; *(Petrović et al., 2004)*; *(Pavlovski, 2004; Pavlovski and Mašić, 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