UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

VOL 30, 2
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2014

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2014

Biotechnology in Animal Husbandry 30 (2), p 175-376, 2014 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 30 (2), p 175-184, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.085.1'636.3
DOI: 10.2298/BAH1402175R

PROTEIN SOURCE IN DIETS FOR RUMINANT NUTRITION

D. Ružić-Muslić, M.P. Petrović, M.M. Petrović, Z. Bijelić, V. Caro-Petrović, N. Maksimović, V. Mandić

Institute for Animal Husbandry, Belgrade-Zemun, 11080, Serbia
Corresponding author: muslic.ruzic@.gmail.com
Review paper

Abstract: The rapid increase in human population leads to increased demand for animal protein. On the other hand, the deficit of protein feeds in the market and rising costs are the most significant obstacles facing animal production. Therefore, most researches have focused on improving the status and utilization of different protein sources in order to reduce costs and maintain optimum performance of animals. The results of our study showed that lambs of MIS population, of average body weight of 18.0 kg, fed diets with different protein sources: sunflower meal, soybean meal, fish meal, realized average daily gain: 0.169, 0.205 and 0.227 kg, respectively. Conversion of dry matter in analogue treatments was: 4.54, 3.71 and 3.30 (kg/kg of gain) and total protein (g/kg): 732, 596 and 549, respectively. It is evident that the fish meal as a protein source improves the growth and utilization of food in lambs. However, given that the European Commission has banned the use of fish meal in diets for animal nutrition, nutritionists' imperative is to investigate the possibility of using "unconventional" sources of protein (peas, beans, lupins) in diets for ruminant nutrition. The aim of this study was to compare the effects of different sources of protein in diets on production performance of ruminants.

Key words: protein sources, ruminants, daily gain, feed conversion

Introduction

Dietary proteins that reach the small intestine of ruminants consist of two protein fractions: microbial and protein undegradable at the rumen level. Microbial protein is produced by the action of the rumen flora, which breaks down the dietary protein to peptides, amino acids and ammonia, after which these materials are used for the synthesis of own proteins *(Ružić-Muslić, 2006)*. In the course of the decomposition and synthesis some losses occur (typically about 20%, but sometimes higher). Thus, reduced amount of amino acids reaches the location where digestion and adoption of proteins occur, which means that the needs of high

yielding meat breeds cannot be satisfied by the microbial protein synthesis from the usual sources of protein and energy (*Ružić-Muslić et al., 2007d, 2011b*). Therefore, in order to ensure optimal pool of amino acids for a particular production, it is necessary to provide protein fraction which avoids degradation of the protein in the rumen (undegradable protein) (*Ružić-Muslić et al., 2007, 2011a).*

According to *Grubić et al. (1991)*, for each level of productivity it is necessary to ensure certain optimal ratio between the proteins that are degraded in the front-stomach / rumen under the action of microorganisms (biodegradable protein-RP), and of proteins which avoid degradation (NP - undegradable protein). The simplest method to influence the extent and rate of degradation of a protein in rumen consists in choosing the correct source of protein *(Grubić et al., 1992). Zeremski (1989)* states that the use of animal-based nutrients that have a low degradability of protein in the reticulum-rumen plays an important role in the utilization of the production potential and intensive fattening of lambs. Protein from these nutrients are considered highly valuable because they contain the essential amino acids necessary for the growth and development of lambs. As an excellent source of high quality protein that is slowly degraded in the rumen are the following: fish meal, meat and bone meal, blood meal and soy meal.

Bearing in mind that the European Commission has banned the use of fishmeal in diets for animals, research has focused on studying the effects of using soybean, sunflower and "unconventional" sources of protein in diets for ruminant nutrition.

Effect of protein source on performance of ruminants

Nutrients whose proteins pass through the reticulum-rumen to a greater extent non-degraded and reach the duodenum, cause greater weight gain in lambs, with the presence of sufficient energy *(Zeremski, 1989).* This was confirmed in studies of *Ružić-Muslić* (*2006, 2007d*) where the protein source has very significantly ($P<0.01$) influenced the ultimate/final result of fattening expressed as average daily gain, and final body weight of fattened lambs, with the best performance achieved by lambs in the treatment with fishmeal.

Table 1. Production performance of fattening lambs (Ružić-Muslić, 2006)

Traits	Protein source		
	Sunflower meal (SWM)	Soybean meal (SBM)	Fish meal (FM)
Initial body weight, kg	18.12±1.03	18.08±1.19	18.17±1.03
Initial age, days	60	60	60
Final body weight, kg	30.78^{a}±1.53	33.52±2.32	35.17^{b}±3.67
Total gain, kg	12.70^{a}±0.61	15.40±1.99	17.00^{b}±2.83
Average daily gain, kg	0.169±0.01	0.205^{a}±0.03	0.227^{b}±0.03
Use of dry matter, kg / kg gain	4.54	3.71	3.30
Use of total proteins, g/ kg gain	732	596	549
Use of NEM, MJ/kg gain	33.77	29.37	26.25

The difference between a and b is significant at the level of ($P<0.01$)

Results similar to ours, in terms of the effect of protein sources on production performance of fattened lambs were obtained by *Orskov et al. (1971), Miller (1978), Grubić et al. (1991), Walz et al. (1998), Beermann et al. (1986).*

Orskov et al. (1971) have established in their study performed on fattening male lambs of 15-50 kg, that with an increase in the proportion of fish meal in the diet from 1 to 6 and 12%, the average daily gain: 0.191, 0.270 and 0.330 kg, respectively.

Miller (1978) states that with the increase of the share of fish meal in diets for lambs of body weight 15-25 kg, their weight gain increases. Treatments were as follows: I control group (2.5% urea), II-2.5% fish meal +1.87% urea, III-5.5% fish meal + 1.25% urea and IV-10% fish meal. Daily weight gain of the treatments was: 0.250, 0.300, 0.330 and 0.350 kg, respectively.

Walz et al. (1998) has examined the impact of iso-protein diets (13% CP) without and with 3% fish meal, on the intensity of growth of lambs of average weight about 25.0 kg. Average daily gain in these treatments was: 0.171 and 0.206 kg.

Researches by *Beermann et al. (1986)* have shown that the replacement of soybean meal with 3% fish meal in diets for crossbred lambs Suffolk x Dorset has resulted in increased daily gain and improved feed conversion (0.441 kg and 3.52 kg) compared to (0.350 kg and 3.90 kg) as realized by animals in the treatment without fish meal.

It is evident that the fish meal, as a protein source, improves growth and feed efficiency in lambs. The explanation lies in the fact that the microbial protein is insufficient to meet metabolic requirements in amino acids necessary for growth of animals, so the use of a protein source with a high content of undegradable protein, results in superior performance.

However, given that the European Commission has banned the use of meat and bone meal and their by-products in diets for farm animals, in order to ensure safe food for consumers, the main source of protein in intensive ruminant production systems is soybean meal. However, its high price, orientation on import, fluctuations in the production, distribution as genetically modified food, have increased consumer interest in alternative sources of protein.

In recent years, the production of sunflower as oil crops has increased (due to the ability to adapt to different soil and climatic conditions), and at the same time production of sunflower meal as a by-product. It contains 36-46% of crude protein and 13-15% of crude fiber *Schingoethe et al., 1977; Nishino et al., 1980)* and it is extensively degraded in the rumen (*NRC, 1985)*. It has a higher content of methionine in comparison to other sources of protein, but is deficient in lysine (*Steen, 1989; Villamide and San Juan, 1998)*. The high fiber content is responsible for limited use in the diet for young ruminants. Therefore, to improve fiber degradation in the rumen the enzyme preparations are used. From the standpoint of the effect of different protein sources on the performance of fattening goats, *Titi (2003*) has examined the effects of three protein sources (soybean meal, sunflower meal, sunflower meal with fibrolytic enzyme supplementation) on the performance of Shami kids of average body weight of 17.0 kg, and has recorded the final body mass values: 30.23 kg, 29.68 kg and 36.38 kg, the average daily gain values: 0.155 kg, 0.142 kg and 0.221 kg, and feed conversion of 6.61, 7.59 and 4.70 kg/kg gain, respectively. Therefore, the use of sunflower meal with fibrolytic enzyme supplementation has caused significantly ($P < 0.05$) higher weight gain and better feed conversion, compared to the other two treatments. Superiority of this treatment is based on the fact that fibrolytic enzymes reduce fiber content and improve dry matter digestibility, which results in the provision of sufficient energy for growth of rumen microflora (*Lewis et al., 1995).* At the same time, kids who consumed soybean and sunflower meal were not significantly different in terms of production performance, although the numerical value of the gain in treatment with soybean meal was slightly higher (0.155: 0.142 kg). The slight difference may be the result of a higher fiber content of sunflower meal and lesser digestion capacity due to incomplete functioning of the rumen (*Schingoethe et al., 1977; Nishino et al*., *1980*). Also, the concentration of energy (MJ/kg of dry matter), and the amino acid composition of protein undegradable at the rumen level of soybean meal is much more favorable than in sunflower meal. However, these differences are not significant and the results indicate that sunflower meal can be used as a substitute for soybean meal in ruminant nutrition (*Stake et al., 1973; Schingoethe et al., 1977; Nishino et al., 1980; Steen, 1989; Isobel et al., 1990; Schingoethe et al., 1996; Economides, 1998; Economides and Koumas,1999).*

A further search for alternative sources of protein has led to increased interest in the use of legumes in ruminant nutrition. Leguminous grains have a high protein content, a considerable concentration of energy and calcium. Their proteins

are highly degradable in the rumen. By comparing some of them, it can be concluded that there is a higher content of proteins in lupine (324-381 g/kg of dry matter), compared to beans (301 g/kg dry matter) and peas (246 g/kg dry matter) *(Degussa, 2006)*. In terms of crude fat content, beans and peas contain from 15-20 g/kg dry matter *(Jezierny et al., 2007*), whereas the lupine has 57-88 g/kg dry matter *(DLG, 1999)*. In regard to starch, beans and peas have from 422-451 and 478-534 g/kg of dry matter, respectively (*Jezierny, 2009*), while lupine contains 42-101 g/kg of dry matter *(Jezierny, 2010)*. Lignin content is low and ranges from 1 to 7 and 6-9 g/kg of dry matter, respectively *(Salgadet et al., 2002a; Jezierny, 2010*). The proteins of peas and beans contain higher proportions of lysine (70 and 80 g of crude protein, respectively) compared to the soybean meal protein (69 g/kg of CP) and lupine (51-54 g/kg CP) *(Degussa, 2006)*.

Di Francia et al. (2007) have assessed the effect of partial replacement of soybean cake with extruded peas in the diet of cows during the first 100 days of lactation, and found that peas construe an attractive source of protein (GMO formulations free) in diets for cows whose production is based on organic principles. The protein content of the peas is about 25-26 % of dry matter. In terms of the amino acid profile, high lysine and low tryptophan content as compared to soybean meal *(Gatel, 1994*). The protein fraction is easily degradable in the rumen while the starch content is outstanding (more than 40 % DM), soluble and easily degradable (*Nocek and Tamminga, 1991). Lanza et al. (2003*) have conducted a comparative study in order to compare the effects of soybean meal and peas (39 and 18 % in the mixture), on fattening performance and meat quality of Barbaresa lambs. Protein source showed no significant impact on average daily gain (0.218, 0.29 and 0.250, respectively), feed conversion ratio (4.7, 4.8 and 4.1 kg, respectively), dressing percentage (50.1, 50.8 and 51.2 %, respectively), as well as the physical and chemical properties of *M. longissimus dorsi*. Obviously, in all diets, protein fraction was sufficient to meet the requirements of lambs in amino acids.

Morbidini et al. (2005) has examined the influence of soybean meal and beans on the performance of Italian Merino lambs. It was found that the use of beans in the diet resulted in a depressive effect on the performance of lambs. The explanation lies in the increasing content of NPN compounds and anti-nutritional factors. Hence, the recommendations for the period upon the early weaning of lambs is: the use of the rumen undegradable protein in the diet, given that in this period, the rumen is not a fully functional protein synthesis and is less efficient. In addition, for the same reason, in this period, the animals are not able to neutralize the possible anti-nutritive factors. The negative side of legume grains is that many of them contain anti-nutritional factors (lecithin, trypsin inhibitors, tannins, saponins, phytase).

Similar studies were conducted by *Cutrignelli et al. (2008b*) on two groups of bulls, average age of 129 days, fed diets based on bean and soybean meal,

weighing up to 620 kg. Source of protein has not significantly affected other parameters, except body weight (173 and 186 kg, in treatment with beans and soybean meal, respectively). The chemical composition of the *Muscullus longissimus thoracis*, amino acid composition, cholesterol content and sensory profile were not affected by the tested treatment. These results show that the beans can be used as an alternative source of protein, with no adverse effects on the biological effectiveness of growth, feed conversion index and meat quality of ruminants.

In view of these results, on the one hand, and instruction to observe and consider the share of undegradable protein, in addition to digestibility, when choosing a source of protein for ruminant rations, the following figure shows the representation of this fraction in individual sources of protein.

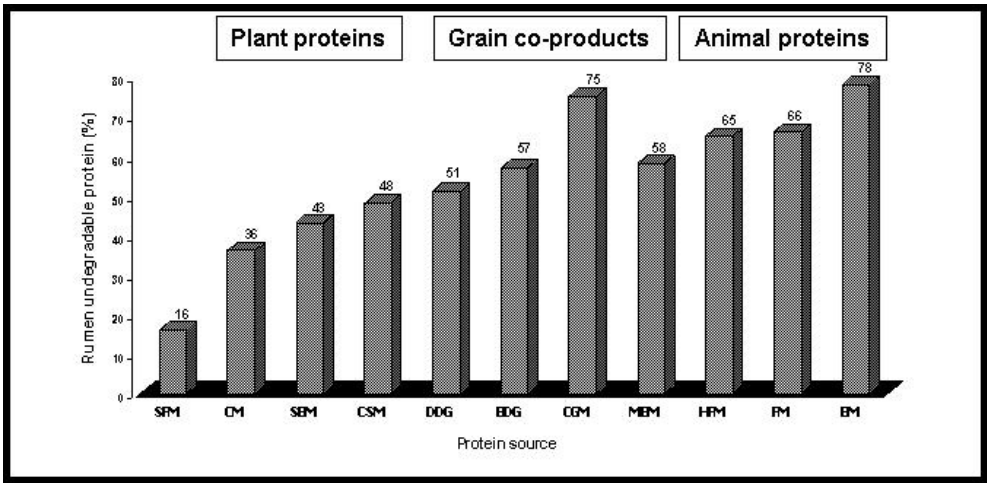


Figure 1. Rumen undegradable protein (%) of various protein sources including: sunflower meal (SFM), canola meal (CM), soybean meal (SBM), cottonseed meal (CSM), distillers dried grains with solubles (DDG), brewers dried grains (BDG), corn gluten meal (CGM), meat and bone meal (MBM), hydrolyzed feather meal (HFM), fish meal (FM) and blood meal (BM) adapted from NRC (2001).

Conclusion

Protein is a critical nutrient for young growing animals and most expensive component of food.

When choosing protein sources in diets of growing ruminants, an important criterion is the share of undegradable protein.

Fish meal is an excellent protein source that is slowly degraded in the rumen and has an excellent amino acid profile. However, due to the prohibition of its use by the European Commission, it is imperative to find other sources.

The high price of soybean, import orientation, fluctuations in the production, distribution as genetically modified food, have increased the consumer interest in alternative sources of protein.

Use of sunflower meal supplemented with fibrolytic enzymes in diets for ruminants resulted in optimal performance, compared with soybean meal.

Peas, beans, lupine can be used as an alternative source of protein without adverse impacts on biological efficiency of growth, feed conversion and meat quality of ruminants.

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

Izvori proteina u obrocima za ishranu preživara

D.Ružić-Muslić, M.P.Petrović, M. M. Petrović, Z.Bijelić, V.Caro-Petrović, N. Maksimović, V. Mandić

Rezime

Brz porast ljudske populacije uslovljava povećanu potražnju za animalnim proteinima. Sa druge strane, deficit proteinskih hraniva na tržištu i porast troškova su najznačajnije prepreke sa kojima se suočava animalna proizvodnja. Zbog toga, većina istraživanja je usmerena na poboljšanje statusa i iskorišćavanja različitih izvora proteina, u cilju smanjenja troškova i održavanja optimalnih performansi životinja. Rezultati naših istraživanja su pokazali da su jagnjad MIS populacije, prosečne telesne mase 18,0 kg, hranjena obrocima sa različitim izvorima proteina: suncokretova sačma; sojina sačma, riblje brašno, ostvarila prosečan dnevni prirast: 0,169 : 0,205 : 0,227 kg, respektivno. Konverzija suve materije na analognim tretmanima, je iznosila: 4,54 : 3,71 : 3,30 (kg/kg prirasta) a ukupnih proteina (g/kg): 732 : 596: 549. Evidentno je da riblje brašno, kao izvor proteina, poboljšava rast i iskorišćavanje hrane kod jagnjadi. Međutim, obzirom da je Evropska komisija zabranila korišćenje ribljeg brašna u obrocima za ishranu životinja, imperativ nutricionista je ispitivanje mogućnosti korišćenjea"nekonvencionalnih" izvora proteina (grašak, pasulj, lupina) u obrocima za ishranu preživara. Cilj ovog

rada je da se uporede efekti različitih izvora proteina u obrocima na proizvodne performanse preživara.

References

BEERMANN D.H., HOGUE D.E., FISHELL V. K., DALRYMPLE R.H., RICN A.C. (1986): Effects of cimaterol and fishmeal on performance, carcass characteristics and skeletal muscle growth in lambs. J. Anim. Science, 62, 370-380.

CUTRIGNELLI M.I., CALABRT S., BOVERA F., TUDISCO R., D'URSO S., MARCHIELLO M., PICCOLO V., INFASCELLI F. (2008b): Effects of two protein sources and energy level of diet on the performance of young Marchigiana bulls. 2. Meat quality. Ital.J.Anim.Sci., 7, 271-285.

DEGUSSA AG. (2006). The amino acid composition of feedstuffs. 5th rev. ed., Degussa AG, Feed Additives, Hanau, Germany

DI FRANCIA A., DE ROSA G., MASUCCI F., ROMANO R., BORRIELLO I., GRASSI C. (2007): Effect of Pisum sativum as protein supplement on buffalo milk production. Ital.J.Anim.Sci., 6, 472-475.

DLG (1999): Kleiner Helfer für die Berechnung von Futterrationen. Wiederkäuer und Schweine. DLG-Verlag, 10. Auflage, Frankfurt am Main, Germany

ECONOMIDES S. (1998): The nutritive value of sunflower meal and its effect on replacing cereal straw in the diets of lactating ewes and goats. Livestock Prod. Sci., 55, 1, 89–97.

ECONOMIDES S., KOUMAS A. (1999): Replacement of soybean meal with peanut meal, sunflower meal, narbon vetch meal or urea in concentrate diets of early-weaned lambs. Agriculture Research Institute, Ministry of Agriculture, Natural Resources, Nicosia, Cyprus.

GATEL F. (1994): Protein quality of legume seeds for non-ruminant animals: a literature review. Anim. Feed Sci. Tech., 45, 317–348.

GRUBIĆ G., ZEREMSKI D., PAVLIČEVIĆ A. (1991): Uticaj razgradivosti proteina hrane na proizvodne rezultate odlučene jagnjadi. Zbornik radova Poljoprivrednog fakulteta, 36, 595, 169-183.

GRUBIĆ G., ADAMOVIĆ M., NEGOVANOVIĆ D. (1992): Razgradivost proteina kao kriterijum za sastavljanje obroka u ishrani preživara. Inovacije u stočarstvu, X, 125-131.

ISOBEL C.V., HILL R., CAMPLING R.C. (1990): A note on the use of rapeseed, sunflower and soybean meals as a protein sources in compound foods for milking cattle. Anim. Prod., 50, 541–543.

JEZIERNY D. (2009): *In vivo* and *in vitro* studies with growing pigs on standardised ileal amino acid digestibilities in grain legumes. Ph.D. Thesis. University of Hohenheim, Stuttgart, Cuvillier Verlag Gцttingen, Germany.

JEZIERNY D., MOSENTHIN R., BAUER E. (2010): The use of grain legumes as a protein source in pig nutrition: A review. Animal Feed Science and Technology, 157, 111–128.
JEZIERNY D., MOSENTHIN R., EKLUND M., RADEMACHER M. (2007): Determination of standardized ileal digestibilities of crude protein and amino acids in legume seeds for growing pigs. Proceedings of the 16th International Science Symposium on Nutrition of Domestic Animals, Radenci, 198–203.
LANZA M., BELLA M., PRIOLO A., FASONE V. (2003): Peas (*Pisum sativum* L.) as an alternative protein source in lamb diets: growth performances, and carcass and meat quality .Small Ruminant Research, 47, 63–68.
LEWIS G.E., SANCHEZ W.K., TREACHER R., HUNT C.W., PRITCHARD G.T. (1995): Effect of direct-fed fibrolytic enzymes on lactational performance of midlactation Holstein cows. Proc., West. Section, Am. Soc. Anim. Sci., 46, 310.
MILLER E.L. (1978): Evaluating the protein contribution of feedsuffs for ruminants. Tech. Bull., 5, IA FMM.
MORBIDINI L., ROSSETTI E., COZZA F., PAUSELLI M. (2005): Different protein source (soybean or faba bean) in post-weaning diets for Apennine and Sopravissana (Italian Merino) light lamb: slaughtering performances. Ital.J.Anim.Sci. 4, 360-362.
NATIONAL RESEARCH COUNCIL (1985): Ruminant Nitrogen Usage. Nutritional Academy of Science. National Academy Press, Washington, DC.
NISHINO S., KONDO S., HAYASHI K. (1980.): Feeding value of sunflower meal as a replacement for soybean meal in lactating cows. J. Coll. Dairying, 8, 2, 275–284.
NOCEK J.E, TAMMINGA S. (1991): Site of digestion of starch in the gastrointestinal tract of dairy cows and its effects on milk yield and composition. J. Dairy Sci., 74, 3598–3629.
NRC (2001): Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001.
ORSKOV E.R., MCDONALD I., FRASER C., CORSE E.L. (1971): The nutrition of the early weaned lamb. III The effect of ad libitum intake of diets varying in protein concentration on performance and on bory composition at differenty live weights. Journal of Agricultural Science, Cambridge, 77, 351-361.
RUŽIĆ-MUSIĆ D., PETROVIĆ P.M., BIJELIĆ Z. (2011a): Undegradable protein-important faktor in balancing of diets for fattening lambs. ***3rd*** International congress on animal husbandry "New perspectives and Challenges of Sustainable Livestock Production", October 2009, Belgrade**.** Biotechnology in Animal Husbandry, 27, 3, 473-485.
RUŽIĆ-MUSLIC D., PETROVIC P.M., PETROVIC M.M.., BIJELIC Z., PANTELIC V., PERIŠIC P. (2011b): Effects of different protein sources of diet on yield and quality of lamb meat. African Journal of Biotechnology, 10, 70, 15823-15829, 9 November, 2011. DOI:10.5897/AJB11.698

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

RUŽIĆ-MUSLIĆ D., GRUBIĆ G., PETROVIĆ P.M., ŽUJOVIĆ M., MUSLIĆ H., NEŠIĆ Z., MARINKOV G., STOJANOVIĆ LJ. (2007d): Efekat izvora proteina u obroku na proizvodne performanse jagnjadi u tovu. Biotechnology in animal husbandry, 23, 1-2, 41-49.

RUŽIĆ-MUSLIĆ D., GRUBIĆ G., PETROVIĆ P.M., NEGOVANOVIĆ D., NEŠIĆ Z., PERIŠIĆ P., ŽUJOVIĆ M. (2007): The effect of the level of undegradable protein on digestibility of nutritive substances in fattening lambs. 2nd International congress on animal husbandry: New perspectives and challenges of sustainable livestock farming, 03.-05. October. Biotechnology in animal husbandry, 23, 5-6, 2, 131-139.

SALGADO P., FREIRE J.P.B., MOURATO M., CABRAL F., TOULLEC R., LALLIS, J.P. (2002a): Comparative effects of different legume protein sources in weaned piglets: nutrient digestibility, intestinal morphology and digestive enzymes. Livest. Prod. Sci., 74, 191–202.

SCCHINGOETHEJ D., ROOK A.J., LUDENS F. (1977): Evaluation of sunflower meal as a protein supplement for lactating cows. J. Dairy Sci., 60, 4, 591–595.

SCHINGOETHEJ D., BROUK J.M., LIGHTFIELD D.K., BAER R.J. (1996): Lactational responses of dairy cows fed unsaturated fat from extruded soybeans or sunflower seeds. J. Dairy Sci., 79, 1244–1249. STAKE E.P., OWENS J.M., SCHINGOETHE J.D. (1973): Rapeseed, sunflower, and soybean meal supplementation of calf rations. J. Dairy Sci., 56, 6, 783–788.

STEEN W.J.R. (1989): A comparison of soybean, sunflower and fish meals as protein supplements for yearling cattle offered grass silage-based diets. Anim. Prod., 48, 81–89.

TITI H. H. (2003): Replacing soybean meal with sunflower meal with or without fibrolytic enzymes in fattening diets of goat kids. Small Ruminant Research, 48, 45–50.

VILLAMIDE J.M., SAN JUAN D.L. (1998): Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. Poult. Sci., 77, 1884–1892.

WALZ L.S.,WHITE T.W., FERNANDEZ J.M.,GENTRY L.R., BLOURN D.C.FROATSCHEL M.A., BROWN T.F., LUPTON C.J., CHAPA A.M. (1998): Effects of fish meal and sodium bentonite on daily gain, wool, growth, carcass characteristics and ruminal and blood characteristics of lambs fed concentrate diets. J. Anim. Sci., 76, 2025-2031.

ZEREMSKI D., PAVLIČEVIĆ A., GRUBIĆ G. (1989): Uticaj sastava obroka na efikasnost tova jagnjadi i šilježadi. XIII Savetovanje o ishrani ovaca, Palić, 115-126.

Received 4 March 2014; accepted for publication 25 May 2014

Biotechnology in Animal Husbandry 30 (2), p 185-192, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.38(5)
DOI: 10.2298/ BAH1402185R

EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH TRAITS IN MAKUIE SHEEP

S. M. Rahimi*, S. A. Rafat, S. Jafari

Department of Animal Science, University of Tabriz, 29 th Bahman Bolvard East Azarbaijan, Tabriz, Iran.
Corresponding author: san_mohamadrahimi@yahoo.com
Original scientific paper

Abstract: The Makuie sheep is a fat-tailed sheep breed which can be found in the Azerbaijan province of Iran. non- genetic parameters obtained from data collected from 1993 to 2012 Makuie sheep research station of West Azerbaijan province in Iran were evaluated in the present study. The non- genetic parameters for birth weight, weaning weight (3 months), 6-month, 9-month and yearling weight traits were estimated based on 19 years of data using SAS software**.** Least squares means were 4.11± 0.88, 21.50±3.50, 27.18±3.53, 28.57±4.44 and 34.21±3.88 kg for weight at birth, 3, 6, 9 and 12 month, respectively. The birth year had a significant effect on all traits while the effect of birth type significantly affected all traits ($p<0.05$). The lamb's sex had a significant effect on all traits ($p<0.05$). The effect of dam's age had a significant effect on all traits except 9- month ($p<0.05$). Results showed that non- genetic factors have an important role in expressing of genetic potential in the lambs.

Keywords: Body weight, non- genetic factors, Makuie sheep.

Introduction

Makuie sheep is a native breed of Iran and can also be found in Turkey (called as Ak Karaman). Its total population is estimated at approximately 2.7 million *(Abbasi and Ghafouri Kesbi, 2011)*. They are fat-tailed sheep with a medium- sized body, white in color with black rings around the eyes, nose and feet *(Saadatnoori and Siahmansoor, 1986)*. They are kept in the Eastern and Western provinces Azerbaijan and their main products are meat, wool and milk *(Saadatnoori and Siahmansoor, 1986)*. Whenever the weather condition is suitable, these animals feed after grazing pasture, alfalfa and clover, while in cold seasons they are fed manually, eating alfalfa, wheat straw, barley straw, barley barn and other extra forages *(Nourian, 2000)*. Investigation and determination of environmental factors that have effect on traits and correction of records for these factors cause estimated genetic parameters and breeding value to show animal's

genetic potential *(Osman, 1965; Rashidi et al., 2008)*.

Effect of birth year, lamb's sex and birth type has been reported significantly in breeds like Kermani *(Rashidi et al., 2008)*, Merino *(Dixit et al., 2001)*, Horro *(Abegaz et al., 2005)* and Sabi *(Matika et al., 2003)* and sheep farm of Institutefor Animal Husbandry, Belgrade-Zemun *(Petrović et al.,2009)*. The effect of dam age has been reported significantly more in breeds such as Baluchi *(Fadilli et al., 2000)*, Zandi *(Kalantar, 2003)*, Kermani *(Rashidi et al., 2008)*, Merino *(Dixit et al., 2001; Ozcan et al., 2005)* and Horro *(Abegaz et al., 2005)*. Reference *(Nourian, 2000, Yazdi et al., 1997, Neser et al., 2001)* reported that herd affects body weight significantly. The objective of this study was to identify the effects of non- genetic factors on weight traits and average daily gain in different ages of Makuie sheep.

Material and method

In order to study the effect of non- genetic factors on growth traits in Makuie sheep, we applied information that was collected from 1993 to 2012 (19 years) in Makuie's Breeding Station. This includes number of animal, birth year, lamb's sex, birth type and age of dam. In addition, records of birth weight (BW), weaning weight (WW), weight at month 6 (6 MW), weight at month 9 (9MW) and weight at yearling (YW). Characteristics of the data structure are summarized in table 2. After birth, the lambs feed manually on alfalfa mill, high quality forage and dams accompany their ewes during grazing in pasture. Weaning was at approximately age of month 3. Mating was controlled and at the birth of lambs register pedigree information (animal code, sire and dam), birth information (date of birth, lambs' sex, birth type) and records (birth weight, weaning weight, and month 6 weight, month 9 weight and yearling weight).

A univariate procedure of SAS was used to check for normality. The SAS software was used for normality test. The data of all traits was normal. Statistical model for studying the effect of these factors were:

$y_{ijklm} = \mu + Y_i + A_j + S_k + T_l + H_m +$ interaction between factors $+ e_{ijklm}$

where y_{ijklm} is records on the different traits, μ = mean, Y_i = effect of birth year (1993- 2012), S_k = effect of lamb's sex (male and female), T_l = effect of birth type (single and twin), A_j = effect of dam age at lambing (2 - 6 years old) details of classes in Table 1, H_m = effect of herd and e_{ijklm} is residual effects.

Table 1. Least squares means and their standard error of mean for dam age at lambing of Makuie sheep

Dam age at lambing	N	BW	WW	6WW
2	14399	4.19±0.81	21.06± 3.19	26.71± 3.39
3	6244	4.22± 0.83	22.31± 2.85	27.42± 3.40
4	2397	4.17± 0.84	21.43± 2.78	27.31± 3.56
5	785	4.74± 0.76	20.90± 2.28	26.47± 3.37
6	237	4.25± 1.48	23.00± 2.20	29.30± 3.25

N: observation in dam age at lambing, BW: birth weight, WW: weaning weight (month 3), 6 MW: month 6 weight.

The age of weighting was used as co-variable for correcting phenotype observation of weaning weight and month 12 weight. This is because the lambs did not give birth at the same time but they were weighted together. Therefore they have different ages. Analysis of variance of non-genetic factors and estimation of least square means with their standard error was carried out by general linear model procedure in SAS software.

Results and discussion

The analysis of variance results, least square means and standard error for BW, WW, 6MW, 9MW, YM in Table 2, estimation of non- genetic factors including birth year, age of dam, lamb's sex and birth type are given in Table3.

The least squares means and standard errors for BW, WW, 6 MW, 9MW and YW are presented in Table 4. Lamb gender, type of birth, age of dam, birth year and herd had significant influences on body weight traits *($p<0.01$)*. In all ages, the male and single lambs were heavier than female and twin lambs.

The effect of birth year on BW, 6 MW, 9MW and YW *($p<0.001$)* and weaning weight (WW) *($P<0.05$)* was significant. These results were consistent with the report on Sabi sheep *(Ozcan et al., 2005)*. It could be due to differences in management, food availability, disease, and climatic condition (rate of rainfall, humidity and temperature) that affect the quality and quantity of pasture forage and raising systems in different years. The age of the dam was significant on birth weight (BW) *($P<0.001$)*, weaning weight (WW) *($P<0.01$)*, 6- month weight and yearling weight *($P<0.05$)* traits. Young ewes tend to produce smaller lambs. Primiparous ewes are not at their mature weight and complement their growth in addition to fetal growth. This could affect the lam weight. It is well known that mothering ability, such as milk yield, increases with parity, as older ewes are usually larger and produce more milk *(Dass and Acharya, 1970)*. However, the age of the dam did not have significant effect on 9MW trait. The same results were

reported on the Moroccan Timahdit sheep *(Fadilli et al., 2000)*. Male lambs were heavier than females at all ages and these differences were significant *(P<0.01)*. The effect of lamb sex on body weight traits at different ages has been reported in various sheep breeds *(Rashidi et al., 2008)*.

Table 2. Basic statistical information about the examined traits of Makuie sheep

Trait	BW	WW	6MW	9MW	YM
Number of records	18967	19297	9957	2407	1231
Mean (kg)	4.11	21.50	27.18	28.57	34.21
Standard deviation (kg)	0.88	3.50	3.53	4.44	3.88
Minimum (kg)	2	10	15	20	28
Maximum (kg)	7	28	33	38	42

BW: birth weight, WW: weaning weight (month 3), 6 MW: month 6 weight, 9MW: month 9 weight, YM: yearling weight.

Table 3. Analysis of variance for Birth weight, weaning weight, 6- month weight, 9- month weight, yearling weight traits in Iranian Makuie sheep

Fixed effects	BW	WW	6MW	9MW	YM
Year	**	***	***	***	*
Age of dam	***	*	**	ns	***
Sex	***	***	***	***	***
Birth type	***	***	***	***	***
Herd	***	***	***	***	***

BW, birth weight; WW, weaning weight; 6MW, 6- month weight; 9MW, 9-month weight; YW, yearling weight. Significant at 0.05 probability level; **significant at 0.01 probability level; ***significant at 0.001 probability level; ns, not significant at 0.05 probability level.

Table 4. Least squares means and their standard error of mean for different levels of factors for traits

Trait	BW	WW	6MW	9MW	YM
Sex					
Male	4.22^{a}±0.87	21.6^{a}±3.51	27.47^{a}±3.57	29.67^{a}±4.36	37.02^{a}±3.52
female	4.02^{b}±0.87	21.04^{b}±3.43	26.94^{b}±3.63	27.64^{b}±4.29	33.28^{b}±3.52
Birth type					
Single	4.23^{a}±0.89	21.77^{a}±3.36	27.62^{a}±3.44	29.24^{a}±4.38	33.55^{a}±3.91
Twins	3.69^{b}±0.72	20.23^{b}±3.97	25.67^{b}±4.36	26.91^{b}±4.29	36.61^{b}±3.92

The effect of birth type was significant on all studied traits. The frequency of single birth type was high compared to other types. A low number of triple birth types were seen so it was not included in the models. The significant effect of birth type on body weight can be due to limited uterine space during pregnancy, nutrition of the dam, especially during late pregnancy (regardless of twin or triple pregnant dams), and competition for milk sucking between multiple birth lambs during the birth to weaning period. Similar results have been reported in other breeds, such as the Hungarian Merino sheep *(Komlosi, 2008)*. According to the equation , 16-40% of traits' phenotypic variances were explained by the established factors in Table 3 and the effects of sex and birth type were the most important of the traits studied (P<0.001).

BirthYear

The effect of birth year on birth weight (BW), 3-month weight (WW), 6-month weight (6MW), 9-month weight (9MW), yearling weight (YW) *(P<0.001)* was significant. These results were consistent with the report on Sabi sheep *(Matika et al., 2003)*. Our results confirmed other reports *(Komlosi, 2008; Kalantar, 2003; Ozcan et al., 2005; Matika et al., 2003)*.

Birth year was significant for all traits. Interaction effects were significant between birth year and lamb's sex, birth year and birth type, birth year and herd. Interaction effect between birth year and age of dam was significant.

Age of Dam

Age of dam was was significant for all traits except 9MW. Results were the same as some of other researchers *(Rashidi et al., 2008; Shahrodi Eftekhar et al., 2003; Shahrodi Eftekhar et al., 2002; Kalantar, 2003; Ozcan et al.,2005; Matika et al.,2003; Dixit et al., 2001)* , but have some contradictions with others *(Rashidi et al., 1994; Fadilli et al., 2000; Abegaz et al., 2005)*. Variations of body mass in lambs depending on the mother's age range in the interval from 4.29 kg to 4.52 kg kg and statistically very significant(P<0.01) *(Petrović et al.,2009)*.

Lamb's Sex

Lamb's sex was significant for all traits. Interaction effects were significant between sex of lambs and birth year and also sex of lambs and herd for all traits. For all traits amount of body weight in male was more than female *(Osman, 1965; Rashidi et al., 2008; Nourian, 2000; Shahrodi Eftekhar et al., 2003; Ahmadi et al., 2004; Abegaz et al., 2005)*.

Birth Type

In this study, type of birth was significant for all traits. Interaction effect was significant between types of birth year for all traits. But Reference *(Shahrodi Eftekhar et al., 2003; Matika et al.,2003)* reported that birth type have no

significant effect on body weight in Kurdish (birth and weaning weights) and Sabi (birth weight and daily gain from birth to weaning) breeds, respectively. Type of birth has also expressed a significant effect on the body mass of lambs at birth and variations range from 4.31 kg (twins) to 4.59 kg (single) *(Petrović et al., 2009)*.

Herd

Herd and its interaction effect with birth year, lamb's sex and age of dam were significant for all traits. The same results were shown by the researchers who investigated the effects of herd on body weight *(Nourian, 2000; Neser et al., 2001; Yazdi et al., 1997)*.

Conclusions

Non- genetic factors were significant sources of variation for growth traits Including body weight and average daily gain and play an important role in expression of genetic potential. Therefore, effects of environmental factors need to account for the estimate of the best linear unbiased predicted value (BLUP) of Makuie sheep.

Uticaj faktora životne sredine na osobine porasta ovaca rase makuie

S. M. Rahimi, S. A. Rafat, S. Jafari

Rezime

Makuie ovca je rasa ovaca sa debelim repom koje se mogu naći u Iranu, provinciji Azerbejdžana. U istraživanju su ocenjivani ne-genetski parametri dobijeni iz podataka prikupljenih od 1993 do 2012. godine, u istraživačkoj stanici za makuie ovce provincije Iran u Zapadnom Azerbejdžanu. Ne-genetski parametri za težinu na rođenju, težinu na odbijanju (3 meseca), težinu u uzrastu od 6 meseci, 9 meseci i godinu dana, procenjeni su na osnovu podataka prikupljenih za 19 godina, korišćenjem SAS softvera. Srednje vrednosti najmanjih kvadrata bile su 4.11± 0.88, 21.50±3.50, 27.18±3.53, 28.57±4.44 i 34.21±3.88 kg za težinu na rođenju, uzrastu od 3, 6, 9 i 12 meseci, respektivno. Godina rođenja imala značajan uticaj na sve osobine dok je tip rođenja značajno uticao sve osobine ($P < 0.05$). Pol jagnjeta imao je značajan uticaj na sve osobine ($p<0,05$). Efekat starosti majke je bio značajan na sve osobine izuzev težinu u uzrastu od 9 meseci ($p <0,05$). Rezultati su pokazali da ne-genetski faktori imaju važnu ulogu u ispoljavanju genetičkog potencijala u jagnjadi.

References

ABBASI M.A., GHAFOURI-KESBI F. (2011): Genetic co(variance) components for body weight and body measurements in Makooei sheep in Asian-Aust, J Anim Sci, 24, 739-743.

ABEGAZ S., VAN WYK JB., OlIVIER JJ. (2005): Model comparisons and genetic and environmental parameter estimates of growth and the Kleiber ratio in Horro sheep, S. Afr. J. Anim. Sci, 35, 30-40.

AHMADI M., ROSHANFEKR A., ASADI KHASHOEI E., MOHAMMADI Y. (2004): The study of genetic and phenotypic parameters the some of growth traits Kermanshah Sanjabi sheep. J Agric Sci, 11, 91-98.

DASS G.S., ACHARYA R.M. (1970): Growth of Bikaneri Sheep. J Anim Sci, 31, 1-4.

DIXIT SP., DHILON JS., SING G. (2001): Genetic and non-genetic parameter estimates for growth traits of Bharat Merino lambs. Small Rumin Res, 42, 101-104.

FADILLI M.E., MICHAUX C., DETILLEUX J., LEROY PL. (2000): Genetic parameters for growth traits of the Moroccan Timahdit breed of sheep. Small Rumin Res, 37, 203-208.

KALANTAR M. (2003): Evalution of some environmental effect on Growth traits in Zandi sheep. J Agric Res, 4, 49-58.

KOMLOSI I. (2008): Genetic parameters for growth traits of the Hungarian merino and meat sheep breeds in Hungary. Appl Ecol Env Res, 6, 77-84.

MATIKA O., VAN WYK JB., ERASMUS GJ, BAKER RL. (2003): Genetic parameter estimates in Sabi sheep. Livest Prod Sci, 79, 17-28.

NESER FWC, ERASMUS G.J., VAN WYK JB. (2001): Genetic parameter estimates for pre-weaning weight traits in Dorper sheep. Small Rumin Res, 40,197-202.

NOURIAN E. (2000): Estimation genetic parameters for pre-weaning in Ghezel sheep.Master Science Thesis, University of Tarbyat Modares, Iran, 96.

OSMAN AH., BRADFORD GE. (1965): Effect Of environmental on phenotypic and genetic variation in sheep. Animal science, 24, 766-774.

OZCAN M., EKIZ B., YILMAZ A., CEYHAN A. (2005): Genetic parameter estimates for lamb growth traits and greasy fleece weight at first shearing in Turkish Merino sheep. Small Rumin Res, 56,215-222.

PETROVIĆ M. P., 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.

RASHIDI A. (1994): Estimation of factors affecting economical traits of Moghani sheep. Master Science Thesis, Ferdowsi University of Mashhad, Iran.

RASHIDI A., MOKHTARI MS., SAFARI JAHANSHAHI A., MOHAMMAD ABDI MR. (2008): Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. 74, 165-171.
SAADATNOORI M., SIAHMANSOOR S. (1986): Principles of sheep industry, 3rd ed. Ashrafi Publ Tehran Iran.
SHAHRODI EFTEKHAR F., BAHRINI MM., VEN DOULK D., DANESH MESGARAN M. (2002): The factor affecting some economical traits in Kermani sheep. J Agric Sci, 33, 395-402.
SHAHRODI EFTEKHAR F., SHIRI A., TWAKOLYAN J., DANESH MESGARAN M. (2003): Estimation of maternal effects on growth traits of Kurdish lamb in north of Khorasan. Pjoohesh Sazandegi, 50, 62-66.
VAEZ TORSHIZI R., EMAM-JOMEH N., NIK KHAH A., HEJAZI M. (1992): A study of pre weaning traits in a Baluchi sheep Flock. J Agric Sci, 23, 33-42.
YAZDI MH., ENGSTROM G., NASHOLM A., JOHANSSON K., JORIANI H., LILJEDAHL LE. (1997): Genetic parameters for lamb weight at different ages and wool production in Baluchi sheep. Small Rumin Res, 40, 197-202.

Received 5 May 2014; accepted for publication 16 June 2014

Biotechnology in Animal Husbandry 30 (2), p 193-201, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.32
DOI: 10.2298/BAH1402193P

RELATIONSHIP BETWEEN BIRTH WEIGHT AND BODY GROWTH CHARACTERISTICS OF LAMBS

M.P. Petrović[1], V.C. Petrović[1], Z. Ilić[2], D. Ružić Muslić[1], M.M. Petrović[1], J. Stojković[2], N. Maksimović[1],

[1]Institute for Animal Husbandry, Belgrade-Zemun
[2]Faculty for Agriculture, University of Priština, Lesak
Corresponding author: milanppet@yahoo.com
Original scientific paper

Abstract: Research was carried out in population of R2 generation Pirot pramenka x Pirot improved sheep during period of three years. Lambs were divided into three groups: I from 2.5 kg to 3.5 kg; II from 3.6 kg to 4.5 kg; III from 4.6 kg to 5.5 kg. Weight of lambs was controlled at birth, with 30, 60 and 90 days of age. Average body weight at birth of the tested lambs was 3.35 kg in the first group, 4.30 kg in the second group and 5.06 kg in the third group. At 30 days of age, the body weight of the lambs was 10.19 kg in the first group, 11.39 kg in the second and 12.49 kg in the third group. All these differences in body weight of lambs at birth were statistically highly significant (P□ 0.01). With 60 days of age, average body weight was 16.48 kg in the first group, 19.01 kg in the second and 20.49 kg in the third group. Differences between groups of lambs at this age were statistically very significant (P□0.01).On the end of experiment at 90 days of lambs age, we have found the following values of the body weight of lambs: 26.35 kg in the first group, when the second 30.49 kg and 28.93 kg in the third group. Differences between groups of lambs at this age were statistically very significant (P□0.01). At the age of 90 days maximum weight of the body was in the second group of lambs, or a group which body weight at birth occupied the mean of the population. Correlations between body weights of lambs vary from weak to mid-sized values. The highest values of correlation coefficients were found between body weight at birth and weight of lambs at 30 days of age.

Key words: lamb, birth weight, body growth, correlations

Introduction

Body weight of lambs at birth has an important role in achieving a good sheep production. In recent years, genetic improvement program for sheep in

Serbia have been primarily directed towards the improvement of growth and carcass traits (*Petrović et al., 2011*). An understanding of the factors which influence the development and growth of lambs will permit changes in the breeding and management schemes to minimize influences, which reduce production efficiency (*Bermejo et al., 2010).*

In addition to the number of lambs which is obtained by sheep, body weight of lambs at birth plays an important role as an initial power factor for the later development of the young organism (*Petrović et al., 2009; Riggio et al., 2008*). Body weight at birth also affect the vitality and mortality of lambs during development (*Morris et al., 2000; Cloete et al., 2001; Berhane and Arendonk, 2006; Vatankhah and Taleb, 2009*).

Information on factors influencing birth weight is of interest to farmers as well as the animal breeders (*Zapasnikiene, 2002; Caro Petrović et al., 2013*).

The objective of the present study was to estimate the effect of birth weight of crossbred lambs on growth during fattening period of 90 days.

Material and methods

Investigations were carried out in the area of Stara Planina. Material for research crossbreds R_2 generation of Pirot Pramenka x Pirot improved sheep. The experiment was conducted in the three-year period with 300 lambs of both sexes (50 female and 50 male). All lambs were kept in the same conditions, fed ad libitum hay and concentrates-18% of protein, and sucked two times a day. Depending on body weight at birth, lambs were divided into three groups: I from 2.5 kg to 3.5 kg; II from 3.6 kg to 4.5 kg; III from 4.6 kg to 5.5 kg. The body weight of lambs was controlled at birth, with 30, 60 and 90 days of age. Data analysis was performed using the statistical software SPSS 20 (2012). Procedures were used, one-way ANOVA, Pearson's correlations and the Dependent t-test (called the Paired-Samples T test).

Results and discussion

The research results are presented in tables 1-5. From table 1 we see that the average body weight at birth of the tested lambs was 3.35 kg in the first group, 4.30 kg in the second group and 5.06 kg in the third group.

Table 1. Descriptive statistics of lambs groups body weight from birth to weaning

Group	Age	Min	Max	Mean	Std. Error
I	1	2.70	3.50	3.3564	.01908
II		4.00	4.50	4.3071	.01728
III		4.80	5.50	5.0600	.01123
I	30	8.60	11.80	10.1911	.07466
II		10.00	13.40	11.3907	.07743
III		10.40	15.00	12.4914	.09551
I	60	14.15	18.70	16.4839	.09511
II		15.30	23.60	19.0143	.14509
III		16.50	24.60	20.4911	.15645
I	90	22.00	33.40	26.3566	.24581
II		24.60	34.80	30.4986	.23807
III		25.00	40.00	28.9336	.16205

From Table 2, we can notice that the difference in body weight of lambs between the first and the second group was 0.95kg, between the first and third groups of 1.70 kg, and the second and third groups of 0.75 kg. All these differences in body weight of lambs at birth were statistically highly significant (P □ 0.01). At 30 days of age, the body weight of the lambs (Table 1) was 10.19 kg in the first group, 11.39 kg in the second and 12.49 kg in the third group. The existing differences in the body weight of lambs in are shown in table 2, and were as follows: Differences between the first and the second group was 1.19 kg, between the first and third groups 2.30 kg, and the second and third groups 1.10 kg. All these differences in body weight of lambs at birth were statistically highly significant (P□ 0.01).

At the end of the second month with 60 days of age, average body weight (Table 1) was 16.48 kg in the first group, 19.01 kg in the second and 20.49 kg in the third group. Differences between groups of lambs at this age (table 2) were statistically very significant (P□0.01).

Table 2. Paired samples test of lambs groups body weight from birth to weaning

Pair of groups	Age	Paired Differences					t	Sig.
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
I- II	1	-.95071	.32018	.02706	-1.00422	-.89721	-35.133	.000
I - III		-1.70357	.25850	.02185	-1.74677	-1.66038	-77.977	.000
II- III		-.75286	.26128	.02208	-.79652	-.70920	-34.094	.000
I- II	30	-1.19964	1.38724	.11724	-1.43145	-.96783	-10.232	.000
I - III		-2.30036	1.65726	.14006	-2.57729	-2.02343	-16.424	.000
II- III		-1.10071	1.46737	.12402	-1.34591	-.85551	-8.876	.000
I- II	60	-2.53036	2.05492	.17367	-2.87374	-2.18698	-14.570	.000
I - III		-4.00721	2.19495	.18551	-4.37400	-3.64043	-21.601	.000
II- III		-1.47686	3.06654	.25917	-1.98928	-.96443	-5.698	.000
I- II	90	-4.14200	4.54922	.38448	-4.90218	-3.38182	-10.773	.000
I - III		-2.57700	3.72146	.31452	-3.19886	-1.95514	-8.193	.000
II- III		1.56500	3.34356	.28258	1.00628	2.12372	5.538	.000

Of particular importance are the results that we got on the end of experiment at 90 days of lambs age. We have found the following values of the body weight of lambs: 26.35 kg in the first group, while the second 30.49 kg and 28.93 kg in the third group. Differences between groups of lambs at this age were statistically very significant (P□0.01).

We can view (Table 1) that at the age of 90 days maximum weight of the body was in the second group of lambs, or a group which body weight at birth occupied the mean of the study population. On this occasion, confirming the biological principle that the vitality and adaptability of living organisms most pronounced in individuals who are in the morphological findings related in the middle of all the possible variants (*Petrović et al., 2011*). To similar findings in terms of the importance of body weight at birth on growth and vitality came from other authors (*Muir et al., 2000; Morris et al.,* 2003; Thompson et al., 2004). A different results found by *Hanford et al., (2003*) which stated that lambs with heavier birth weights have heavier weaning weights.

Table 3. Correlation between body weights of lambs in first group

Age of lambs					
		1	30	60	90
1	r	1	.176*	.020	.028
	Sig.		.037	.816	.742
30	r	.176*	1	-.048	.103
	Sig.	.037		.575	.225
60	r	.020	-.048	1	-.022
	Sig.	.816	.575		.798
90	r	.028	.103	-.022	1
	Sig.	.742	.225	.798	
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

According to the research of *London and Weniger (1995*) higher body weight of lambs at birth affects the higher birth weight of lambs at 60 days of age, while *Greenwood et al. (2002*) stated that, the low birth weight lambs are less mature than high birth weight lambs in aspects of metabolic and endocrine development, which may enhance their capacity for growth.

Correlation between body weights of lambs from birth to weaning may give a clearer picture of physical development of lambs. In tables 3, 4 and 5 are given the values of obtained correlation coefficients and their significance.

Table 4. Correlation between body weights of lambs in second group

Age of lambs					
		1	30	60	90
1	r	1	.389**	.262**	-.133
	Sig.		.000	.002	.117
30	r	.389**	1	-.066	-.310**
	Sig.	.000		.438	.000
60	r	.262**	-.066	1	.344**
	Sig.	.002	.438		.000
90	r	-.133	-.310**	.344**	1
	Sig.	.117	.000	.000	
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

From Table 3 we can observe that the lambs of the first group have significant correlation (P<0.05) only between the body weight of lambs at birth and the age of 30 days.

In other groups of lambs correlation coefficients have intermediate intensity values, a significance was determined between weight of the lambs at birth and 30 days and birth and 60 days (P<0.01), as well as the body weight of lambs between the 30 and 60 days, respectively, and 90 to 60 days (P <0.01).

Table 5. Correlation between body weights of lambs in third group

Age of lam	lambs				
		1	30	60	90
1	r	1	.342**	.052	-.014
	Sig.		.000	.545	.868
30	r	.342**	1	.405**	.177*
	Sig.	.000		.000	.036
60	r	.052	.405**	1	.216*
	Sig.	.545	.000		.010
90	r	-.014	.177*	.216*	1
	Sig.	.868	.036	.010	
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

Correlations between body weights of lambs in third group vary from weak to mid-sized values. Statistical significance (P<0.01) was found between body weight at birth and 30 days, weight of the 30 days and weight at 60 days, and between body weight at 30 days and 90 days of age (P<0.05). The research results we obtained in the third group of lambs show that there is a correlation between body weight at the age of 60 and 90 days (P<0.05).

The highest values of correlation coefficients of the body weight of lambs, as indicated by all three tables, were found between body weight at birth and weight of lambs at 30 days of age. This could be interpreted as a benefit greater weight at birth, in other words, the higher body weight of lambs at birth gives greater potential force for growth in the first month, in contrast to lambs with less weight and thus less power of growth (*Petrović et al., 2013*).

In the literature there are various research results related to this subject. *Sawalha et al. (2007*) reported a weak genetic correlation (0.21) between lamb viability and birth weight. *Hanford et al. (2003)* reported that correlation between birth weight and weaning weight was moderate for the Columbia breed. Birth weight and weaning weight were highly correlated on the direct and maternal genetic levels (*Cloete et al., 2003*). The association between body weight of lambs

at weaning and weight gain during the previous period ages have studied by many authors. *Ghafouri-Kesbi et al. (2011*) reported a positive correlation and secondary qualities of body weight of lambs. *Matika et al. (2001*) found that genetic correlations between birth weight and other weights up to 18 months were high (0.75-0.85) whilst the relationship between weaning, 12 month and 18 month weight was close to unity. *Fogarty (1995*) reported lower correlations between birth weight and later weights.

Conclusion

The research results influence of body weight of lambs at birth on the subsequent development of body weight showed the following. Higher body weight at the end of the experimental period-at the age of 90 days were the lambs' second groups. These are the lambs whose body weight at birth had the population average. This is a very important conclusion for science and farmers, as information that in selection should not force direction of getting lambs with high body weight at birth. Specifically, all variants of the left and right of the average population are less desirable, which means that it confirms the old wisdom of man "middle is gold."

Povezanost između mase tela pri rođenju i karakteristika porasta jagnjadi

M.P. Petrović, V.C. Petrović, Z. Ilić, D. Ružić-Muslić, M.M. Petrović, J. Stojković, N. Maksimović

Rezime

Istraživanja su sprovedena u populaciji R_2 generacije Pirotska pramenka x Pirotska oplemenjena ovca tokom perioda od tri godine. Jagnjad su bila podeljeni u tri grupe: I od 2,5 kg do 3,5 kg; II od 3,6 kg do 4,5 kg; III od 4,6 kg do 5,5 kg. Masa jagnjadi je kontrolisana na rođenju, sa 30, 60 i 90 dana starosti. Prosečna telesna težina na rođenju testiranih jagnjadi bila je 3,35 kg u prvoj grupi, 4,30 kg u drugoj grupi i 5,06 kg u trećoj grupi . Sa 30 dana starosti ,telesna masa jagnjadi bila je 10,19 kg u prvoj grupi, 11,39 kg udrugoj i 12,49 kg u trećoj grupi. Sve ove razlike u telesnoj masi jagnjadi na rođenju su visoko statistički značajne (P□0,01). Sa 60 dana starosti, prosečna telesna masa je bila 16,48 kg u prvoj grupi, 19,01 kg udrugoj i 20,49 kg u trećoj grupi. Razlike između grupa jagnjadi u ovom uzrastu

su statistički vrlo značajne (P□0,01). Na kraju eksperimenta sa 90 dana starosti, pronašli smo sledeće vrednosti telesne mase jagnjadi: 26.35 kg u prvoj grupi, 30,49 kg u drugoj grupi i 28,93 kg u trećoj grupi. Razlike između grupa jagnjadi u ovom uzrastu su statistički vrlo značajne (P□0,01). U uzrastu od 90 dana maksimalna masa tela je registrovana u drugoj grupi jagnjadi, ili grupi čija telesna masa na rođenju zauzima srednju vrednost populacije. Korelacija između telesne mase jagnjadi variraju od slabe do srednje vrednosti. Najveće vrednosti koeficijenata korelacije su pronađene između telesne mase na rođenju i mase jagnjadi sa 30 dana starosti.

References

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

BERMEJO L.A., MELLADO M., CAMACHO A., MATA J., ARÉVALO J.R., DE NASCIMENTO L. (2010): Factors Influencing Birth and Weaning Weight in Canarian Hair Lambs. J. Appl. Anim. Res., 37, 29-31.

CARO PETROVIC V., ILIC Z.Z., TENEVA A., PETROVIC M.P., SPASIC Z. LJ., PETROVIC M.M., RUZIC MUSLIC D. (2013): Study of the growth traits relationship of lambs in the postnatal development. Bulgarian Journal of Agricultural Science, 19, 4, 647-651.

CLOETE S.W.P., OLIVIER J.J., VAN WYK J.B., ERASMUS G.J., SCHOEMAN S.J. (2003): Genetic parameters and trends for birth weight, birth coat score and weaning weight in Merino lines divergently selected for ewe multiple rearing ability. South African J. Anim. Sci., 33, 248-256.

CLOETE S.W.P., GREEFF J.C., LEWER R.P. (2001): Environmental and genetic aspects of survival and early live weight in Western Australian Merino sheep. South Afr. J. Anim. Sci., 31, 123-130.

FOGARTY N.M. (1995): Genetic parameters for live weight, fat and muscle measurements, wool production and reproduction in sheep: a review. Animal Breeding Abstracts, 63, 101-143.

GHAFOURI-KESBI F., ALI ABBASI M., AFRAZ F.,BABAEI M., BANEH H., ROSTAM ABDOLLAHI ARPANAHI R.A. (2011): Genetic analysis of growth rate and Keleiber ratio in Zandi sheep. Tropical Animal Health and Production, 43, 6, 1153-1159.

GREENWOOD P.L., HUNT A.S., SLEPETIS R.M., FINNERTY K.D., ALSTON C., BEERMANN D.H., BELL A.W. (2002): Effects of birth weight and postnatal nutrition on neonatal sheep: III. Regulation of energy metabolism. J Anim Sci., 80, 2850-61.

HANFORD K.J., VAN VLECK L.D., SNOWDE G.D. (2003): Estimates of genetic parameters and genetic change for reproduction, weight, and wool characteristics of Targhee sheep. J. Anim. Sci., 81, 630–640.
LONDON C.J., WENIGER H. (1995): Investigations into traditionally managed Djallonké-sheep production in the humid and sub-humid zones of Asante, Ghana III. Relationship between birth weight, pre-weaning growth, and post-weaning growth of lambs. Journal of Animal Breeding and Genetics, 112, 431-453.
MATIKA O., VAN WYK., J.B., ERASMUS G.J., BAKER R.L. (2001): Phenotypic and genetic relationships between lamb and ewe traits for the Sabi sheep of Zimbabwe. South African Journal of Animal Science. 31, 215-222.
MORRIS S.T., KENYON P.R., BURNHAM D.L., EVERETT-HINCKS J.M. (2003): The effect of sward height on twin and triplet lamb birth weights and survival rates to weaning. Proceedings of the New Zealand Society of Animal Production, 63, 152-154.
MUIR P.D., SMITH N.B., WALLACE G.L., FUGLE C.J., BOWN M.D. (2000): Maximizing lamb growth rates. Proceedings of the New Zealand Grassland Association, 62, 55-58.
PETROVIĆ M.P., ILIC Z., CARO-PETROVIĆ V. (2013): Sheep and goat breeding (Ovčarstvo i kozarstvo). Institute for Animal Husbandry, 520 p.p.
PETROVIĆ M.P., 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., 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.
RIGGIO V., FINOCCHIARO R., BISHOP S.C. (2008): Genetic parameters for early lamb survival and growth in Scottish Blackface sheep. J. Anim. Sci., 86, 1758-1764.
SAWALHA R.M., CONINGTON J., BROTHERSONE S., VILLANUEVA B. (2007): Analyses of lamb survival of Scottish Blackface sheep. Anim., 1, 151-157.
THOMPSON B.C., MUIR P.D., SMITH N.B. (2004): Litter size, lamb survival, birth and twelve week weight in lambs born to cross-bred ewes. Proceedings of the New Zealand Grassland Association, 66, 233–237.
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.
ZAPASNIKIENE B. (2002): The effect of age of ewes and lambing season on litter size and weight of lambs. Veterinaria Zootechnika, 41, 112-115.

Received 6 May 2014; accepted for publication 26 June 2014

Biotechnology in Animal Husbandry 30 (2), p 203-214, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.043'636.3
DOI: 10.2298/BAH1402203Y

DIETARY INFLUENCE ON FATTY ACID CHARACTERISTICS OF LAMB CARCASS IN RELATION TO PROTEIN SOURCE

M. R. Yossifov

Department „Animal nutrition and feed technologies", Institute of Animal Science, Kostinbrod, Republic of Bulgaria
Corresponding author: m_vet@abv.bg
Original scientific paper

Abstract: The aims of our study were to evaluate the effect of different protein supplements on fatty acid (FA) composition (%), profile (ratios and indices), etc. of carcass in lambs fed iso-caloric, iso-nitrogenous and equal in PDI and Ca: P ratio high- concentrate rations. Regional breed (Bulgarian Synthetic Dairy Population) lambs were fed cereal-based diets with different protein supplement - control diet with sunflower meal (SFM) or a DDGSc diet containing dried distillers' corn grains with solubles (DDGSc). Animals were slaughtered after 87-d feedlot period. Fat tissue extracted from carcass was analyzed for FA profile. There were significantly higher ($p< 0.01$) performance (FBW= 38.90 vs. 35.1 kg and HCW= 5.24 vs. 4.67 kg) of DDGSc diet on lamb performance. Feeding 37.6 % (DM basis) DDGSc significantly increased the content of C18:2 ($p< 0.05$) but decreased n_3 PUFA and total long chain n_3 FA ($p< 0.05$) compared with control group. DDGSc increased n_6 ($p= 0.06$), PUFA ($p= 0.07$) and PUFA / SFA ratio ($p= 0.10$), but decreased MUFA ($p= 0.10$). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and are characterized with significant Pearson's correlation coefficients ($R> 0.56$). In regards to obtained results, dietary DDGS inclusion altered the fatty acid profile and indices of lipids of lamb carcass.

Key words: Lamb, Protein Source, Carcass, DDGSc, Fatty Acids Profile and Indices

Introduction

The Bulgarian Synthetic Dairy Population (*BSDP*) is the major breed of total sheep population – more than 1 million head or over 70 % (*www.noa.bg*).

Protein supplements, fed to improve growth rate at intensive feeding system, affecting dietary lipid composition in concentrate- based diets (*Webb and O'Neill, 2008*), e.g. influenced meat quality (*Wood et al., 2003*). Ruminants disposed gather of feedstuffs enrished in PUFA with different protection against rumen biohydrogenation. Such forage is dried distillers' corn grain with solubles – nutrient-dense by-product, rich in protected amino and fatty acids. Latter, by-passing the rumen, were in regards to increasing proportions of monounsaturated (MUFA), polyunsaturated (PUFA) FA and n_6/n_3 PUFA ratio in ruminant meat (*Williams, 2000; Palmquist, 2009*).

The fatty acid profile of animal products plays an important role in human nutrition. High- quality meat, stamped as healthful, is high in UFA/SFA and DFA/OFA ratios. Simultaneously, the demand for low-fat meat poor in SFA and rich in CLA has been increased in order to avoid health risk associated with excessive fat intake (*Scollan et al., 2006*). The FA with a potential negative effect on human health is saturated FA (SFA), unlike favourable proportions of beneficial FA such as C18:1, conjugated linoleic acid (CLA) and PUFA (especially n_3).

The present study is an extension of our wide investigation on DDGSc and its nutritive, metabolic and productive peculiarities as a protein and / or energy supplement and report effects on fatty acid profile and indices of lamb meat (*Yossifov, 2012; Yossifov, 2012a; Yossifov and Kozelov., 2012; Yossifov and Kozelov., 2012a; Yossifov et al., 2012; Yossifov, 2013; Yossifov, 2013a; Yossifov, 2014*).

Analyses on the fatty acid profile and indices of carcasses obtained from ruminants, fed DDGSc- based diets are still limited and needs more thoroughness. In this regards, our hypothesis was that DDGSc lipid profile would be affected the fatty acid content of lamb carcass.

Material and methods

Animals and management. Detailed descriptions of experimental design, animals and diets composition were reported in a companion paper (*Yossifov et al., 2012*). Briefly, weaned lambs (Bulgarian Synthetic Dairy Population, n= 32, age = 59 d, initial BW= 16.69±2.53 kg) were randomly allotted by BW, sex, type of litter. Dietary treatments (table 1) were iso-caloric, iso-nitrogenous and equal in PDI and Ca: P ratio – 1./ control (CON) – with sunflower meal (SFM), and 2./ experimental (EXP) – with DDGSc. Lambs were fed twice daily to approximately 5 % weigh-back to ensure *ad libitum* consumption. The concentrate (offered at 8.00 and 14.00 h) and forage (offered at 10.00 and 16:00 h) were fed separately throughout the experimental period.

Slaughter and sample collection. On d 87,5 male lambs per diet were randomly selected, weighted in two consecutive days (to calculate final BW) and slaughtered (*Yossifov et al., 2012*). Dressed carcass was weighted (hot carcass weight (HCW), kg) and after 24 h cold storage (at 4 ^{o}C) was divided into halves. The right carcass half was weighed and dissected into compound tissues (meat, fat and bone), expressed as absolute and relative values of the right half. Dissected meat and fat were mixed, ground and sampled for consecutive FA analysis. Concentrates and forage were also evaluated for individual FA.

Table 1. Diet composition[1] and nutrient content (% DM) of sunflower meal (CON) and DDGSc (EXP) protein supplemented diets

	CON	EXP
Meadow hay	36.6	35.6
Sunflower meal	26.3	-
DDGSc	-	37.6
Triticale	17.2	16.7
Corn	17.2	6.7
Vitamin-mineral premix	2.7	3.4
Feed units for gain, FUG	1.2	1.2
CP, %	19	18
PDI, % CP	62	62
Ca/P ratio	2	2

[1] Yossifov et al., 2012

DDGSc-dried distillers' corn grains with solubles; CP-crude protein; PDI-protein digestible in small intestines; Ca-calcium; P-phosphorus.

Measurements and calculations. Total lipids extraction (*Bligh and Dyer, 1959*) and fatty acid methyl esters (FAME) isolation (*Christie, 1973*) of the samples were described in details at *Yossifov* (*2014*). Samples lipids were extracted by homogenising in chloroform: methanol: water (1:2:8 v/v/v) and FAME were prepared for 14 h with 0.01 % solution of sulphuric acid in dry methanol. The FA profile of triacylglycerols was determined by GLC analysis with chromatograph C Si 200 equipped with a 60 m capillary column (TR-FAME) with 0.25 mm inner diameter and coating thickness of 0.25 μm. Hydrogen was used as a carrier gas. The temperature programme started at 160 ^{o}C (held for 0.2 min) and increasing at a rate of 5 ^{o}C.min^{-1} to 220 ^{o}C, where it's maintained for 5 min. Injector' and detector' temperature set points were stated at 200 ^{o}C. Individual FAME peaks were identified by comparison with reference methyl esters. FAs were expressed as a weight percentage of total FAs (*Christie, 1973*). A number of indices (fatty acid and healthy) and enzyme activities were calculated (*Yossifov, 2014*): total of saturated FA (SFA), hypercholesterolemic FA (OFA), total amount of

monounsaturated fatty acids (MUFA), n_6 fatty acids, n_3 fatty acids, total of polyunsaturated fatty acids (PUFA); total of long-chain n_3 fatty acids (*total LC* n_3), total of unsaturated fatty acids (UFA), sum of desirable fatty acids (DFA), atherogenicity index (AI), thrombogenic index (TI), hypocholesterolemic / hypercholesterolemic *index* (h/H), index of D_9 desaturase enzyme activity on the conversion of C16:0 and C18:0 to C16:1 n_9 and C18:1 n_9 ($IDSA_{16:0}$ and $IDSA_{18:0}$), stearoyl CoA desaturase (SCD) and elongase activity (EAI).

Statistical analyses. Carcass FA data were analysed by Statistical Package (*Microsoft Office, 2007*). Diet was used as the treatment effect, with individual animal as the experimental unit. All obtained data are offered as mean, standard deviation (SD), and simple variance (Var). The results were submitted to calculate standard error of mean (SEM) to assess the influence of dietary protein source (DDGSc vs. SFM) on the meat FA profile. Means were compared throughout the Student t-test and differences with level of significance below $p< 0.05$ were considered as significant. Pearson's correlation coefficient between variables was also calculated as a measure of the strength and direction of the linear relationship between two variables.

Results and discussion

Animal performance. A summary of diet composition (table 1) and animal performance (table 2 and 3), as reported by *Yossifov et al.* (*2012*), illustrated the effect of DDGSc inclusion. Feed intake was increased (6 %), but protein intake (CP) was similar (22 %). Nutrient differences between the protein supplements affected fat intake – 1.8 vs. 7.3 % for CON and EXP, respectively.

Table 2. Mean daily intake of diet DM and nutrients (g)

Item	Forage		Concentrate		Total ration	
	CON	EXP	CON	EXP	CON	EXP
DM	327.31	353.81	764.36	799.16	1091.70	1152.98
CP*	32.68	35.32	188.40	180.40	221.10	215.70
EE*	4.21	4.55	13.30	68.70	17.50	73.20
CF*	138.20	149.40	95.63	51.92	233.80	201.30

* g.kg DM^{-1}

DM-dry matter; CP-crude protein; EE-ether extract; CF-crude fiber; CON-control diet; EXP-experimental diet

Final body weight ($p<0.01$) and hot carcass weight ($p<0.05$) were significantly increased (35.10 and 16.29 vs. 38.90 and 18.45 kg for CON and EXP,

respectively). Meat (5.24 vs.4.67 kg) and fat yield (1.45 vs. 1.15 kg) were higher in DDGSc- based diet compared with CON (table 3).

Table 3. Lamb performance fed DDGSc vs. SFM- based diets (kg)

Item	Treatment								SEM	P-value
	SFM				DDGSc					
	Avr.	%	SD	Var	Avr.	%	SD	Var		
Final body weight	35.10	100.0	3.13	9.80	38.90	110.8	1.98	3.92	1.01	<0.01
Hot carcass weight	16.29	100.0	2.25	5.06	18.45	113.3	1.04	1.08	0.63	<0.05
Meat yield[1]	4.67	58.3	0.64	0.41	5.24	58.4	0.35	0.04	0.05	0.98
Separable fat[1]	1.15	15.2	0.18	0.03	1.45	16.2	0.27	0.07	0.10	0.56

[1] Right side slaughter weight ; SFM-sunflower meal; DDGSc-dried distillers' corn grains with solubles

Diet FA composition. Fatty acid composition of diets is presented in table 4. Percentages of FAME were similar among the diets, but greater levels of EE (1.8 vs. 7.3 %) increased the differences at ingested FA. Weights of C16:0, C18:1, C18:2, as well as SFA, DFA, OFA, MUFA, PUFA, UFA and C18:2/C18:3 ratios were greater in DDGSc- based diets. Established concentrations of the weight percentages of FAME are presented in table 5. The lipid content of the lamb carcass for the respective diets was 3.9 g/100 g (CON) and 4.6 g/100 g (EXP), but the differences were not significant (p= 0.12, SEM= 2.3) (*Yossifov, 2013*).

There was a tendency for EXP diet (table 5) to increase PUFA levels compared to CON (p=0.07). C18:2 concentrations were the most eminent PUFA member, significantly affected by DDGSc supplement (p<0.05) than the other treatment. DDGSc altered rumen environment and higher amounts of C18:2 escaping biohydrogenation in rumen, resulting in less C18:0 and C18:1 in carcass. Thus confirmed the other reports (*Kim et al., 2007*). There was also a hardy trends of increased n_6 elongation and desaturation products, e.g. increased n_6 FA when feeding DDGSc (p= 0.06) compared to the SFM (table 5). It's in agreement with some authors (*Gill et al., 2008*). In contract, n_3 FA and total LC n_3 FA contents of the CON diet were significantly higher (p<0.05) compared to the EXP. The numeric decreases of C18:0 in EXP differed from data obtained by others (*Gill et al., 2008; Depenbusch et al., 2009a*).

DDGSc increased C18:3, MUFA/SFA, PUFA/SFA, C18:2/C18:3, C18:2/CLA but decreased C18:1, MUFA and SCD.

Table 4. Fatty acid content of sunflower meal (CON) and DDGSc (EXP) protein supplemented diets

Item	FA profile (% identified FA)			Ingested FA ($g.head^{-1}.d^{-1}$)	
	Hay	CON	EXP	CON	**EXP**
C14:0	1.16	ND	ND	0.05	**0.06**
C16:0	22.05	12.12	14.80	2.49	**9.56**
C18:0	2.18	2.30	2.86	0.38	**1.74**
C18:1	10.38	28.67	29.65	3.93	**17.44**
C18:2	29.76	54.90	50.99	7.98	**30.60**
C18:3	34.47	2.01	1.71	1.85	**2.72**
SFA	25.39	14.42	17.66	2.92	**11.36**
OFA	23.21	12.12	14.80	2.54	**9.62**
MUFA	10.38	28.67	29.65	3.93	**17.44**
PUFA	64.23	56.91	52.70	9.84	33.32
UFA	74.61	85.58	82.35	13.76	50.76
MUFA/SFA	0.41	1.99	1.68	1.35	1.54
PUFA/SFA	2.53	3.95	2,98	3.37	2.93
UFA/SFA	2.94	5.93	4.66	4.71	4.47
DFA	76.79	87.88	85.21	14.14	52.51
(C18:0+C18:1)/C16:0	0.57	2.56	2.20	1.73	2.01
C18:2/C18:3	0.86	27.31	29.82	4.30	11.25

CON-control diet; EXP-experimental diet; FA-Fatty acids; SFA-saturated FA; OFA-Undesirable FA; DFA-Desirable FA; MUFA-Monounsaturated FA; PUFA-Polyunsaturated FA; UFA-Unsaturated FA; LC-Long-chain; IDSA16:0-Index of D9 desaturase enzyme activity on the conversion of C16:0 to C16:1 n9; IDSA18:0-Index of D9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n9; CLA-Conjugated linoleic acid; AI-Atherogenicity Index; TI-Thrombogenic index; h/H-Hypocholesterolemic/hypercholesterolemic index; SCD-Stearoyl CoA desaturase; EAI-Elongase activity; ND – not detectable

The values of human healthy indices, as AI and TI, were higher in EXP (0.81, 1.09 and 0.99, 1.30, respectively) compared with CON. Contrary, h/H value was lower (1.70 vs. 1.51, respectively).

The indices of D_9 desaturase enzyme activity on the conversion of C16:0 and C18:0 to C16:1 and C18:1 was greater in CON than EXP. The lower desaturase activity and higher CLA supported lower substrate (C18:1) availability (Table 5). EAI and SCD values were lower in EXP (0.49 and 0.58) than CON (0.52 and 0.66). In regards to obtained FA profile and indices, more or less carcass FA reflects ruminal FA profile (*Vasta et al., 2009*).

Table 5. Effect of protein supplement on FA profile and indices of lamb carcass

	CON			EXP			SEM	P-value
	Avr.	± SD	Var.	Avr.	± SD	Var.		
C14:0	4.30	0.70	0.49	5.36	1.70	2.88	0.43	0.23
C15:0	0.77	0.25	0.06	0.83	0.25	0.06	0.07	0.72
C16:0	24.59	2.41	4.82	26.89	2.19	3.55	1.09	0.32
C16:1	1.93	0.90	0.82	1.78	0.52	0.27	0.22	0.75
C17:0	1.90	0.41	0.17	1.54	0.35	0.12	0.13	0.18
C18:0	15.94	2.21	4.90	14.95	2.80	7.85	0.77	0.55
C18:1	42.80	2.13	4.53	38.07	1.99	2.88	1.55	0.13
C18:2	5.44	1.04	1.07	8.32	2.21	4.90	0.70	< 0.03
C18:3	0.43	0.07	0.01	0.51	0.08	0.01	0.03	0.15
Total CLA	0.71	0.09	0.01	0.78	0.20	0.04	0.05	0.48
C20:4	0.98	0.40	0.16	0.83	0.35	0.12	0.12	0.55
C20:5	0.11	0.03	0.01	ND			0.01	< 0.001
C22:5	0.15	0.06	0.01	0.13	0.08	0.01	0.02	0.71
SFA	47.50	2.39	5.70	49.57	3.61	13.04	0.98	0.31
OFA	31.55	1.47	2.10	34.62	2.30	4.73	1.60	0.37
DFA	67.78	3.37	11.30	64.59	6.24	38.94	1.59	0.34
MUFA	44.73	1.90	3.60	39.85	1.55	3.84	1.48	0.10
n_6	6.85	1.31	1.72	9.66	2.52	6.33	0.76	0.06
n_3	0.26	0.09	0.01	0.13	0.08	0.01	0.03	< 0.04
PUFA	7.11	1.36	1.84	9.79	2.53	6.40	0.75	0.07
(C18:0+C18:1)/C16:0	2.41	0.33	0.11	2.05	0.62	0.39	0.16	0.28
Total LC n_3	0.26	0.09	0.01	0.13	0.08	0.01	0.03	< 0.04
UFA	51.84	2.27	5.16	49.64	3.54	4.56	0.96	0.28
MUFA/SFA	0.95	0.09	0.01	0.81	0.17	0.03	0.04	0.15
PUFA/SFA	0.15	0.03	0.01	0.20	0.04	0.01	0.01	0.10
UFA/SFA	1.10	0.11	0.01	1.01	0.14	0.02	0.04	0.31
C18:2/C18:3	12.58	1.89	2.59	16.49	2.25	3.07	1.18	0.10
C20:4/C20:5	11.27	1.85	2.42	NA			0.58	< 0.001
C18:2/CLA	7.70	1.16	1.34	11.48	4.66	21.70	1.19	0.12
C18:3/CLA	0.62	0.07	0.01	0.68	0.19	0.03	0.04	0.48
AI	0.81	0.12	0.01	0.99	0.30	0.09	0.07	0.24
TI	1.09	0.16	0.02	1.30	0.34	0.11	0.09	0.24
h/H	1.70	0.22	0.05	1.51	0.39	0.15	0.10	0.37
IDSA16:0	7.13	3.17	10.10	6.10	0.94	0.89	0.72	0.51
IDSA18:0	72.93	2.73	7.45	71.87	1.60	2.58	0.69	0.47
SCD	0.52	0.02	0.01	0.49	0.04	0.00	0.01	0.12
EAI	0.66	0.13	0.02	0.58	0.19	0.04	0.05	0.48
ND – not detectable; NA-not available								

Carcass concentration of C18:1, C18:2 and C18:3 have been shown to be highly correlated (*Nuernberg et al., 2005; Aldai et al., 2009*).

Pearson's correlation coefficients. The results of the regression analysis of the experimental data are presented graphically (Fig. 1, 2, 3 and 4). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and as could be seen below, are characterized with significant Pearson's correlation coefficients (R> 56).

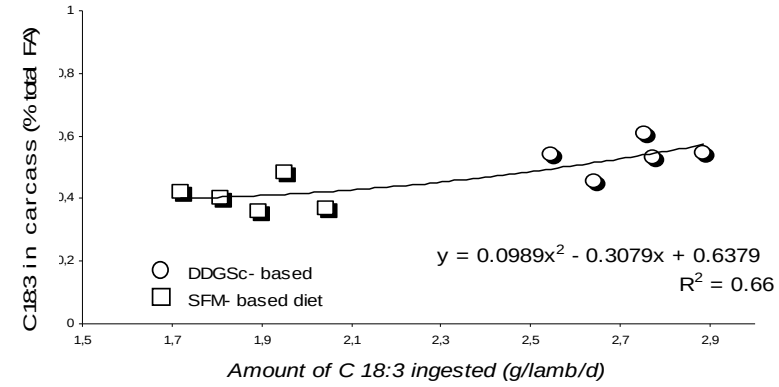


Figure 1. Relationship between the level of carcass C18:3 (% total FA) and amount of C18:3 ingested (g.lamb^{-1}.d^{-1})

In the first graph (Fig. 1) the amount of C18:3 as percentage of total FA in carcass displayed high coefficient of determination (R= 0.66) with concentrations of ingested C18:3 (g.lamb^{-1}.d^{-1}).

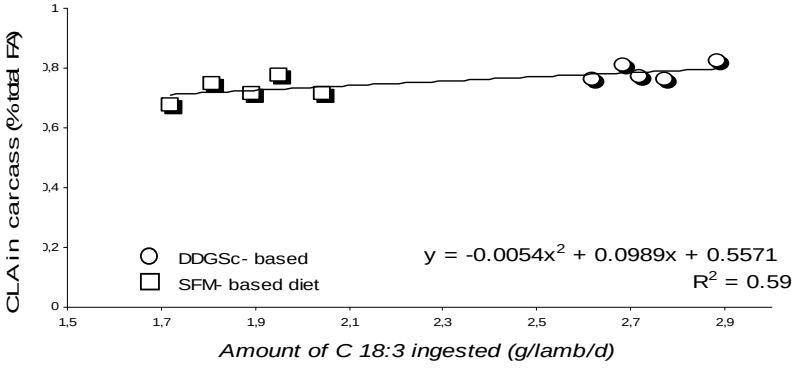


Figure 2. Relationship between the level of CLA in lipids of carcass (% total FA) and amount of C18:3 ingested (g.lamb^{-1}.d^{-1})

Similar trends were observed between total amount of carcass CLA (% total FA) and level of ingested C18:1 (R= 0.56) or C18:3 (R= 0.59) – fig. 2 and 3.

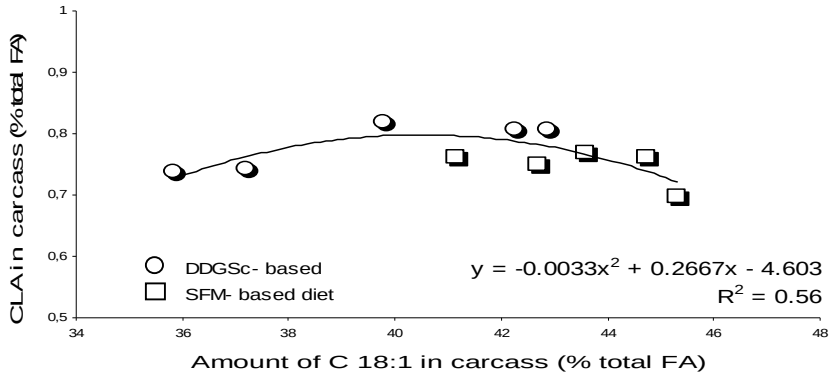


Figure 3. Relationship between the level of CLA in lipids of carcass (% total FA) and C18:1 (% total FA)

The relationship between the values of CLA in lamb carcass and index of D_9 desaturase enzyme activity on the conversion of C18:0 to C18:1 n_9 is revealed with high coefficient of determination (R= 0.73) – fig. 4.

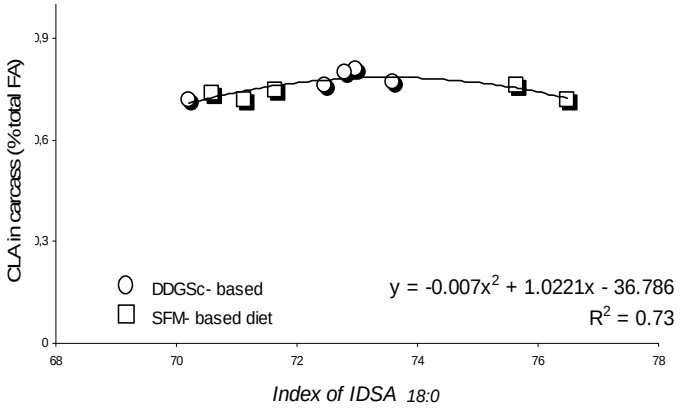


Figure 4. Relationship between the level of CLA in lipids of carcass (% total FA) and IDSA $_{18:0}$

Conclusion

The tested protein supplements altered fatty acid profile and indices (% and ratios) of lamb carcass. In this regards, differences in ruminal biohydrogenation

were related to the supplement source' FA profile as well as factors altered rumen condition, e.g. rumen environment. DDGSc- based diet significantly increased the content of C18:2 ($p< 0.05$) but decreased n_3 PUFA and total long chain n_3 FA ($p<0.05$) compared with control. DDGSc increased n_6 ($p= 0.06$), PUFA ($p= 0.07$) and PUFA / SFA ratio ($p= 0.10$), but decreased MUFA ($p= 0.10$). Examined relationships between ingested FA and carcass FA in slaughtered lambs shows good parity and are characterized with significant Pearson's correlation coefficients ($R> 0.56$). In regards to obtained results, dietary DDGS inclusion altered the fatty acid profile and indices of lipids of lamb carcass.

Acknowledgement

This work was accomplished in order to project Ж 82, Bulgarian Agricultural Academy. Author would like to thanks to barn stuff (sin. exp. *Ganka Boycheva*) and Dept. "Ecology and quality" for support during the FA analysis.

Uticaj obroka na profil masnih kiselina jagnjećih trupova u odnosu na izvor proteina

M. R. Yossifov

Rezime

Cilj našeg istraživanja je bio da se ispita uticaj različitih proteinskih dodataka na sastav masnih kiselina (FA) (% , profil (koeficijenti i indeksi), itd, trupa jagnjadi hranjenih izo-kaloričnim, izo-azotnim koncentratnim obrocima, obrocima sa jednakim PDI i odnosom Ca : P. Jagnjad regionalna rase (Bugarska sintetička mlečna populacija) su hranjena obrokom na bazi žitarica sa različitim proteinskim dodacima - kontrolni obrok sa suncokretovom sačmom (SFM) ili obrok sa DDGSc koji sadrži sušenu destilovana kukuruza zrna sa rastvorljivim materijama (DDGSc). Životinje su zaklane posle 87 dana tova. Masno tkivo ekstrahovano iz trupa je analizirano na FA profil. Proizvodne performanse jagnjadi na DDGSc ishrani su bile značajno veće ($p< 0,01$) (FBV = 38,90 u poređenju sa 35,1 kg i HCW = 5,24 prema 4,67 kg). Ishrana sa 37,6 % (na bazi SM) DDGSc značajno je povećala sadržaj C18:2 ($p<0,05$), ali je smanjena n_3 PUFA i ukupne masne kiseline dugog lanca n_3 FA ($p<0,05$) u poređenju sa kontrolnom grupom. DDGSc je uticao na povećanje n_6 ($p=0,06$) PUFA ($p=0,07$) i PUFA/SFA odnosa ($p=0,10$), ali je uticao na smanjenje MUFA ($p=0,10$). Ispitivani odnosi između unete FA i FA u trupovima zaklane jagnjadi pokazuje dobar paritet i karakterišu ih značajni koeficijenti Pearsonov-e korelacije ($R>0,56$). U vezi sa dobijenim

rezultatima, uključivanje DDGS u obrok je uticalo na menjanje profila masnih kiselina i indeksa lipida jagnjećeg trupa.

Reference

ALDAI N., DUGAN M., ROLLAND D., KRAMER J. (2009): Survey of the fatty acid composition of Canadian beef: Backfat and longissimus lumborum muscle. Canadian Journal of Animal Science, 89, 3, 315–329.

BLIGH E., DYER W. (1959): A rapid method of total lipid extraction and purification. Canadian Journal of Biochemical Physiology, 37, 911.

CHRISTIE W. (1973): Lipid analysis. Pergamon Press, New York.

DEPENBUSCH B., COLEMAN C., HIGGINS J., DROUILLARD J. (2009a): Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. Journal of Animal Science, 87, 2653–2663.

GILL R., VANOVERBEKE D., DEPENBUSCH B., DROUILLARD J., DICOSTANZO A. (2008): Impact of beef cattle diets containing corn or sorghum distiller's grains on beef colour, fatty acid profiles, and sensory attributes. Journal of Animal Science, 86, 923–935.

KIM S., ADESOGAN A., BADINGA L., STAPLES C. (2007): Effects of dietary n-6:n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. Journal of Animal Science, 85, 706–716.

VASTA V., MELE M., SERRA A., SCERRA M., LUCIANO G., LANZA M., PRIOLO A. (2009): Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. Journal of Animal Science, 87, 2674–2684.

NUERNBERG K., NUERNBERG G., ENDER K., DANNENBERGER D., SCHABBEL W., GRUMBACH S. (2005): Effect of grass vs. concentrate feeding on the fatty acid profile of different fat depots in lambs. European Journal of Lipid Science and Technology, 107, 10, 737–745.

SCOLLAN N., HOCQUETTE J., NUERNBERG K., DANNENBERGER D., RICHARDSON I., MOLONEY A. (2006): Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Science, 74, 17-33.

PALMQUIST D. (2009): Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. Professional Animal Scientist, 25, 3, 207–249.

WWW.NOA.BG

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

WILLIAMS C. (2000): Dietary fatty acids and human health. Annals Zootechnie, 49, 3, 165-180.
WOOD J., RICHARDSON R., NUTE G., FISHER A., CAMPO M., KASAPIDOU E., SHEARD P., ENSER M. (2003): Effects of fatty acids on meat quality: a review. Meat Science, 66, 21-32.
YOSSIFOV M. (2012): Influence of different protein supplements on sheep milk quantity and quality. XV International Feed Technology Symposium "Feed-to-food", Novi Sad, 3 – 5. 10.12, Available in: http://*www.uns.ac.rs*.
YOSSIFOV M. (2012a): Establishment feeding value of wheat dried distillers' grains with solubles (DDGSw) as protein source in small ruminants' diets. Proceedings of International Scientific Conference „Traditions and modernity in Veterinary Medicine", 29.11-02.12.12, Yundola, Bulgaria, University of Forestry.
YOSSIFOV M., KOZELOV L. (2012): Ingredient profile and nutritive value: corn dried distillers' grains (DDGc) vs. corn dried distillers' grains with solubles (DDGSc). Proceedings of 6-th CEFood Congress, Novi Sad – Serbia, 23-26.05.12, 1475-1481, Available in: http://www.*cefood2012.rs/ uploads/.../1st-announcement.p...*
YOSSIFOV M., KOZELOV L., DIMOV K. (2012): Effect of Dried Distillers' Grains with Solubles from corn fed on fattening lambs. Agricultural Science and Technology, 4, 3, 223-227.
YOSSIFOV M. (2013): Utilization by-products of biofuel industry in sheep diets. Ph. D. Thesis (Abstract), Available at *http://ias.bg/images/PDF/ Avtoreferat_Iosifov.pdf*.
YOSSIFOV M. (2013a): Nutritional characteristics and cumulative effects in dry grind bioethanol processing chain: corn – dried distillers' grains with solubles from corn (DDGSc). I. Mineral components, Proceedings of International Scientific Conference „Traditions and modernity in Veterinary Medicine", 29.11-0112.13 Yundola, Bulgaria, University of Forestry.
YOSSIFOV M. (2014): Effect of dietary rapeseed meal on fatty acid profile of lamb carcass. Animals, S-14-00189.

Received 24 February 2014; accepted for publication 12 April 2014

Biotechnology in Animal Husbandry 30 (2), p 215-223, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.32
DOI: 10.2298/BAH1402215I

SURVIVABILITY OF LAMBS IN RELATION TO THEIR DAM'S HAEMOGLOBIN VARIANTS

A.O. Iyiola-Tunji[1], G.N. Akpa[2], B.I. Nwagu[3], I.A. Adeyinka[3]

[1]National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria
[3]National Animal Production Research Institute, Ahmadu Bello University, Zaria, Nigeria
Corresponding author: tunjiyiola@yahoo.com
Original scientific paper

Abstract: A total of 65 Yankasa, 23 Uda and 16 Balami ewes were mated to 4 Yankasa, 3 Uda and 3 Balami rams in a diallel breeding pattern to produce 192 lambs within 9 genotypes, which were used to study survivability of lambs in relation to their dam's haemoglobin variants.Blood samples (5ml) were collected from 104 ewes and 10 rams through jugular venepuncture. Electrophoresis was carried out in a Shandon electrophoresis tank on cellulose acetate strips.Each of the 9 lamb genotypes had very high proportion of HbAB. The dam's haemoglobin type BB (HbBB) were only found in YK X YK, UD X UD, YK X UD, BL X YK and BL X UD lambs at birth and 90-Day. Survivability of lamb that were given birth to by dams with haemoglobin type AB (HbAB) is highest in the studied populations from birth to 360-Day. Lambs with HbAB should be selected for improved survivability of sheep in Northern Nigeria.

Keywords: Nigerian sheep, haemoglobin variants, electrophoresis, survivability

Introduction

Livestock breeds, including the domestic sheep (*Ovisaries*), have been characterized for variations in the major blood proteins and enzymes which are used as genetic markers. Such proteins are albumin (*Margetin and Malik, 1982*), ceruloplasmins (*Graetzeret al., 1964; Bhat, 1986*), vitamin D-binding protein (*Ibeagha-Awemu and Erhardt, 2004),* haemoglobin and transferrin *(Braend, 1972; Baruah and Bhat, 1980; Margetin and Malik, 1982; Bhat, 1986; Henkeset al., 1994; Di Stasio, 1997).* Some of the enzymes are amylase *(Bhat, 1986),* carbonic anhydrase *(Margetin and Malik, 1982),* malic enzyme, NADH diaphorase and catalase *(Tsunoda and Douge, 1990; Henkeset al., 1994).*

Many studies in sheep have already linked these markers to production traits and environmental adaptation *(Vicovan and Rascu, 1989; Charon et al., 1996; Salakoet al., 2007).* Information on blood proteins has also been extensively used for parentage control *(Francois et al., 1992)* and to study the genetic relationships among sheep breeds (*Buis and Tucker, 1983; Ordas and San Primitivo, 1986; Mwacharoet al., 2002).* However, the polymorphism of these important blood proteins had not being linked to survivability traits in the populations of sheep of Northern Nigeria (Balami, Uda and Yankasa sheep). Study of the influence of variations in haemoglobin genotype and its effect on survivability of different lamb genotype can help in selection of sheep on the basis of lambs with the best survival rate. This study was therefore aimed at monitoring the level of survivability of lambs in relation to their dam's haemoglobin variants.

Materials and Methods

This study was conducted at the Sheep Project Unit of Small Ruminant Research Programme (SRRP) of National Animal Production Research Institute (NAPRI), AhmaduBelloUniversity, Shika-Zaria. Three breeds of sheep that are found predominantly in Northern Nigeria were used for this study. They were Balami, Uda and Yankasa. A total of 65 Yankasa, 23 Uda and 16 Balami ewes were mated to 4 Yankasa, 3 Uda and 3 Balami rams in a diallel breeding pattern to produce 192 lambs within 9 genotypes.

Blood samples (5ml) were collected from 104 ewes and 10 rams through jugular venepuncture. The blood samples were placed in ethylene diamine tetra-acetic acid (EDTA) tubes to prevent coagulation and were transported in ice-pack to the Genetic and Breeding Laboratory, Department of Animal Science, University of Ibadan, Nigeria.

Red blood cells (RBCs) were prepared from the erythrocyte fraction of blood by centrifuging at 3000 rpm for 10 minutes at 4^{o}C. The supernatant was decanted leaving the sediment (RBCs). The RBCs were washed in saline (0.155M NaCl) three times and centrifuged at 3000 rpm for 5 minutes at 4^{o}C. The RBCs were lysed by using haemolysing reagent (0.3g EDTA; 2 ml potassium cyanate and 120 ml distilled water) to release haemoglobin. Subsequently, 0.5 ml of the haemolysing reagent was added to individual animal sample's sediment in a test tube to produce the haemolysates. Electrophoresis was carried out in a Shandon electrophoresis tank on cellulose acetate strips 34.5 x 150 mm with 0.26 M Tris buffer (pH 8.4) at both anode and cathode. The strips were ran for 40 minutes at a constant voltage of 350V according to the procedure described by *Riken (2006)* and *Akinyemi (2010).*

On separation, the strips were stained with Ponceau-S, later washed with 5% glacial acetic acid, and dried using filter paper. Interpretations were made based on the relative mobility of the haemoglobin bands towards the anode, with

haemoglobin AA (single band) being the fastest while haemoglobin BB (single band) was the slowest and haemoglobin AB (double band) having slow and fast bands (*Abdussamadet al., 2004; Riken, 2006; Akinyemi, 2010)* as shown in Plate I.

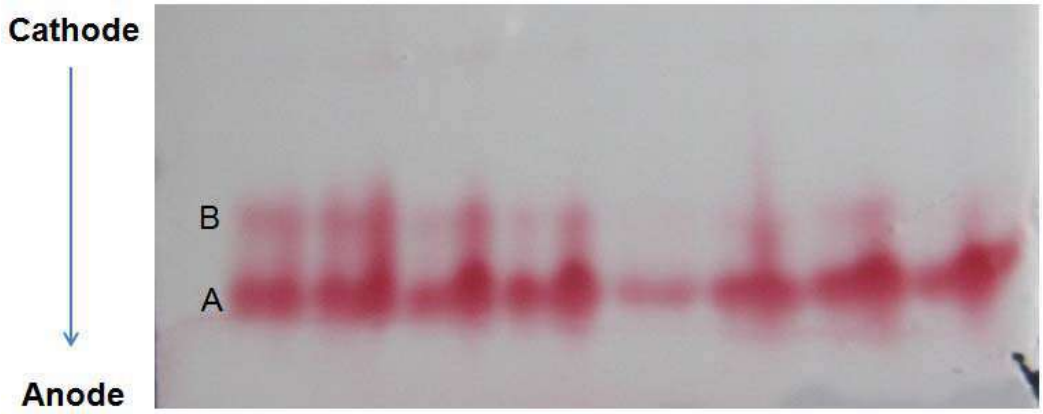


Plate I: Electropherogram of haemoglobin variants

Genotype frequency was calculated thus:

$$AA = \frac{\text{Number of AA}}{\text{Total number of samples}} \times 100$$

$$AB = \frac{\text{Number of AB}}{\text{Total number of samples}} \times 100$$

$$BB = \frac{\text{Number of BB}}{\text{Total number of samples}} \times 100$$

Results and discussion

Figures 1 to 4 show the distribution of dam's haemoglobin types within lamb genotype at birth, 90, 180 and 360-Days, respectively. Each of the 9 lamb genotypes had very high proportion of HbAB. The dam's haemoglobin type BB (HbBB) were only found in YK X YK, UD X UD, YK X UD, BL X YK and BL X UD lambs at birth and 90-Day (Figures 1 and 2). At 180 and 360-Day (Figures 3 and 4), HbBB were completely lost to mortality except for a very small proportion that were found only in YK X YK (8.0%) and UD X UD (16.67%) at 360-Day.

The abundance of HbAB in the populations of this study suggests a better adaptation of the haemoglobin type to the region. It also suggests that genotype HbAB is favoured through natural selection in ruminants of Northern Guinea savannah Zone of Nigeria. *Akinyemi (2010)* however reported higher frequency of HbBB in population of West African Dwarf sheep at low altitude (about 200m above sea level) in South West Nigeria (Forest Zone). However, lambs that were given birth to by dams with HbBB were unable to survive up till 360 days except for those of pure Yankasa and Uda lambs. *Evans et al*. (1958) had earlier suggested that allele A of haemoglobin type has a selective advantage at high altitudes because it constituted the most common allele in highland breeds of English and Scottish sheep. It has been established that the affinity of allele A for oxygen is 30 to 50% greater than allele B (*Chamley and Holland, 1969).*

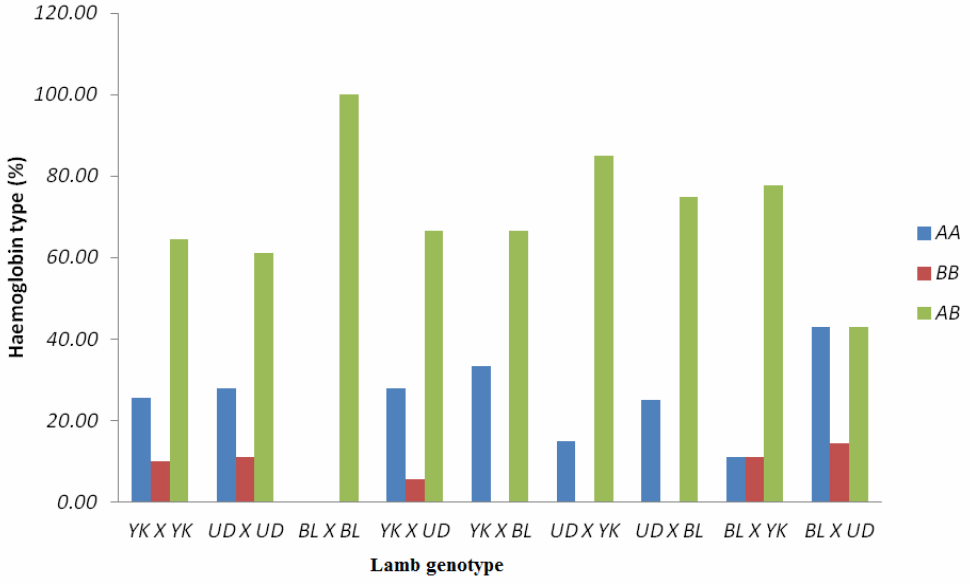


Figure 1. Distribution of dam's haemoglobin types within lamb genotype at birth

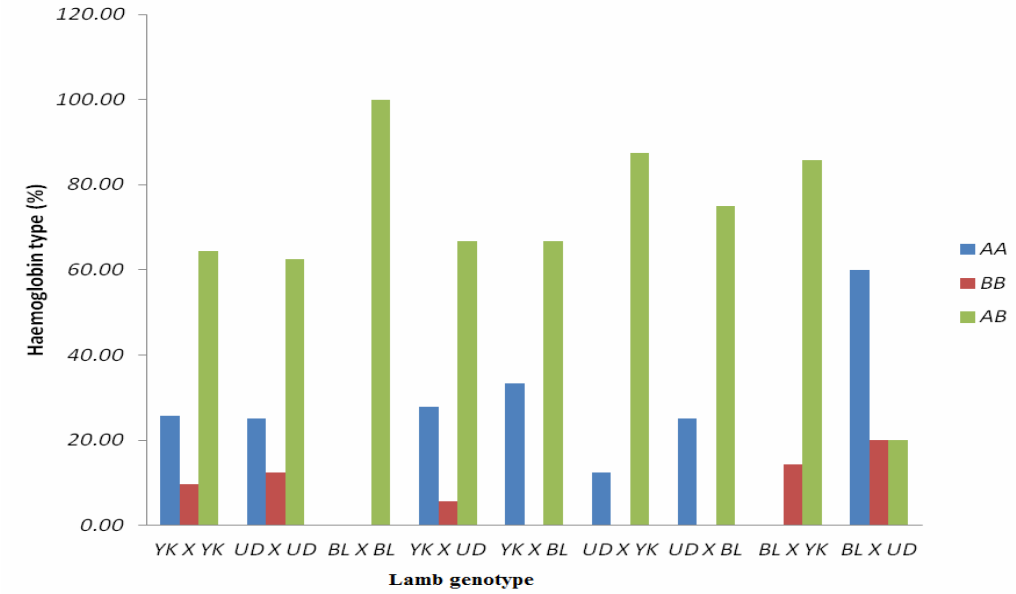


Figure 2. Distribution of haemoglobin types within lamb genotype at 90-days

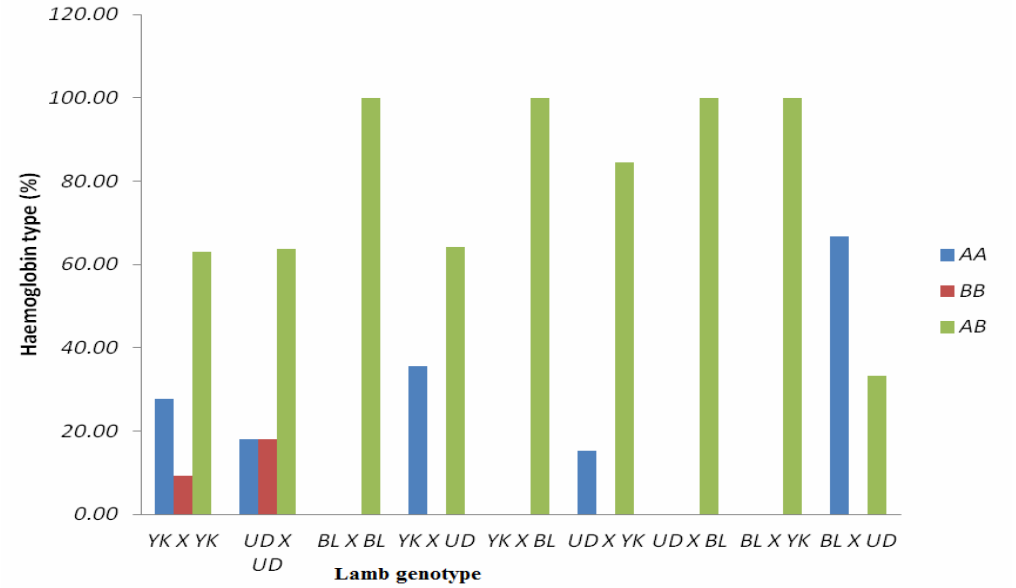


Figure 3. Distribution of haemoglobin types within lamb genotypes at 180-days

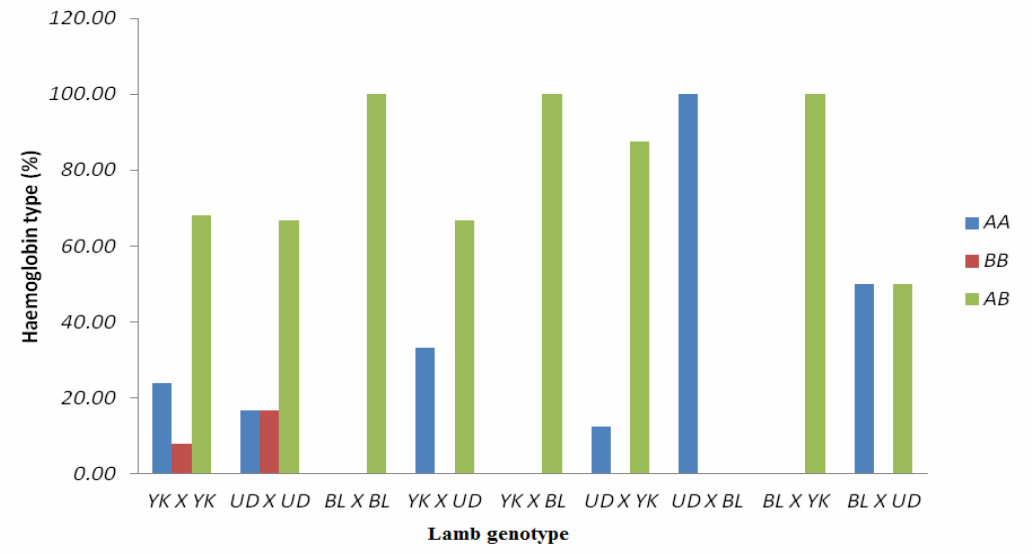


Figure 4. Distribution of haemoglobin types within lamb genotypes at 360-days

Conclusion and Recommendation

Survivability of lamb that were given birth to by dams with haemoglobin type AB (HbAB) is highest in the studied populations from birth to 360-Day. Lambs with HbAB should be selected for improved survivability of sheep in Northern Nigeria.

Acknowledgements

This is part of doctoral study "Genetic analysis of growth and some reproductive traits of sheep of Northern Nigeria and their crosses", which was conducted at the National Animal Production Research Institute (NAPRI) and Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria. The authors appreciate the Director of NAPRI for the permission to conduct this study in NAPRI.

Uticaj hemoglobin varijante majki na preživljavanje jagnjadi

A.O. Iyiola-Tunji, G.N. Akpa, B.I. Nwagu and I.A. Adeyinka

Rezime

Ukupno 65 jankasa, 23 uda i 16 balami ovaca su uparene sa 4 jankasa, 3 uda i 3 balami ovna u jednom dialelnom obrascu parenja i dobijeno je 192 jagnjadi u okviru 9 genotipova, koji su korišćeni za proučavanje preživljavanje jagnjadi u odnosu na hemoglobin varijante njihovih majki. Uzorci krvi (5ml) su prikupljeni od 104 ovce i 10 ovnova punktiranjem vratne vene. Elektroforeza je izvedena u Shandon rezervoaru za elektroforezu, na celuloza acetat trakama. Svaki od 9 genotipova je imao vrlo visok procenat HbAB. Tip hemoglobina majki BB (HbBB) je utvrđen samo kod jagnjadi YK x YK, UD x UD, YK x UD, BL x YK and BL x UD na rođenju i uzrastu od 90 dana. Preživljavanje jagnjadi koja su imale majke sa hemoglobin tipom AB (HbAB) je najviša u ispitivanim populacijama od rođenja do uzrasta of 360 dana. Jagnjad sa HbAB treba izabrati za poboljšanje preživljavanja ovaca u severnoj Nigeriji.

References

ABDUSSAMAD A.M., ESIEVO K.A.N., AKPA G.N. (2004): Haemoglobin types in the Nigerian Zebu and their crosses in Zaria. In: Proceedings of the 29th Annual Conference of the Nigerian Society for Animal Production. 21st – 25th March, 2004. Usman Dan Fodio University, Sokoto, Nigeria., 31-37.

AKINYEMI M.O. (2010): Morphological and biochemical diversity in Balami, Uda and Yankasa sheep of Nigeria. Unpublished Ph.D Thesis, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria. 174pp.

BARUAH P., BHAT P.P (1980): Note on the genetics of haemoglobin and transferrin polymorphism in three breeds of Indian goats. Indian Journal of Animal Sciences, 50, 7, 576-579.

BHAT P.P. (1986): Genetic markers in Jumunapari and Sirohi goat breeds. Indian Journal of Animal Sciences, 56, 4, 430-433.

BRAEND M. (1972): Studies on the relationships between cattle breeds in Africa, Asia and Europe: Evidence obtained by studies of blood group and protein polymorhisms. World Review of Animal Production, 8, 1, 9-14.

BUIS R.C. AND TUCKER E.M. (1983): Relationships between rare breeds of sheep in The Netherlands as based on blood-typing. Animal Blood Groups and Biochemical Genetics, 14, 17–26.

CHAMLEY J.H., HOLLAND R.A.B. (1969): Some respiratory properties of sheep haemoglobins A, B and C. Respiratory Physiology, 7, 3, 287-294.
CHARON K.M., LIPECKA C., SIUDEK T., SWIDEREK W. (1996): Relationship between transferrin and globin antigen polymorphism and sheep resistance to mastitis. Journal of Applied Genetics, 37, 161–172.
DI STASIO L. (1997): Biochemical genetics. In: The Genetics of Sheep (Piper, L. And Ruvinsky, A. Editors). CAB International, UK, 133–148.
DODGSON S.J., HOLLAND R.A.B. (1983): The reaction kinetics of four sheep haemoglobins with identical α-chains. Respiratory Physiology, 53, 1, 31-45.
EVANS J.V., KING J.W.B., COHEN B.L., HARRIS H., WARREN F.L. (1956): Genetics of haemoglobin and blood potassium differences in sheep. Nature, 178, 849-850.
FRANÇOIS D., BOSHER M., MÉRIAUX J.C., NGUYEN T.C. (1992): Les controles de filiation dans les populations. INRA Productions Animales (hors série), 273–276.
GRAETZER M.A., HESSELHOLT M., MOUSTGAARD J., THYMANN M. (1964): Studies on protein polymorphism in pigs, horses and cattle. In: Proceedings of the 9th European Animal Blood Group Conference (First Conference arranged by E.S.A.R), Prange, August 18-22., 279-283.
HENKES L.E., WEIMER T.A., MORAES J.C.F. (1994): Genetic markers and fertility gene (Fec^B) in a ¾ Romney Marsh X ¼ Merino Booroola flock. Small Ruminant Research, 14, 55-59.
IAN K. (1998): Introduction to animal physiology. BIOS Scientific Publishers Ltd, United Kingdom.pp 122.
IBEAGHA-AWEMU E.M., ERHARDT G. (2004): Genetic variations between African and German sheep breeds, and description of a new variant of vitamin D-binding protein. Small Ruminant Research, 55, 33–43.
MARGETIN M., MALIK J. (1982): A study of the genetic structure of sheep on the basis of biochemical polymorphism. VedeckePraceVyskumnehoUstavaOvelarskeho v Trechie, 11, 13-23.
MWACHARO J.M., OTIENO C.J., OKEYO A.M. (2002): Genetic variations between indigenous fat-tailed sheep populations in Kenya. Small Ruminant Research, 44, 173–178.
ORDÁS J.G., SAN PRIMITIVO F. (1986): Genetic variations in blood proteins within and between Spanish dairy sheep breeds. Animal Genetics, 17, 255–266.
RIKEN (2006): Genetic quality monitoring by biochemical isozymes. RIKEN Bioresources Center. http://www.riken.go.jp/engn/
SALAKO A.E., IJADUNOLA T.O., AGBESOLA Y.O. (2007): Hemoglobin polymorphism in Nigerian indigenous small ruminant populations - preliminary investigation. African Journal of Biotechnology, 6, 22, 2636-2638.

TSUNODA K., DOUGE H. (1990): Association between red cell NADH-diaphorase types and blood parameters related to the iron metabolism in sheep. Japanese Journal of Zoo-technical Sciences, 61, 7, 633-639.
VICOVAN G., RASCU D. (1989): Types of haemoglobin in sheep related to environmental adaptation. Archiva Zootechnica, 1, 33–44.

Received 12 March 2014; accepted for publication 27 April 2014

Biotechnology in Animal Husbandry 30 (2), p 225-232, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'636.32
DOI: 10.2298/BAH1402225K

HISTOLOGY OF UTERUS OF DUBSKA PRAMENKA DURING SEXUAL SEASON

A. Katica, N. Mlaćo, R. Avdić, F. Tandir, V. Ćutahija, P. Bejdić, N. Hadžiomerović

Veterinary Faculty, University of Sarajevo, Department for Anatomy, Histology and Embryology, Sarajevo, B&H
Corresponding author: amela10katica@yahoo.com
Original scientific paper

Abstract: Bosnia and Herzegovina has always had a developed sheep production, at least from the aspect of the number of sheep per capita. Today, the ratio is 1 sheep per 4 persons, because the cattle production, globally looking, is decimated by war. Thanks to the geographic location of the country, the quality of mountain pastures and environment that is still healthy, we believe that with increased investments in sheep production we could increase the number of heads, which would have positive effects on production of meat and milk of exceptional quality. The study involving the uterus of Dubska pramenka during sexual season under nomadic conditions of holding, demonstrated that, in adequate zoo hygiene conditions (holding, feeding, treatment of animal), the sheep showed increased reproductive parameters as well as parameters manifested in meat and milk production. In our studies, microstructure of uterus of Dubska pramenka during sexual season shows extremely positive characteristics for nidation of the egg cell and normal development of the embryo. Epithelium of the uterus is in a form of high-prismatic cells, which points to significant cell activity; perfusion and development of myometrium are visible. The uterine glands are extremely well developed and their histological structure indicates increased secretion and preparation of the uterus for gravidity.

Key words: Dubska pramenka, uterus, sexual season

Introduction

In Bosnia and Herzegovina, the share of domestic Pramenka in sheep breeding is 90%; it is bred in the extensive system, in the hill and mountain area. It should be emphasised that the domestic Pramenka breed, in struggles to sustain

itself, managed to keep its reproductive capacity relatively well, because under certain conditions it is possible to get 100 lambs from 100 sheep on a regular basis.

Sheep production in these areas (Central Bosnia Canton) represents a significant income and in some way it contributes to the development of these areas. Considering the vast pasture areas, B&H had substantial opportunities to increase the number of sheep. However, after the war, the huge losses are not even remotely covered, and the organisation of the sheep production has suffered many changes.

Sheep is a short-day animal, which means that oestrus occurs naturally in late autumn, when the number of daylight hours during the day is reduced. Sexual cycle lasts between 16 and 17 days. The signs of oestrus include mating readiness, growth and rupture of ovary follicles (ovulation), excretion of cervical secretion, hyperaemia and perfusion of the vaginal mucosa, redness and swelling of the vulva. Potential reproductive capacities of the Pramenka sheep are significantly higher under favourable feeding and holding conditions; it is not mono-seasonal animal, which is proven by off-season responsiveness of its ovaries and possibility to induce oestrus, fertility in the off-season phase. It should be underlined that success in inducing off-season oestrus and fertility is largely dependent on resolving the feeding problem.

Materials and Methods

The material required was taken in the field, Central Bosnia Canton, (Dubska Pramenka is indigenous sheep, village Dub, the Travnik area), and the study was done at the Faculty of Veterinary of the University of Sarajevo. The study of histological characteristics of Dubska pramenka was done during sexual season. The total number of animals involved is eight (8). Samples of uterus were taken from several places, closely paying attention not to crush the tissue of uterus. The samples were stored in plastic cups with lid, filled with 10% formalin, until the moment of moulding in paraffin blocks. Moulding in paraffin blocks was done in a way that the samples of uterus were placed in 70% of alcohol for two days, then in 96% of alcohol for one day, and in 100 % of alcohol for one day. The materials were then transferred to a mixture of 100% alcohol and toluol for two hours, and then only in toluol for four hours. The prepared samples were placed in paraffin I for five hours and paraffin II for twelve hours, and the paraffin moulding process was completed.

The paraffin blocks with the moulded samples of uterus were cut using digital microtome - several series cuts, 0.5 to 1.5 micron thick. The cuts were placed on glass slides, stained with haematoxylin eosin and azan, covered with cover glass and glued with Canada balsam. Histological assays were done using light microscope, under magnification of 100, 200 and 400 times. The microscopic

examination included the entire uterus in order to get a full picture of the organs examined over given periods of time. The results were presented using descriptive interpretation of histological preparations, making sure that the comparative presentation of histological preparations is representative of our studies.

Results and Discussion

The womb is a hollow, muscular organ; it has mucosa -Tunica mucosa, *Endometrium*; the middle layer-Tunica muscularis, *Myometrium*, and the outer layer-tunica serosa with subserosa, *Perimetrium*.

Histological structure of the Dubska pramenka uterus during sexual season points to excellent characteristics for nidation of a fertilised egg cell and smooth development of the embryo. Epithelium is in a form of high-prismatic cells with clearly visible oval nuclei. Among the dominant high-prismatic cells, there are also stem- basal cells, and the full structure of the endometrial surface indicates excellent cell activity (Figure 1. Epithelium of the endometrium during sexual season; haematoxylin eosin; x 400). It is important to emphasise that our results are fully compatible with the results of *Miljkovic (1986); Mitic (1984); Katica et al. (2010),* who also highlight that sexual season in sheep, in terms of the uterine microstructure, is characterised by significant cell activity of the endometrium. Hyperplasia, an increase in number of cells and hypertrophy, an enlargement of the size of the cell components, occurs under the influence of estrogen *(Dellmann, Brown, 1976).*Perfusion is also pronounced, which is clearly seen in a well-developed connective tissue, while the uterine glands are clearly visible; they are of limited lumen with high-prismatic cells in a form of larger groups, which points to the increased secretion and the preparation of the uterus for gravidity. Progesterone induces hypersecretion, i.e. increased secretion (Figure 2. Uterine glands during sexual season; haematoxylin eosin x 200). Some sites in the lamina propria are well-vascularised; they do not contain glands; they represent the carunculae, *Kozarić Z., (1997)* to which, after insemination and development of placenta, cotyledons attach, forming the placentomes.

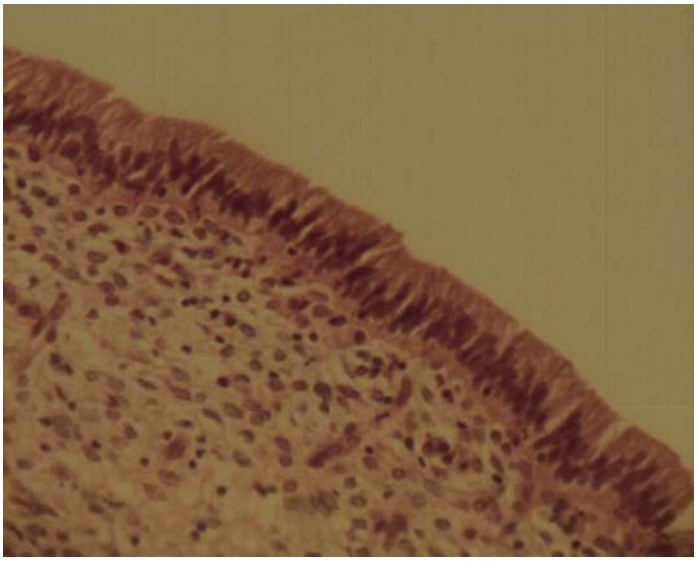


Figure 1. Epithelium of the endometrium during sexual season; haematoxylin eosin; x 400

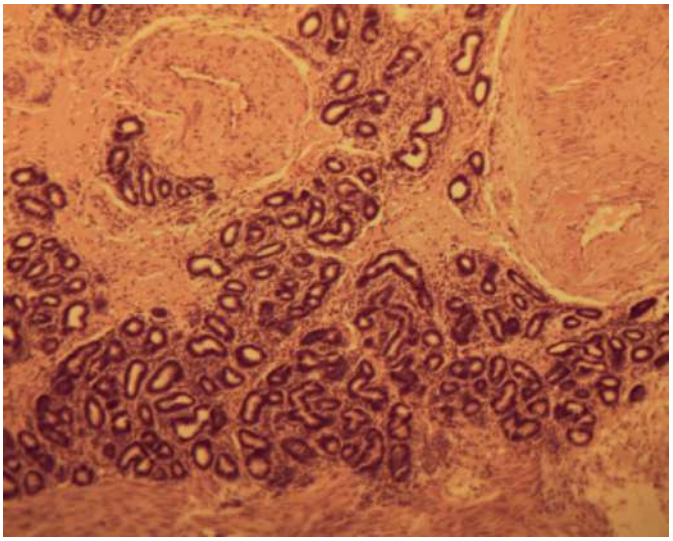


Figure 2. Uterine glands during sexual season; haematoxylin eosin x 200

Myocyte - myometrium is well developed, and spindle-shaped smooth muscle cells are arranged in longitudinal and circulatory layer, with clearly visible nuclei and tapered ends adding on to each other (Figure 3. Myometrium during sexual season; haematoxylin eosin x 200). The studies of other authors correspond to these results *(Gutic M. et al. 2006.; Okljesha B., 1957., Mutevelic A. et al. 2003.)*, who highlight the significant development of the muscle layer of the uterus

in sexual season, not only in sheep but in other ruminants, bovine or goat, for instance.

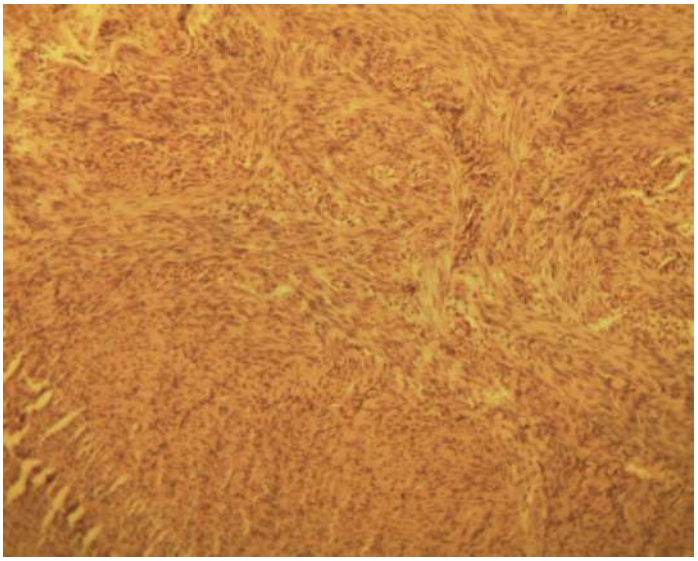


Figure 3. Myometrium during sexual season; haematoxylin eosin x 200

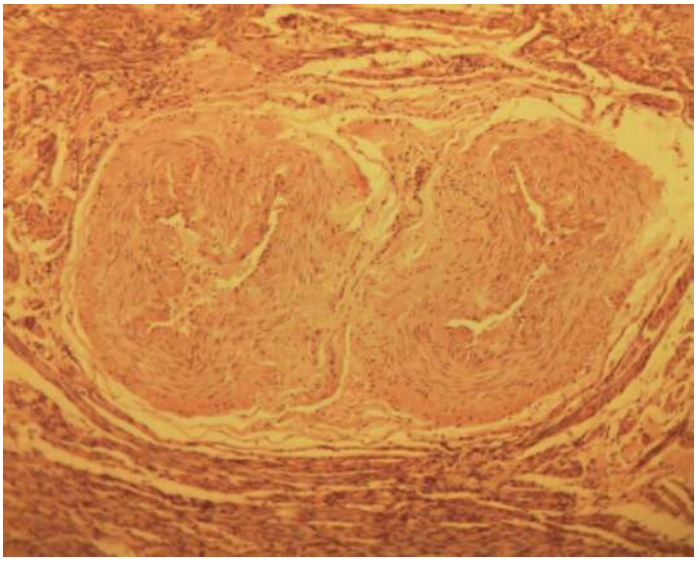


Figure 4. Ligamenta viva uteri; haematoxylin eosin x 200

The studies also showed clearly visible so called "ligamenta viva", *ligamenta viva uteri*, i.e. branches of art. uterine, *arteriae arcuatae*, located in the

muscle layer of the uterus, in larger or smaller groups and with rather thick walls, with not so visible lumen due to thickness of the blood vessel wall, i.e. there is an increased number of smooth muscle cells in the walls of the blood vessels, and their contraction is increased and under the influence of estrogen. (Figure 4. Ligamenta viva uteri; haematoxylin eosin x 200), with which other authors such as *Sobotta (2004); L.C. Junquera, J. Carneiro (2005)* are also concurrent.

In our case, the perimetrium was clearly separated from the myometrium; it is of somewhat lighter colour and is somewhat thicker than usual**.** There is a clear layer of connective tissue and a layer of squamous cells separated from each other with their edges (Figure 5. Perimeterium; haematoxylin eosin x 100).

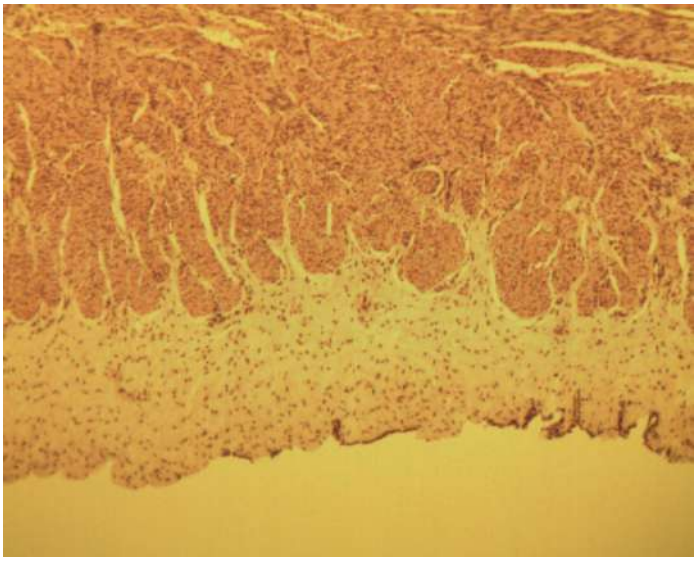


Figure 5. Perimeterium; haematoxylin eosin x 100

Conclusion

The descriptive histological assays of the Dubska pramenka uterus during sexual season concludes that, regardless of nomadic holding of the indigenous breed of the sheep Dubska pramenka, but with adequate feeding and care, the microstructure of the uterus shows favourable conditions that allow for proper development of a healthy embryo. The conditions include:

- High-prismatic epithelium indicating an increased secretion and significant hormonal activity;
- Presence of carunculae, protrusion of mucosa, the embryo nidation site;
- Development of the uterine glands, which is closely related to the progesterone phase of the sexual cycle, i.e. preparation of the uterus for

embryo nidation Developed muscle layer of uterus with significant strong musculature that ensures gravidity, injected lig. lata uteri

Histologija uterusa dubske pramenke u polnoj sezoni

A.Katica, N. Mlaćo, R. Avdić, F. Tandir, V.Ćutahija, P. Bejdić, N. Hadžiomerović

Rezime

Bosna i Hercegovina je uvek imala razvijenu ovčarsku proizvodnju, bar gledano na osnovu broja ovaca na ukupan broj stanovnika. Danas je odnos 1 ovca na 4 čoveka, jer je stočarska proizvodnja, globalno gledajući , ratom desetkovana. Zahvaljujući geografskom položaju naše države, te kvalitetu planinskih pašnjaka i još uvek zdravoj okolini, smatramo da povećanim ulaganjem u ovčarsku proizvodnju, možemo povećati i ukupan broj grla, što bi se pozitivno reperkutiralo na proizvodnju mesa i mleka, izuzetnog kvailteta.

Naša istraživanja uterusa dubske pramenke u polnoj sezoni u nomadskom načinu držanja, pokazala su da ovca uz adekvatne zoohigijenske uslove (držanje, ishrana, odnos prema životinji) znatno pakazuje povećane reproduktivne parametre kao i parametre iskazane u proizvodnji mesa i mleka.

Mikrostruktura uterusa pramenke u polnoj sezoni, našim istraživanjima, pokazuje izuzetno pozitivne karakteristike za nidaciju jajne ćelije i normalan razvoj ploda. Epitel uterusa je u formi visokoprizmatičnih ćelija, što ukazuje na znatnu ćelijsku aktivnost, prokrvljenost je uočljiva i razvijenost miometrijuma. Glandule uterine su izvrsno razvijene i po svojoj histološkoj strukturi ukazuju na pojačanu sekreciju i pripremu uterusa za graviditet.

References

GUTIĆ M. (2006): Ovčarstvo tehnologija proizvodnje, Agronomski fakultet, Čačak

JUNQUEIRA C.L., CARNEIRO J. (2005): Osnovi histologije, tekst i atlas, Data status, Beograd, Novi Sad

KATICA A., MLAĆO N., HASANBAŠIĆ D., HAMZIĆ E. (2010): Osnove veterinarske histologije, Amela Katica, Nadžida Mlaćo

MILJKOVIĆ V. (1986): Reprodukcija i vještačko osjemenjavanje ovaca i koza, Zavod za udžbenike i nastavna sredstva-Beograd

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

MUTEVELIĆ A., FERIZBEGOVIĆ J., MUTEVELIĆ T. (2003): Reprodukcija domaćih životinja, Veterinarski fakultet, Sarajevo
OKLJEŠA B. (1957): Porodiljstvo domaćih životinja, Poljoprivredni nakladni zavod, Zagreb
WELSCH ULRICH S. (2004), Citologija, histologija, mikroskopska anatomija, Histološki atlas, Naklada Slap za hrvatsko izdanje
KOZARIĆ Z. (1997): Veterinarska histologija, Naklada Karolina
DELLMANN H-D, BROWN E.M. (1976): Textbook of Veterinary Histology

Received 28 February 2014; accepted for publication 10 April 2014

Biotechnology in Animal Husbandry 30 (2), p 233-242, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'636.32
DOI: 10.2298/BAH1402233S

HAPLOTYPE ASSOCIATION OF OVINE LEPTIN GENE ON BREEDING VALUE OF BODY MEASUREMENTS IN MAKOOEI SHEEP BREED

S. Sadeghi[1], A. Hajihosseinlo[2], M. Bohlouli[1]

[1]Young Researchers and Elite Club, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran
Corresponding author: m.bohlouli@tabrizu.ac.ir
Original scientific paper

Abstract: The research was undertaken to find association of genetic variation in the exon 3 of the leptin gene and breeding value of body weight traits in Makooei sheep breed using single strand conformation polymorphism (SSCP). The PCR product was obtained to encompass exon 3 of leptin gene corresponding to ovine leptin gene. The PCR fragments were subjected to electrophoresis to reveal the SSCP patterns. Among the total of 130 sheep, five SSCP patterns (haplotypes) were identified for amplified fragment. The frequencies of SSCP patterns of polymorphic fragment were 0.09, 0.17, 0.37, 0.14 and 0.23.The relation between the different haplotypes and body measurements including body length (BL), heart girth (HG), height at withers (HW), height at back (HB), rump length (RL) and scrotal circumference (SC) were ascertained in all of the analyzed animals. According to our results, there is significant association between the different haplotypes of this fragment with additive estimated breeding value for the HG and RL traits. These results confirmed the potential usefulness of leptin gene in marker-assisted selection programs for sheep breeding in Makooei sheep breed.

Key words: Body measurements, leptin, polymorphism, breeding value, Makooei sheep

Introduction

Leptin is a 16 kDa polypeptide hormone and secreted mostly by adipose tissue. Leptin acts as a satiety factor by inhibiting neuropeptide Y in the hypothalamus, provides a satiety signal with subsequent increase of energy expenditure and metabolic processes intensification. Hence, the leptin gene has gained much attention as a key regulator of biological processes such as appetite and metabolism that are related to very important productive traits, such as feed intake, fat content and meat quality in farm animal (*Houseknecht et al., 1998;*

Geary et al., 2003; Van der Lende et al., 2005). However, some evidences indicate that Leptin also functions as acytokine, mediating thymichomestasis and both the innate and adaptive immune systems (*La Cava and Matarese, 2004*). Leptin is also known to play a role in different parts of the body, such as the male and female reproductive organs, the mammary gland, bone mineral density, the gut, the kidney, and the lung (*Baratta, 2002*).In turn, polymorphisms in this gene have been proposed as predictors of relative differences among individuals for those traits (*Nkrumah et al., 2004; Schenkel et al., 2005*).

Several polymorphisms have been found in leptin gene that some of them may affect either activity or expression of leptin. While polymorphism in the leptin gene has been thoroughly investigated in human, pig and bovine, but limited information is available on genetic variation in the ovine leptin gene. Polymorphism in the human leptin gene is reported to be associated with low leptin levels (*Hager et al., 1998*), overweight or obesity and non-insulin-dependent diabetes mellitus (*Van der Lende et al., 2005*). *Kuryl et al. (2003)* studied the relationship of leptin genotype and the quality of fatness of various pig breeds and indicated significant differences in dressing percentage, content of meat in ham, and mass of loin in different leptin genotypes. Genetic variations in the bovine leptin gene have been extremely described and the associations were reported with feed intake (*Lagonigro et al., 2003; Zwierchowskiet al., 2001*), milk production (*Liefers et al., 2002; Buchanan et al., 2003*), somatic cell count (*Kulig et al., 2009*) and carcass and meat quality traits (*Schenkel et al., 2005; Zwierchowski et al., 2001*). Genes encoding leptin was mapped to ovine chromosome 4 (*Perucatti et al., 2006*) and contains three exons. *Zhou et al. (2009)* reported four single nucleotide polymorphisms (SNPs) in exon 3 of the ovine leptin gene that three of these SNPs were non-synonymous and resulted in amino acid changes at codon positions 105, 120 and 144.Therefore, the objective of this study was to estimate the association of different genotypes of ovine exon3 with breeding value of body measurements in Makooei sheep breed.

Materials and Methods

Blood samples were obtained from 130 unrelated Makooei sheep stored in EDTA-coated tubes. Genomic DNA was extracted from 0.3 ml blood using the genomic DNA purification kit (Cat. No 0512, Fermentas, EU) according to manufacturer's instructions.

Two PCR primers, LEP-up (5-AGGAAGCACCTCTACGCTC-3) and LEP-dn (5′-CTTCAAGGCTTCAGCACC-3′), targeting a fragment of 471 bp were employed as described (Zhou et al., 2009). The PCR were carried out in 50 µl volumes using PCR master mix kit (Cinnagen, Iran) containing 2.5 units Taq DNA polymerase in reaction buffer, 4 mM $MgCl_2$, 50 µM each of dNTP, 0.5 µM of each primer and 100 ng of extracted DNA as a template. The thermal profile consisted

of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 59°C and 30 s at 72°C, with a final extension of 5 min at 72°C.

For single-strand conformation polymorphism (SSCP) analysis, several factors were tested to optimize the methodology: amount of PCR product (4 – 15 µL), dilution in denaturing solution (20 - 85%), denaturing solution (A: 95% of formamide, 10mM NaOH, 0.05% xylene-cyanol and 0.05% bromophenol blue; B: same as A, plus 20mM of EDTA), Acrylamide concentration (6 - 14%), percentage of cross linking (1.5 to 5%), presence (10%) or absence of glycerol, voltage (100 - 350 V), running time (2-12 h) and running temperatures(4, 6, 10 and 15 °C). Each PCR reaction was diluted in denaturing solution, denatured at 95 °C for 5 min, chilled on ice and resolved on non-denaturing polyacrylamide gel. The gels were subsequently fixed in 10% ethanol, stained with 0.15% AgNO3 and revealed with 1.5% Na2CO3.

Statistical Analysis

The analyzed traits were yearling weight (*YW*) and six body dimensions measured at yearling age: body length (*BL*), heart girth (*HG*), height at withers (*HW*), height at back (*HB*), rump length (*RL*) and scrotal circumference (*SC*). The following fixed effects model was employed to estimate breeding value (*BV*) with *DFREMEL* software.

Model: $y_{ijklmn} = \mu + YR_i + SX_j + BT_k + AD_l + AN_m + e_{ijklmn}$

Where:

y_{ijklmn}= dependent variable evaluated on the i^{th} level of the random factor; μ = overall mean for each trait; year (YR_i, i=1, 2, 3,…, 21), the j^{th} level of the fixed factor; sex (SX_j , j=1 and 2), the k^{th} level of the fixed factor; number of offspring in each birth (BT_k , k= 1,2 and 3), the i^{th} level of the fixed factor; mother age (AD_l , l=1,2,…,7), m^{th} level of the random additive genetic effect (AN_m, m= number of animal for each trait) and e_{ijklmn} is the random error effect. The estimated parameters according to model were: phenotypic variance (σ_p^2), direct additive genetic variance (σ_a^2), residual variance (σ_e^2) and direct heritability (h_a^2) was equal to $\frac{\sigma_a^2}{\sigma_p^2}$.

For the association studies, the traits of interest were analyzed using the general linear model (*GLM*) procedure of the *SAS 9.1 (2002)*, program according to the following statistical model:

$y_{ijk} = \mu + G_i + S_j + e_{ijk}$

y_{ijk}= breeding value of body measurements, μ = the overall mean, G_i= the fixed effect of the i^{th} genotype for leptin gene (i= 1, 2… 5), S_j= the fixed effect of sex (j = 1, 2), e_{ijk} is the random error. Effect of sex was removed for the *SC* trait (scrotal circumference).

Results

The PCR-SSCP analysis of amplified fragment from ovine leptin gene revealed five distinct patterns. Our study confirmed polymorphic nature of ovine leptin exon3 as revealed by *Zhou et al., (2009)*.The frequencies of the observed genotypes were 0.09, 0.17, 0.37, 0.14 and 0.23 for L1, L2, L3, L4 and L5, respectively (Figure 1).

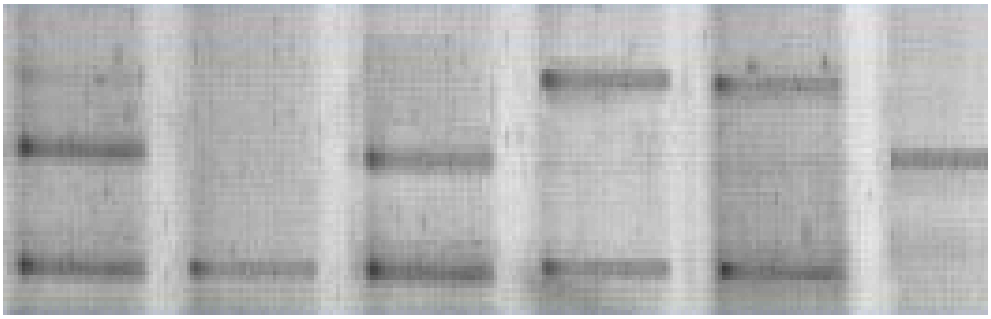


Figure 1. Different SSCP patterns of amplified fragment from ovine exon 3 leptin gene.

Variance and covariance components were estimated based on animal model with the restricted maximum likelihood (*REM*L) approach using a derivate-free (*DF*) algorithm (*Meyer, 1989*). Estimates of variance components and genetic parameters for yearling weight, body length, heart girth, height at withers height at back and scrotal circumference from the single-trait analyses are indicated in Table 1. The direct heritability estimates varied between 0.10 and 0.27 for development traits.

The relationship between these identified haplotypes with body length, heart girth, height at withers, and height at back, scrotal circumference and yearling weight were evaluated. Results indicated that different patterns in this fragment had a significant association with additive estimated breeding value for the HG ($P<0.01$) and RL ($P< 0.05$) traits (Table 2).

Table 1. Parameter estimated of analyzed traits

Traits	σ_a^2	σ^2_e	σ^2_p	h^2
BL	1.235	9.537	10.772	0.105
HG	8.071	29.643	37.714	0.214
HW	2.981	9.563	12.544	0.238
HB	3.452	9.323	12.775	0.270
SC	1.612	4.559	6.171	0.261
RL	2.137	7.429	9.566	0.223
YW	3.228	11.63	14.95	0.221

Body length (BL), heart girth (HG), height at withers (HW), height at back (HB), scrotal circumference (SC), rump length (RL) and yearling weight (YW).

Animal expressing L4genotype had significantly lower breeding value for *GH* (-0.43) relative to the others. Also, Makooei sheep expressing L3 haplotype showed higher breeding value for RL. There was no significant difference between detected genotypes and HB, HW, BL, SC and YW traits, but the combined genotypes of amplified fragment revealed to tend significant differences (P=0.073) between observed genotypes and the breeding value for YW trait (Table 3).

Table 2. Least square means of the EBVs of Makooei sheep according to the different leptin pattern

Leptin	Body measurements estimated breeding values						
	HB	HW	HG	BL	SC	RL	YW
L1	0.3050	0.0001	0.2505^{ab}	0.8827	0.2102	-0.0146^{a}	0.783
L2	0.0560	-0.0004	0.0192^{ab}	0.3734	0.1262	0.2439^{ab}	0.927
L3	0.5947	0.0007	1.1172^{a}	0.3965	0.6247	0.6111^{b}	0.195
L4	0.0087	-0.0004	-0.4352^{b}	0.7740	0.2983	0.1556^{a}	0.965
L5	0.2786	-0.0010	0.1960^{ab}	0.8502	0.2462	0.3617^{ab}	0.544
SEM	0.174	0.0021	0.249	0.183	0.126	0.119	0.292
Prob.	0.317	0.972	0.0068	0.482	0.264	0.0281	0.073

[abc] Means in a column with different superscripts differ statistically (P< 0.05),
Height at back (HB), height at withers (HW), heart girth (HG), Body length (BL), scrotal circumference (SC), rump length (RL) and yearling weight (YW).

Discussion

Considerable interest exists in determining the relationship between SNP for different genes with productive and health traits of economic importance for the livestock industry. Genetic differences in the leptin gene were first reported in mice and humans (*Ohshiro, 2000; Halaas et al., 1995*). In recent years studies have been performed on the association between leptin gene polymorphisms and production traits in farm animal (*Yazdani et al., 2010; Hajihosseinlo et al., 2012*). Most association studies involving leptin gene have focused on carcass composition and beef quality traits (*Zwierchowski et al., 2001*). Our finding for ovine genetic

variation of exon3 are similar to *Zhou et al. (2009)*, who reported five SSCP patterns for this fragment and indicated the high potential of ovine exon3 as an effective DNA marker for marker assisted selection (*MAS*) in sheep.

To date, this was the first study that attempted to detect allele variation in the ovine exon3 of leptin gene and its association with development traits in Iranian sheep breeds. In the analyzed population significant statistical results were found in additive estimated breeding value for the *GH* and *RL* traits. The L3 genotype was associated with low breeding value for HG, and L3 haplotype was associated with high breeding value for *RL*. The observed association between the ovine exon 3 genotypes and sheep body measurement traits in the present study has not been previously reported. It is, however, unclear why and how exon 3 haplotypes would affect sheep body measurement traits, but sequence analysis of these haplotypes revealed four SNPs in ovine exon 3 that three of these SNPs were non-synonymous and resulted in amino acid changes at codon positions 105, 120 and 144. Based on the results for the overall effects of the ovine exon 3 haplotypes in the present study, we can assume that leptin locus or genes linked to it affect body measurement traits in sheep. Few studies have been undertaken to evaluate the association of leptin gene polymorphism with performance traits in sheep. For example, significant associations were found with same haplotypes of this study and additive estimated breeding value for weaning weight and six month weight in Makooei sheep (*Hajihosseinlo et al., 2012*). In addition, in the Suffolk breed, the leptin genotypes were associated with reduced muscle thickness and loin eye area and with increased shear forces, pH and cross-sectional area of the slow-twitch oxidative fibers of the L muscle (*Boucher et al., 2006*). *Tahmoorespur et al. (2009)* analyzed the association of leptin polymorphism with average daily gain (*ADG*) in Baluchi sheep breed. They reported a significant association between leptin polymorphism and *ADG* at birth to 3 months of age. The association of different leptin genotypes with the growth traits in Kermani sheep indicates that the growth traits are significantly affected by the genotypes (*Shojaei et al., 2010*).

However, research on associations between leptin gene polymorphism and performance traits in sheep is rather scanty, but several studies confirmed association between bovine leptin gene polymorphism and performance traits in cattle. For example, significant associations were reported between the polymorphism in exon 2 of bovine leptin gene with grade fat and average fat, with the *T* allele associated with higher fat, but with no significant association with carcass marbling score (*Buchanan et al., 2002*). *Schenkel et al. (2005)* also reported that the two SNPs in the exon 2 of the bovine leptin gene were associated with fat, lean yield and grade fat. Three single nucleotide polymorphisms (the R4C polymorphism in exon 2, the Sau3AI polymorphism in intron 2 and the A59V polymorphism in exon 3) were genotyped in Jeresy cows and significant associations were found between the R4C and Sau3AI polymorphisms with somatic cell count (*Kulig et al., 2009*). An association of the bovine leptin

polymorphism increased chest girth in Anatolian Black cattle breed (*Kaygisiz et al., 2011*).

Conclusion

In conclusion, these results indicate that different genotypes of ovine exon 3 contributed to variation in the traits analyzed and may lead to the use of these haplotypes as genetic marker in marker-assisted selection and sheep breeding programs. However, to prove this, additional investigations are required with bigger sheep populations of different breeds. In addition, there are no data on regulation of ovine leptin expression. Therefore, this raises the questions of what influence these haplotypes might have on the regulation of ovine leptin expression. Hence, gene expression analysis also needs to investigate the functional role of these identified haplotypes in the exon 3 of ovine leptin gene.

Acknowledgment

We thank the Makooei sheep breeding station staff for providing us the data used in this study. Also we are grateful to the head of the institute of biotechnology, Urmia University for providing laboratory facilities.

Haplotipska veza leptin gena ovce i priplodne vrednosti telesnih mera ovaca rase makooei

S. Sadeghi, A. Hajihosseinlo, M. Bohlouli

Rezime

Istraživanje je imalo za cilj da se pronađu veze između genetskih varijacija u egzonu 3 leptin gena i priplodne vrednosti osobine telesne težine ovaca rase Makooei, koristeći jedinstveni lanac usaglašenosti polimorfizma (Single strand conformation polymorphism - SSCP). PCR proizvod koji je dobijen obuhvata egzon 3 leptin gena koji odgovara ovčijem leptin genu. PCR fragmenti su podvrgnuti elektroforezi kako bi se otkrili SSCP obrasci. Od ukupno 130 ovaca, pet SSCP obrazaca (haplotipova) je identifikovano za amplifikovane fragmente. Učestalosti SSCP obrazaca polimorfnog fragmenta su bile 0,09, 0,17, 0,37, 0,14 i 0,23. Odnos između različitih haplotipova i telesnih mera, uključujući dužinu tela (BL), obim (HG), visinu grebena (HV), visinu krsta (HB), dužinu krsta (RL) i obim testisa (SB) je utvrđen kod svih analiziranih životinja. Prema našim rezultatima,

postoji značajna povezanost između različitih haplotipova ovog fragmenta sa aditivima procenjene priplodne vrednosti za HG i RL osobine. Ovi rezultati potvrdili su potencijalnu korisnost leptin gena u programima marker asistirane selekcije u ovčarstvu za odgoj Makooei rase ovaca.

References

BARATTA M. (2002): Leptin-from a signal of adiposity to a hormonal mediator in peripheral tissues. Med. Sci. Monitor., 8, 282–292.

BOUCHER D., PALIN M.F., CASTONGUAY F., GARIEPY C., POTHIER F. (2006): Detection of polymorphisms in the ovine leptin (LEP) gene: Association of a single nucleotide polymorphism with muscle growth and meat quality traits. Canadian: Journal of Animal Science, 86, 31-35.

BUCHANAN F.C., VAN KESSEL A.G., WALDNER C., CHRISTENSEN D.A., LAARVELD B., SCHMUTZ S.M. (2003): An association between a leptin single nucleotide polymorphism and milk and protein yield. Journal of Dairy Science, 86, 3164-3166.

GEARY T.W., MCFADIN E.L., MACNEIL M.D., GRINGS E.F., SHORT R.R., FUNSTON R.N., KEISLER D.H. (2003): Leptin as a predictor of carcass composition in beef cattle. Journal of Animal Science, 81, 1-8.

HAGER J., CLEMENT K., FRANCKE S., DINA C., RAISON J., LAHLOU N., RICH N., PELLOUX V., BASDEVANT A., GUY-GRAND B., NORTH M., FROGUEL P. (1998): A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. Inter. Journal of Obesity. 22, 200–205.

HAJIHOSSEINLO A., HASHEMI A., SADEGHI S. (2012): Association between polymorphism in exon 3 of leptin gene and growth traits in the Makooei sheep of Iran. Livestock Research for Rural Development, 24, 9.

HALAAS J.L., GAJIWALA K.S., MAFFEI M., COHEN S.L., CHAIT B.T., RABINOWITZ D., LALLONE R.L., BURLEY S.K., FRIEDMAN J.M. (1995): Weight reducing effects of the plasma protein encoded by the obese. Genetic Science, 269, 543-546.

HOUSEKNECHT K.L., BAILE C.A., MATTERI R.L., SPURLOCK M.E. (1998): The biology of leptin: a review. Journal of Animal Science, 76, 1405-1420.

KAYGISIZ A., BENGI C., CILEK S. (2011): Investigation of Leptin gene polymorphisms in east Anatolian, Red Anatolian and black cattle and determination of genetic distance from Brown Swiss cattle. Journal of Animal and Plant Science, 21, 121-125.

KULIG H., KMIE M., (2009): Association between Leptin gene polymorphisms and growth traits in Limousin cattle Russian. Journal of Genetics, 45, 738-741.
KURYL J., KAPELANSKI W., PIERZCHALA M., BOCIAN M., GRAJEWSKA S. (2003): A relationship between genotypes at the GH and LEP loci and carcass meat and fat deposition in pigs. Anim. Sci. Papers and Reports, 21, 15-26.
LA CAVA A., MATARESE G. (2004): The weight of leptin in immunity. Nature Reviews Immunology, 4, 371–379.
LAGONIGRO R., WIENER P., PILLA F., WOOLLIAMS J.A., WILLIAM J. (2003): A new mutation in the Coding region of the bovine leptin gene associated with feed intake. Anim. Genetics, 34, 371-374.
LIEFERS S.C., TE PAS M.F., VEERKAMP R.F., CHILLIARD Y., DELAVAUD C., GERRITSEN R., VAN DER LENDE T. (2003): Association of leptin gene polymorphisms with serum leptin concentration in dairy cows. Mammalian Genome, 14, 657–663.
Meyer K. (1989): Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm. Genetics Selection Evolution, 21, 317-340.
NKRUMAH J.D., LI C., BASARAB J.B., GUERCIO S., MENG Y., MURDOCH B., HANSEN C., MOORE S.S. (2004): Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake feed efficiency growth feeding behavior carcass quality and body composition. Canadian: Journal of Animal Science, 84, 211-219.
OHSHIRO Y., UEDA K., NISHI M., ISHIGAME M., WAKASAKI H., KAWASHIMA H., FURUTA H., SASAKI H., SANKE T., TAKASU N., NANJO K. (2000): A polymorphic marker in the Leptin gene associated with Japanese morbid obesity. J. Molecular Medicine, 78, 516-520.
PERUCATTI A., DI MEO G.P., VALLINOTO M., KIERSTEIN G., SCHNEIDER M.P.C., INCARNATO D., CAPUTI JAMBRENGHI A., MOHAMMADI G., VONGHIA G., SILVA A., BRENIG B.B., IANNUZZ L. (2006): FISH-mapping of LEP and SLC26A2 genes in sheep, goat and cattle R-banded chromosomes: comparison between bovine, ovine and caprine chromosome 4 (BTA4/OAR4/CHI4) and human chromosome 7 (HSA7). Cytogenetic and Genome Research, 115, 7-9.
SAS INSTITUTE. (2002): Statistical analysis system, systems for windows SAS Institute Inc, Cary.
SCHENKEL F.S., MILLER S.P., YE X., MOORE S.S., NKRUMAH J.D., LI C., YU J., MANDELL IB., WILTON J.W., WILLIAMS J.L. (2005): Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. Journal of Animal Science, 83, 2009-2020.
SHOJAEI M., MOHAMMADABADI M.R., ASADI FOZI M., ESMAILIZADEH K.A., FERDOWSI M.H., TORABI A., TAYYARZADEH M., MIRZAKHANI H.

(2010): Using PCR-SSCP technique to investigate polymorphism of leptin gene in Kermani sheep. Animal Science Research, 20, 115-122.
TAHMOORESPUR M., NASSIRY M.R., ANSARY M., HERAVIMOUSAVI A., VAFAYE VALE M., EFTEKHARISHAHROUDI F. (2009): Analysis of leptin gene polymorphism and their association with average daily gain trait in Baluchi sheep. Prod. 3rd Conf. of animal science. Mashhad. Iran: [in Persion].
VAN DER LENDE T., TE PAS M.F., VEERKAMP R.F., LIEFERS S.C. (2005): Leptin gene polymorphisms and their phenotypic associations. Vitamins and Hormones, 71, 373–404.
YAZDANI H., RAHMANI H.R., EDRIS M.A., DIRANDEH E. (2010): Association between A59V polymorphism in exon 3 of leptin gene and reproduction traits in cows of Iranian Holstein. African: Journal of Biotechnology, 9, 5997-6000.
ZHOU H., HICKFORD H., GONG H. (2009): Identification of allelic polymorphism in the ovine leptin gene. Molecular Biotechnology, 41, 22–25.
ZWIERCHOWSKI L., OPRZADEK J., DYMNICKI E., DZIERZBICKI P. (2001): An Association of growth hormone k-casein, β-lactoglobulin, leptin and Pit-I loci polymorphism with growth rate and carcass traits in beef cattle. Animal Science Papers and Reports, 19, 65-78.

Received 5 May 2014; accepted for publication 20 June 2014

Biotechnology in Animal Husbandry 30 (2), p 243-250, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.04'636.32
DOI: 10.2298/BAH1402243G

CHEMICAL COMPOSITION AND BIOLOGICAL PROTEIN VALUE OF MILK OF TSIGAI SHEEP AND THEIR F2 CROSS-BREEDS OF CHIOS

G. Gerchev[1]. T. Iliev[2]. S. Slavkova[1]. G. Mihaylova[2]

[1]Research Institute of Mountain Stockbreeding and Agriculture, Troyan. Bulgaria
[2]Trakia University, Stara Zagora, Bulgaria
Corresponding author: gercho_g@abv.bg
Original Scientific paper

Abstract: The composition of milk was studied from purebred Tsigai sheep and F2 cross-breeds with Chios breed from 4th to 6th month of lactation, raised on a mountain pasture. The chemical analysis samples were taken from each sheep in the period of April-June. The content of fat, protein and amino acids was determined in individual samples and total sample formed by the milk yield from all animals, proportional to the daily milk yield from each individual. There is a tendency for higher milk yield and content of total protein and casein in milk of F2 cross-breeds of Chios in comparison with Tsigai sheep. Milk sheep from F2 cross-breeds of Chios had higher content of Lysine, as well as glutamic acid, methionine and leucine, in comparison to that form Tsigai sheep, respectively: 0.458±0.011. 1.389±0.040. 0.084±0.005 and 0.572±0.013. The milk chemical index in both groups of sheep was comparatively low and it constituted respectively 38.3% in Tsigai sheep and 35.4% in F2 cross-breeds, as a result of low concentrations of methionine and cysteine in milk. Biological value of milk obtained from Tsigai breed sheep and F2 milk cross-breeds of Chios had close values - respectively 92.01 and 91.87%. Results showed that the sward composition and the vegetation stage had an influence over the essential amino acids content in purebred Tsigai sheep and their cross-breeds with Chios breed.

Key words: sheep, milk, chemical composition, amino acids

Introduction

The amount of milk proteins and their amino acid content have been studied profoundly in different animal species. Milk is the only source of food for the new-born and depending on the specific nutritional needs it has a certain composition. The differences among species in the amount of protein in the chemical composition are influenced by the nutritional requirements of the new-

born, the difference in postnatal growth rate, stage of maturity, body composition at birth, the influence of environment etc.

The interest for milk proteins is related to their biological and nutritional full value or degree of retention of nitrogen in body (*Gachev, 1995*). Amino acid composition of proteins in sheep milk has been studied in dairy sheep breeds by a number of authors (*Velev, 1986; Tanev et al., 1986; Stancheva, 2002*). Comparing amino acid composition of milk from Karakachanska and Tsigai sheep. *Mihaylova et al. (2006)* found higher content of proline, valine and isoleucine, as well as higher biological value in the first breed.

The objective of this study was to determine the chemical composition and protein biological value of milk from Tsigai sheep and their F2 cross-breeds with Chios breed raised in the region of the Central Balkan Mountain.

Materials and methods

The study was conducted with Tsigai sheep – I group and their F2 cross-breeds – II group, at the time of grazing period in April, May and June, raised on a mountain pasture.

The milk samples for the analysis were taken during the controls for each animal once a month from April till June including. As a total, sample was taken from each group. The basic chemical composition was determined on Milko-skan 133B. The principle of ion exchange column chromatography was used to determine amino acids of total milk protein. For that purpose the sample was treated by acidic hydrolysis with 6 n solution of hydrochloric acid at 110º C for 24 hours. Dissolution of the residue was performed in buffer of pH = 2.2. Sulphur-containing amino acids (methionine and cystine) were determined after sample oxidation with a mixture of carbon peroxide and performic acid. The different amino acids (except for tryptophane) were separated on an Amino analyzer (Amino Acid Analyzer T 339 M. Mikrotehchna – Praha) and their quantity was calculated from their elution volume and standard mixture.

The obtained data was processed by variance statistical methods of Statistica for Windows *(Release. 4.3. stat. soft. Inc. 1994)*.

Results and discussion

The results of the study on milk yield and the basic chemical composition of milk during the period are given in Table 1. From the reported data could be seen that the total milk quantity in the course of the experimental period was higher in sheep cross-breeds 13 l ($P<0.001$), but the dry matter content was by 0.24% lower in comparison with that in Tsigai sheep irrespective of higher values of the standard

deviation. Our results are close to those of *Unal et al. (2002)* and *Esen and Ay (2003)* in F1 cross-breeds of Chios. Inversely proportional to the milk, which had already been milked, was the content of dry matter, with a lower percentage (0.24%) was that from cross-breeds without the difference to be reliable. In F1 cross-breeds of Chios, *Pacinowski et al. (1999)* found a tendency for higher content of dry matter in milk, while in our results the values were lower. The low content of dry matter is determined by the low percentage of milk fat as was recorded a lowering by 0.55% in its concentration. Contrary to fats, a tendency for higher content of proteins in milk was recorded in the cross-breeds. The increase is due rather to high percentage of caseine in milk from the cross-breeds ($P<0.05$) than to non-caseine protein (NCP), where the differences between groups were minimal. The higher values of proteins in milk of F2 cross-breeds also determined the higher dry fat-free residue (DFR). At similar breeding conditions *Odzhakova et al. (2002)* found lower content of dry fat-free residue (11.57%) in cross-breeds of Rhodope Tsigai with rams of Bulgarian White Milk breed.

Table 1. Chemical indicators of milk

Indicators	Tsigai sheep	cross-breeds F2 sheep (Ts x Chios)
	x±Sx	x±Sx
Milk-yield during milking period. l	41.22±0.90	54.18±1.80***
Dry matter. %	17.08±0.28	16.86±0.69
Milk fat. %	5.51±0.26	4.96±0.36
DFR. %	11.58±0.12	11.90±0.12
Proteins. %	5.65±0.11	6.02±0.28
Casein. %	4.08±0.08	4.54±0.18*
NCP. %	1.48±0.03	152±0.03
Ca. mg %	0.200±0.005	0.204±0.007
Curdling. s	250±36	270±44

DFR – Dry fat rescue
NCP – Non caseine proteins
* $p<0.05$
*** $p<0.001$

The average values of milk amino acids content are represented in Table 2. The proteins of both groups have almost equal content of the amino acids, such as aspartic and serine, slightly higher values have threonine, proline and alanine and milk of Tsigai sheep, while the difference in glutamatic acid is in favour of F2 cross-breeds sheep ($P>0.05$). It is known that both amino acids - aspartic and glutamic, improve the brain function. In the sulphur-containing amino acid glycine, the percentage in milk has close values, while in methyonine it is higher in sheep from the second group without reliable difference. The latter participates in the synthesis of complex lipids and choline. Our results of the amino acids reported above are higher than those announced by *Mihaylova et al. (2006)* for Tsigai and Karakachan

sheep with the exception of glutamic acid. The same author reported considerably higher values for sulphur-containing amino acids in milk of Tsigai and Karakachan sheep respectively (methionine 0.184 and 0.170mg% and glycine - 0.118 and 0.113 mg %). According to us, that's due to the sward composition on which the animals are bred.

The amino acids leucine, izoleucine and valine are included into the composition of proteins and they have an important biological significance for the animal organism. The differences between groups are minimal, but the advantage is for the milk cross-breeds. Our data are higher than those found by *Sabahelkheir et al. (2012)* in the sheep milk respectively 108.1, 72.4 and 71.9 mg/g from the total content of amino acids. The cyclic group of amino acids phenylalanine, tyrosine and histidine have a functional role for the organism, as the tyrosine participates in the synthesis of the hormone of the thyroid. Their content in the milk of both groups is of close values.

Table 2. Amino acids in milk during the milking period

Amino acids %	Tsigai sheep x±Sx	Sheep cross-breeds F2 (Ts x Chios x±Sx
Aspartic acid	0.482±0.025	0.481±0.008
Threonine	0.236±0.013	0.229±0.006
Serine	0.222±0.011	0.224±0.006
Glutamic acid	1.346±0.061	1.389±0.040
Proline	0.559±0.028	0.547±0.023
Cysteine	0.059±0.004	0.060±0.003
Glycine	0.099±0.005	0.099±0.025
Alanine	0.216±0.010	0.209±0.006
Valine	0.395±0.018	0.399±0.011
Methionine	0.075±0.012	0.084±0.005
Izoleucine	0.277±0.013	0.281±0.007
Leucine	0.556±0.023	0.572±0.013
Tyrosine	0.215±0.013	0.221±0.004
Phnylalanine	0.263±0.013	0.268±0.006
Histidine	0.175±0.008	0.172±0.004
Lysine	0.411±0.014	0.458±0.011*****
Arginine	0.195±0.009	0.191±0.006
Total	5.78±0.241	5.88±0.184

* $p<0.05$

The lysine and arginine are basic amino acids, as they contain two amino groups and one carboxylic, they serve the cell nucleus of the animal organism and especially are favourable for the proper growth and bone formation. The lysine can be found in greater quantity in the milk of diary cross-breeds at a low reliability of the difference ($P<0.05$), while as for arginine the values are close. Our results in relation to lysine are close to those of *Tanev et al. (1986)* for diary sheep breeds

(8.30 and 7.77 g/100g), but they are a little lower than the general values of *Alexieva et al. (1986)* for the amino acids lysine (571 mg/100g milk) and arginine (260 mg/100 g milk).

The values, which we obtained, for the different amino acids in sheep milk from both studied groups are higher than results found by *Stancheva (2002)* in high milk yield population of sheep.

The difference among groups of amino acids in milk from Tsigai sheep and their F2 cross-breeds are insignificant (Table 3). The amount of nonessential amino acids in milk is by 24.5% and 22.4% respectively for I and II groups, more in comparison with that of the essential amino acids (EAA). For both groups the amount of monoaminomonocarbonic acids is the highest, then follows the monoaminodicarbonic and cyclic amino acids, and the lowest is the amount of diaminomonocarbonic.

The content of essential amino acids that we found in both groups is higher than the average data of *Stancheva (2002)* for the high milk yield sheep population (19.03 g/l).

Table 3. Amino acid groups in the milk

Amino acid group %	Tsigai sheep x±Sx	Sheep cross-breeds F2 (Ts x Chios) x±Sx
Σ Essential	2.487±0.113	2.572±0.066
Σ Non-essential	3.294±0.129	3.312±0.118
Σ MAMC	2.135±0.109	2.157±0.082
Σ MADC	1.828±0.066	1.979±0.048
Σ DAMC	0.606±0.023	0.649±0.017
Σ CAA	1.212±0.060	1.208±0.037

MAMC – monoaminomonocarbonic amino acids
MADC – monoaminodicarbonic
DAMC – diaminomonocarbonic
CAA – cyclic amino acids

The biological value of a particular product could be measured as the results of the determined amino acids composition are compared to the so called 'ideal amino acid profile', which correspond to completely balanced amino acid protein. The method of amino acid indicator was based on that comparison *(Ganchev, 1995; Makova, 1988)*. In table 4 are compared the obtained values for the different essential amino acids in sheep milk for the studied groups with referent values for the 'ideal' and the whole egg protein.

Table 4. Biological value of sheep s protein

Essential amino acids g/100 g Total protein	Reference pattern (FAO/ WHO)	Whole egg Protein	Tsigai sheep	Index %	cross-breeds F2 sheep (Ts x Chios)	Index %
Threonine	4.0	4.8	4.10	102.5	3.89	97.3
Leucine	7.0	8.8	9.62	137.4	9.73	139.0
Isoleucine	4.0	6.7	4.79	119.8	4.78	119.5
Valine	5.0	7.2	6.83	136.6	6.79	135.8
Methionine +	3.5	5.2	1.34	66.3	1.24	70.0
Cysteine						
Lysine	5.5	6.2	7.11	129.3	7.79	141.6
Phenylalanine +	6.0	5.7	8.27	137.8	8.32	138.7
Tyrosine						
Tryptophane	1.0	1.6				
Eessential amino acids	36.0	46.3	42.06		42.54	
Chemical index	100%		38.29		35.42	
Biological value		97%	92.01		91.87	

The milk of both groups surpassed the egg protein in relation to its content of almost all EAA, total phenylalanine + tyrosine with the exception of methionine + cysteine. The chemical index in sulphur-containing methionine + cysteine was lower in milk from F2 cross-breeds - 35.42 than that of Tsigai sheep - 38.29.

Biological value of sheep milk obtained from Tsigai sheep and their F2 cross-breeds of Chios, compared to that of egg protein, which is accepted for 97 % was respectively 92.01 and 91.87%, respectively in Tsigai sheep and the cross-breeds and the chemical index is respectively 38.29 and 35.42.

Conclusions

A tendency was found for higher milk yield and content of total protein and casein in milk from F2 cross-breeds of Chios in comparison with Tsigai sheep.

Milk from sheep F2 cross-breeds of Chios had higher content of Lysine, as well as glutamic acid, methionine, leucine, in comparison with that from Tsigai breed.

The milk sheep chemical index in both groups of sheep was comparatively low and it constituted respectively 38.3% in Tsigai sheep and 35.4% in F2 cross-breeds, as a result of low concentrations of methionine and cysteine in milk. Biological value of milk, obtained from Tsigai breed sheep and F2 milk cross-breeds of Chios had close values - respectively 92.01 and 91.87%.

Hemijski sastav i biološka vrednost proteina u mleku ovaca rase cigaja i njihovih F2 meleza sa rasom hios

G. Gerchev, T. Iliev, S. Slavkova, G. Mihaylova

Rezime

Sastav mleka je ispitivan kod čistokrvnih cigaja ovaca i F2 meleza sa rasom Hios, odgajanih na planinskom pašnjaku, od 4. do 6. meseca laktacije. Uzorci za hemijsku analizu su uzeti od svake ovce u periodu april-jun. Sadržaj masti, proteina i amino kiselina određivan je u pojedinim uzorcima i ukupnom uzorku formiranom od mleka od svih životinja, srazmerno dnevnom prinosu mleka od svake pojedinačne životinje.

Postoji tendencija za veći prinos mleka i sadržaj ukupnih proteina i kazeina u mleku kod F2 meleza sa rasom hios u poređenju sa cigaja ovcama. Mlečna grla F2 melezi sa rasom hios su imala veći sadržaj lizina, kao i glutaminske kiseline, metionina i leucina, u odnosu na cigaja ovce, odnosno: 0,458 ± 0,011, 1,389 ± 0,040, 0,084 ± 0,005 i 0,572 ± 0,013.

Hemijski indeks mleka u obe grupe ovaca bio je relativno nizak i respektivno predstavlja 38,3 % kod cigaja ovaca i 35,4 % kod F2 meleza, kao rezultat niskih koncentracija metionina i cisteina u mleku. Biološka vrednost mleka dobijenog od cigaja rase ovaca i mleka F2 meleza sa rasom hios imaju bliske vrednosti - odnosno 92,01 i 91,87 %.

Rezultati su pokazali da su sastav travnjaka i faza vegetacije imali uticaj na sadržaj esencijalnih aminokiselina kod čistokrvnih cigaja ovaca i njihovih meleza sa rasom hios.

References

ALEXSEEVA N., ARISTO SEREBYANNIKOVA R.V., TOSHEV YU.V., FETISOV A.E., SHIDLOVSKAYA P.V. (1986): Composition and characteristics of milk as resource for milk industry. Moscow. Agroprmizda.

VELEV S. (1986): Amino acid composition of milk of local sheep in the region of Stara Zagora. Animal Sciences, 7, 74-77.

GACHEV E. (1995): Proteins as food. Biochemistry for physicians and dentists. University Publishing House ‘St. Kliment Ohridski’, 541-545.

ODZHAKOVA TS., KAFEDZHIEV V., MIHAYLOVA G. (2002): Comparative study on changes of some indicators of milk from Tsigai sheep and their cross-breeds during lactation period. Animal sciences, 3, 48-52.

MARKOVA M. (1988): Milk and health. Medicine and physical culture, Sofia, 63.
MIHAYLOVA G., GERCHEV. G., BEEV G. (2006): Protein biological value in milk of Tsigai and Karakachan sheep. Animal sciences, 3, 34-37.
STANCHEVA N. (2002): Phenotype and genotype parameters of selection traits in newly created high milk yield population in the country. Dissertation. Sofia.
TANEV G., VASILEVA. A., SIMEONOVA R., NIKOLOV H. (1986): Differences among breeds in the amino acid composition of complete α and β-caseine from sheep milk. Animal Sciences, 6, 67-72.
ESEN F., AY G. (2003): Fertility and milk yield characteristics in Chios x White Karaman (F1 and B1) crossbred sheep under semi-intensive conditions. saqlk – Bilimleri-Dergist.-Firat- Uniwersitesu – Veteriner, 17, 3, 161-165.
PACINOVSKI N., KOZAROVSKI N., PALASEVSKI B., DJABIRSKI V., ADAMOV M., NALETOVSKI Z., MATEVA N. (1999): Quality Characteristiks of the milk producied by the F1-gen of crossbreeds between the milk improved domestic breed with Chios and improved domestic breed with Sardinia breed. 7 International Conferenc for Ovine and Caprine Production, Macedonia, 32.
SABAHELKHEIR M.K., FAT EN M.M., HASSAN A.A. (2012): Amino Acid Composition of Human and Animal's Milk (Camel. Cow. Sheep and Goat). Journal of Science and Technology, 2.
UNAL N., ATASOY F., AYTAC M., AKCAPNAR K. (2002): Milk production in Akkaraman. Sakz (Chios) x Akkaraman F1. Kvrsk x Akkaraman F1 and Sakz(Chios) x Karayaaka B1 ewes during the first lactation. Turk Veterinerlik ve Hayvanclk Dergisi, 26, 3, 617-622.

Received 15 April 2014; accepted for publication 5 June 2014

Biotechnology in Animal Husbandry 30 (2), p 251-260, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'636.237
DOI: 10.2298/BAH1402251P

THE EFFECT OF GENETIC AND NON-GENETIC FACTORS ON PRODUCTION TRAITS OF SIMMENTAL COWS

V. Pantelić[1*], M.M. Petrović[1], D. Ostojić-Andrić[1], D. Ružić-Muslić[1], D. Nikšić[1], Ž. Novaković[2], M. Lazarević[1]

[1]Institute for animal husbandry, 11080 Zemun, Serbia
[1]Institute of science application in agriculture, 11000 Belgrade, Serbia
* Corresponding author:Vlada Pantelić, e-mail: vladap4@gmail.com
Original scientific paper

Abstract: This study covered 737 controlled first calving Simmental cows with, lactations concluded within one year. All first calving animals were on the farms of individual farmers in the municipalities of Kragujevac and Mladenovac. The research included the influence of bull sires, year and season of calving on milk yield and fertility. Least squares method was used to determine the average duration of lactation of 323.74 days. For a period of 305 days, heifers produced 3701.67 kg of milk, or 3644.58 kg of 4% FCM. The average production of milk fat was 144.26 kg and milk fat content was 3.88%. The interval from calving to first insemination lasted in average 124.19 days, and the animals were first calved at the age of 789.95 days. The bull sires had a highly significant effect ($P<0.01$) on the duration of lactation, milk yield and 4% FCM, milk fat yield and content and age of cows at first calving. The duration of service period was not under significant effect ($P>0.05$) of bull sires. Year of calving had a significant effect ($P<0.01$) on the duration of lactation, production of milk, milk fat and 4% FCM. Milk fat content, service period and age at first calving did not show significant variation due to the impact of different years of calving ($P>0.05$). Season of calving of cows demonstrated highly significant effect ($P<0.01$) on the production of milk and 4% FCM, and significant ($P>0.05$) on the production of milk fat, however it had no effect ($P>0.05$) on the milk fat content, duration of lactation and service period and age at calving.

Key words: Simmental breed, milk performance, fertility, bulls, year, season

Introduction

Milk production is important from the biological aspect as well as from the aspect of production. Together with reproduction it represents the material basis for

maintaining of the species. In order to achieve high milk production and production of milk fat, it is necessary, in addition to strict selection, to provide adequate living conditions, particularly with regard to nutrition, rearing and care.

Simmental cows in the Republic of Serbia are reared in semi-intensive conditions - housing, management and feeding, i.e. on small farms with few animals to farms with a few dozen cows in the more intensive systems of production. Genetic improvement of Simmental cattle in our country is achieved by selection and breeding in pure breed. At one time there were attempts to introduce genes of red Holstein breed to improve milk production traits and milking properties *(Petrović M.M. et al.2009).*

Kučević et al.(2005) have analysed milk traits of daughters of same Simmental bull sires, in Germany and Serbia. First calving heifers born and reared in our conditions had 3.796 kg of milk yield and milk fat content of 144.8 kg or 3.81%.

Presenting the results of the application of technology of genetic improvement of Simmental cattle population in Serbia, *Petrović et al.(2006)* have obtained following milk performance results in the study with 2.134 first calving cows. The average milk production was 4.413 kg with 3.88% milk fat contetn or 170 kg milk fat quantity. The yield of 4% FCM was 4.213 kg.

Pantelić et al.(2008a) have analysed the phenotypic variability of milk production traits of Simmental first calving cows in Serbia. Using the method of least squares, average values are obtained for the lactation period - 317.94 days, milk yield - 3872.65 kg, milk fat content - 3.87%, the milk fat yield - 149.78 kg and yield of 4% FCM - 3795.82 kg.

Nikšić et al.(2011) have investigated the production potential of first calving Simmental cows calved in the period 2007-2010 in the individual sector in Serbia, and found the average milk production level of 4.348 kg of milk with 3.93% milk fat content and milk fat yield of 171.1 kg.

Given the fact that the economic importance of fertility traits in cattle is considerable, it is necessary to know some factors that affect the fertility of cows. Fertility of cows is significantly more influenced by environmental factors because most of the variation of reproductive traits are affected by them. The impact of individual factors on the fertility of cows varies, and also a complex effect of multiple factors is possible. Comprehensive knowledge of the impact of certain reproductive traits can be used to improve cow fertility (*Trifunović et al.2004).*

In his research by *Đurđević (2001),* an average value for the age of the animals at first calving of 831.94 days was determined. Service period between the first calving and second fertilization was in average of 96.81 days.

Analysing the production and reproductive performance of Simmental bull dams in our country, *Pantelić et al.(2005)* have found the average age at first conception of 517.61 days, and the duration of the service period of 108.98 days.

In the research of the variability of age at calving and service period of first calving Simmental *Pantelić et al.(2008b)* have established the average service period of 115.19 days and age at first calving of 795.53 days. Year and season of calving have demonstrated highly statistically significant effect ($P<0.01$) on age at first calving and duration of service period.

Pantelić et al.(2013) list some of the indicators of milk production and fertility of Simmental cattle population: the duration of lactation - 311.45 days, milk yield - 5754.49 kg, milk fat percentage - 3.98% and milk fat quantity -230.24 kg, and 5755.47 kg – yield of 4 % FCM. The average duration of gestation of studied animals is 286.31 days, 110.37 days of service period, and calving interval of 398.44 days.

Material and methods

The present study covered 737 controlled first calving Simmental cows with lactations concluded within one year. All first calving cows were on the farms of individual farmers in the municipalities of Kragujevac and Mladenovac. The total number of bull sires included in the study was 33.

In order to more precisely determine the effects of different non-genetic factors, all first calving cows included in this study were classified into four groups according to the season, and three groups according to year of calving.

Months of calving of cows, i.e. beginning of lactation, were divided into four seasons, namely:

- I - winter (December, January, February)
- II - spring (March, April, May)
- III - summer (June, July, August)
- IV - autumn (September, October, November)

The following properties/traits were included in the study:

1. Milk performance:

- Duration of lactation (days)- DL
- Milk yield in standard lactation (kg)- MY
- Milk fat content in standard lactation (%)- MFC
- Milk fat yield in standard lactation (kg)- MFY
- Yield of 4% FCM in standard lactation (kg)- 4%FCM

2. Fertility:

- Age at first calving (days)-AFC
- Duration of service period (days)-DSP

The reduction of lactation to the standard lactation of 305 days was carried out according to the coefficients by *Nenadović, 1974*. Correction of milk yield to

4% FCM was performed using the Gaines-Davidson formula as follows: 4% FCM = 0.4 M + 15 F

where: M – milk yield, kg

F – milk fat yield, kg

The method of least squares LSMLMW *(Harvey 1990)* was used in the analysis of the collected data. This method allows optimal assessment of the impact of many of the studied traits (bull sires, year and season of calving). Mixed model for assessing the phenotypic variability of milk yield and fertility traits in standard lactation for the entire sample was set according to the following equation:

$$Y_{iklm} = \mu + B_i + G_k + S_l + b_1(x_1 - \overline{X}_1) + b_2(x_2 - \overline{X}_2) + e_{iklm}$$

Y_{ijklm} = expression of milk trait of cow *m*, daughter of bull sire *i*, which produced milk in region *j*, and calved in year *k,* in season *l*

μ = general average

B_i = random effect of *i* bull sire

G_k = fixed effect of *k* calving year

S_l = fixed effect of *l* calving season

b_1 = linear regression effect of duration of service period

b_2 = linear regression effect of age at calving

e_{ijklm} = random error

Results and discussion

The overall average, Least squares mean values, the variability of production indicators by year and season, and F-test of examined impacts are presented in Tables 1, 2, 3, 4, and 5.

Table 1. General average (μ) and standard errors (SE) for milk and fertility traits in standard lactation and F-test of studied factors (n=737)

Traits	μ	SE	F – test of studied factors		
			Bull	Year	Season
			$df_1=32$ $df_2=699$	$df_1=2$ $df_2=699$	$df_1=3$ $df_2=699$
DL, days	323.74	9.45	3.682**	31.876**	1.675^{ns}
MY, kg	3701.67	277.96	2.930**	7.594**	4.637**
MFC, %	3.88	0.10	9.245**	1.018^{ns}	1.701^{ns}
MFY, kg	144.26	13.17	3.840**	6.738**	3.779*
4%FCM, kg	3644.58	305.69	3.432**	7.136**	4.141**
DSP, days	124.19	10.32	0.728^{ns}	1.982^{ns}	0.552^{ns}
AFC, days	789.95	48.58	3.609**	0.645^{ns}	2.498^{ns}

The Least squares method was used to determine the average duration of lactation of 323.74 days. For a period of 305 days first calving cows produced 3701.67 kg of milk, i.e. adjusted to 4% FCM - 3644.58 kg. The average production of milk fat was 144.26 kg and milk fat content 3.88%. The interval from calving to first insemination lasted in average 124.19 days, and the animals were first calved at the age of 789.95 days.

Very similar results for milk traits were obtained by *Pantelić et al.(2008a), Kučević et al.(2005)*, and higher values are reported by *Nikšić et al.(2011), Petrović et al.(2006)* and *Pantelić et al.(2013).* Regarding the results of reproductive traits, they are very similar to those obtained in the research by *Pantelić et al.(2008, 2013),* and slightly higher than the results of *Đurđević (2001).*

The bull sires had a highly significant effect (P<0.01) on the duration of lactation, milk yield and yield of 4% FCM, on milk fat yield and content and age of cows at first calving. The bull sires did not show a significant effect (P>0.05) on the duration of the service period.

Stojic (1996) has examined the correction factors of milk production traits and their contribution to the evaluation of breeding value of bulls and cows. In this study, the bull sires have influenced highly significantly (P<0.01) the properties of milk yield, milk fat and 4% FCM in the first standard lactation, but no significant effect (P>0.05) is observed on the duration of lactation and milk fat content. The influence of bull-sires on age at first calving is highly significant (P<0.01), but no significant effect (P> 0.05) on the service period is manifested.

Studying the variability of estimated linear type and milk traits of first calving Black and White cows *Živanović (2002)* have found a highly significant effect (P<0.01) of bulls-sires on milk production, the quantity of milk fat, milk fat content and yield of 4% FCM. On the duration of lactation bulls-fathers did not show a significant effect (P>0.05).

Table 2. Least square means (lsm) and standard errors (Slsm) for milk and fertility traits in standard lactation by calving years

Traits	μ	SE	1		2		3	
			lsm	Slsm	lsm	Slsm	lsm	Slsm
DL, days	323.74	9.45	368.89	14.27	309.39	8.70	292.94	9.04
MY, kg	3701.67	277.96	2976.03	454.38	4178.82	248.67	3950.16	262.32
MFC, %	3.88	0.10	3.84	0.12	3.89	0.10	3.92	0.10
MFY, kg	144.26	13.17	115.37	19.62	162.81	12.17	154.60	12.63
4%FCM, kg	3644.58	305.69	2921.07	472.50	4113.64	279.20	3899.03	291.48
DSP, days	124.19	10.32	165.31	30.47	104.74	2.88	102.51	7.26
AFC, days	789.95	48.58	783.92	73.85	784.78	44.62	801.14	46.45

Year of calving had a significant effect (P<0.01) on the duration of lactation, production of milk, milk fat and 4% FCM. Milk fat content, service period and age at first calving did not show significant variation due to the impact of different years of calving (P>0.05).

Table 3. Deviation of least square (ĉ) and deviation errors (Sĉ) for milk and fertility traits in standard lactation by calving years

Traits	μ	SE	1		2		3	
			ĉ	Sĉ	ĉ	Sĉ	ĉ	Sĉ
DL, days	323.74	9.45	45.15	7.72	-14.35	3.98	-30.80	4.22
MY, kg	3701.67	277.96	-725.64	259.47	477.15	133.88	248.49	141.71
MFC, %	3.88	0.10	-0.04	0.05	0.01	0.03	0.03	0.03
MFY, kg	144.26	13.17	-28.89	10.50	18.55	5.42	10.34	5.74
4%FCM, kg	3644.58	305.69	-723.51	260.08	469.06	134.19	254.45	142.04
DSP, days	124.19	10.32	41.13	20.69	-19.45	10.68	-21.68	11.30
AFC, days	789.95	48.58	-6.03	40.15	-5.17	20.72	11.19	21.93

Variability of duration of lactation ranged from 292.94 days in the third year to 368.89 days in the first year. However, the highest milk production per cow was recorded in the second year – 4.178.82 kg, with a deviation from the general average of 477.15 kg. In the first year, lactation had the longest duration, but milk production was the lowest 2.976.03 kg, with a deviation of -725.64 kg. Similar oscillations were identified for the production of 4% FCM.

In this example, the impact of the year on the performance traits of cows is obvious, which is primarily related to the quantity and quality of feed in the diet of cattle.

Year of calving caused significant variation of milk fat yield, so the highest production was realized in the second (deviation 18.55 kg), and the lowest in the first year (deviation -28.89 kg).

Service period and age at calving did not have a significant variation due to the impact of factor of calving year.

Season of calving of cows demonstrated highly significant effect (P<0.01) on the production of milk and 4% FCM, significant (P>0.05) on the production of milk fat and the milk fat content, but it had no effect on the duration of lactation and service period, and age at calving (P>0.05).

In this region, grazing and summer diet of cows, the use of pastures and free ranges showed positive effect on milk production which was the highest in the summer season (June, July, August) - 3807.75 kg with a deviation of 106.08 kg.

Lack of quality hay and silage, which usually occurs in late March, influenced that in the second season (March, April, May) the lowest quantity of milk with 3606.78 kg was produced with a deviation of -94.89 kg. The calving season caused significant deviations in the quantity of milk fat, which was the highest in the third season - 148.06 kg, and the lowest in the second - 140.58 kg.

Pantelić et al.(2008b) have found a statistically significant effect (P<0.01) of the calving year and season on age at first calving and duration of service period.

Table 4. Least square means (lsm) and standard errors (Slsm) for milk and fertility traits in standard lactation by calving seasons

Traits	μ	SE	I (n=148)		II (n=191)		III (n=213)		IV (n=185)	
			lsm	Slsm	lsm	Slsm	lsm	Slsm	lsm	Slsm
DL, days	323.74	9.45	326.19	9.48	322.94	9.53	322.02	9.53	323.81	9.51
MY, kg	3701.67	277.96	3670.32	279.08	3606.78	281.29	3807.75	281.01	3721.83	280.34
MFC, %	3.88	0.10	3.90	0.10	3.89	0.10	3.88	0.10	3.87	0.10
MFY, kg	144.26	13.17	143.71	13.21	140.58	13.28	148.06	13.27	144.70	13.25
4%FCM, kg	3644.58	305.69	3623.72	306.72	3551.40	308.74	3743.98	308.48	3659.21	307.87
DSP, days	124.19	10.32	126.46	10.51	123.21	10.88	121.15	10.83	125.93	10.72
AFC, days	789.95	48.58	799.43	48.73	779.30	49.04	782.46	49.00	798.61	48.90

Table 5. Deviation of least squares (ĉ) and deviation errors (Sĉ) for milk and fertility traits in standard lactation by calving seasons

Traits	μ	SE	I (n=148)		II (n=191)		III (n=213)		IV (n=185)	
			ĉ	Sĉ	ĉ	Sĉ	ĉ	Sĉ	ĉ	Sĉ
DL, days	323.74	9.45	2.45	1.26	-0.80	1.08	-1.72	1.01	0.07	1.06
MY, kg	3701.67	277.96	-31.35	42.31	-94.89	36.23	106.08	33.99	20.16	35.63
MFC, %	3.88	0.10	0.02	0.01	0.00	0.01	-0.01	0.01	-0.01	0.01
MFY, kg	144.26	13.17	-0.55	1.71	-3.68	1.47	3.80	1.38	0.44	1.44
4%FCM, kg	3644.58	305.69	-20.86	42.41	-93.17	36.31	99.40	34.07	14.63	35.71
DSP, days	124.19	10.32	2.27	3.37	-0.98	2.89	-3.04	2.71	1.74	2.84
AFC, days	789.95	48.58	9.48	6.55	-10.65	5.61	-7.49	5.26	8.66	5.51

Milk production in Serbia is still mainly realized on relatively small farms, often with less than 10 cows, but it is not negligible in terms of the total quantity

of milk that is produced, nor in terms of the number of people engaged in this production. In terms of social transition, milk production, even for farmers with fewer cows is an important source of livelihood.

Conclusion

Knowledge of the impact of external factors on milk and fertility traits is very important in view of their importance in achieving breeding goals and good economic results.

The basis for selection work is the knowledge of the quality of bull sires used for insemination, and the mode of transmission of important traits to offspring. The bull-sires exhibited a highly significant effect ($P<0.01$) on all production traits, except for service period.

The impact of the year on the production traits is manifested mainly through the production and preparation of food, as well as its use in the diet of cattle during a year. Climatic factors have a very significant impact on the production of food, which is reflected in its quality and nutritional value that directly affects the quality of the diet of cows, and therefore the production and reproduction traits.

Season of calving of cows and differences between the seasons of the year can have a significant impact on the production of milk and meat. Differences between the seasons is reflected in the specific climatic conditions and the differences in diet, housing and care of animals.

Uticaj genetskih i negenetskih faktora na proizvodne osobine krava simentalske rase

V. Pantelić, M.M. Petrović, D. Ostojić-Andrić, D. Ružić-Muslić, D. Nikšić, Ž. Novaković, M. Lazarević

Rezime

Ovim istraživanjem je obuhvaćeno 737 kontrolisanih prvotelki simentalske rase, sa laktacijama zaključenim u toku jedne godine. Sve prvotelke su se nalazile na imanjima individualnih poljoprivrednih proizvođača. Istraživanja su obuhvatila uticaj bikova očeva, godine i sezone telenja na osobine mlečnosti i plodnosti. Metodom najmanjih kvadrata ustanovljeno je prosečno trajanje laktacije od 323,74 dana. Za vremenski period od 305 dana prvotelke su proizvele 3.701,67 kg mleka, odnosno, korigovano na 4%MKM 3.644,58 kg. Prosečna proizvodnja

mlečne masti iznosila je 144,26 kg a sadržaj mlečne masti 3,88%. Interval od telenja do prve inseminacije trajao je u proseku 124,19 dana, a grla su se prvi put telila u uzrastu od 789,95 dana.

Bikovi-očevi su imali visoko signifikantan uticaj (P<0,01) na trajanje laktacije, prinos mleka i 4%MKM, proizvodnju i sadržaj mlečne masti i uzrast krava pri prvom telenju. Na dužinu servis perioda bikovi-očevi nisu ispoljili značajnije dejstvo (P>0,05).

Godina telenja imala je visoko značajan uticaj (P<0,01) na trajanje laktacije, proizvodnju mleka, mlečne masti i 4%MKM. Sadržaj mlečne masti, trajanje servis perioda i uzrast pri prvom telenju nisu pokazali značajnija variranja usled uticaja različitih godina telenja (P>0,05). Sezona telenja krava je visoko značajno uticala (P<0,01) na proizvodnju mleka i 4%MKM, značajno (P>0,05) na proizvodnju mlečne masti, a na sadržaj mlečne masti, trajanje laktacije i servis perioda, uzrast pri telenju nije imala značajnijeg uticaja (P>0,05).

References

ĐURĐEVIĆ, R. (2001): Genetička analiza mlečnosti i reprodukcijskih svojstava krava simentalske rase. Doktorska disertacija. Poljoprivreni fakultet, Novi Sad.

KUČEVIĆ, D., WAEHNER, M., PETROVIĆ, M.M, PANTELIĆ, V. (2005): Ispitivanje osobina mlečnosti kćeri istih bikova simentalske rase u Nemačkoj i Srbiji. Biotehnologija u stočarstvu 21 (1-2), p. 21-27

NIKŠIĆ, D., OSTOJIĆ-ANDRIĆ, D., PANTELIĆ, V., PERIŠIĆ, P., NOVAKOVIĆ, Ž., ALEKSIĆ, S., LAZAREVIĆ, M. (2011): Production potential of first calving Simmental heifers in Serbia. 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production", Belgrade, October 5th to 7th, 2011, Biotechnology in Animal Husbandry, Vol. 27 (3), p. 1033-1043.

PANTELIĆ, V., PETROVIĆ, M.M, ALEKSIĆ, S., SRETENOVIĆ, LJ., OSTOJIĆ-ANDRIĆ, D., NOVAKOVIĆ, Ž (2008a)**:** Phenotypic variability of milk traits in first calving Simmental cows in Serbia, Journal of Mountain Agriculture on the Balkans, Vol. 11, No. 4, 660-672. Troyan, Bulgaria.

PANTELIĆ, V., PETROVIĆ, M.M, ALEKSIĆ, S., SRETENOVIĆ, LJ., OSTOJIĆ-ANDRIĆ, D., NOVAKOVIĆ, Ž (2008b)**:** PANTELIĆ, V., PETROVIĆ, M.M, ALEKSIĆ, S., SRETENOVIĆ, LJ., OSTOJIĆ-ANDRIĆ, D., NOVAKOVIĆ, Ž (2008)**:**Varijabilnost uzrasta pri telenju i servis perioda prvotelki simentalske rase. Savremena poljoprivreda, vol. 57, (3-4), p. 131-136.

PANTELIĆ, V., RUŽIĆ-MUSLIĆ, D., PETROVIĆ, M.M., NIKŠIĆ, D., OSTOJIĆ-ANDRIĆ, D., ALEKSIĆ, S., LAZAREVIĆ, M (2013): The phenotypic variability of production traits in the population of Simmental cows. 10th

International Symposium Modern Trends in Livestock Production, Hotel Park, Belgrade, Serbia, october 2-4, 2013, Proceedings, 26-36.
PANTELIĆ, V., SKALICKI, Z., PETROVIĆ, M.M, KUČEVIĆ, D. (2005): Reproductive characteristics of Simmental breed bull dams. Biotechnology in Animal Husbandry 21 (1-2), p. 13-20.
PETROVIĆ, M. M., SRETENOVIĆ, LJ., BOGDANOVIĆ, V., PERIŠIĆ, P., ALEKSIĆ, S., PANTELIĆ, V., PETROVIĆ, D. M., NOVAKOVIĆ, Ž. (2009): Quantitative analysis of genetic improvement of milk production phenotypes in simmental cows. Biotechnology in Animal Husbandry, vol. 25, (1-2), 45-51.
PETROVIĆ, M.M, LJILJANA SRETENOVIĆ, PANTELIĆ, V., ALEKSIĆ, S., MIŠČEVIĆ, B., BOGDANOVIĆ, V., DUŠICA OSTOJIĆ, PETROVIĆ, M. (2006): Results of the application of the technology of genetic improvement of Simmental cattle population in Serbia. Biotechnology in Animal Husbandry 22 (1-2), p. 1-8.
STOJIĆ, P. (1996): Faktori korekcije osobina mlečnosti i njihov doprinos oceni priplodne vrednosti bikova i krava. Doktorska disertacija. Poljoprivredni fakultet, Beograd.
TRIFUNOVIĆ, G., LATINOVIĆ, D., MEKIĆ, C., ĐEDOVIĆ RADICA, PERIŠIĆ, P., BUNEVSKI, G., NIKOLIĆ, R. (2004): Uticaj nivoa prinosa mleka na osobine plodnosti goveda. Biotehnologija u stočarstvu 20 (5-6), p. 35-40.
ŽIVANOVIĆ LJ. (2002): Varijabilnost linearno ocenjenih osobina tipa i mlečnosti prvotelki crno-bele rase. Magistarska teza. Poljoprivredni fakultet, Beograd.

Received 23 May 2014; accepted for publication 20 June 2014

Biotechnology in Animal Husbandry 30 (2), p 261-279, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.082'636.234
DOI: 10.2298/BAH1402261M

ESTIMATION OF GENETIC PARAMETERS AND COMPARISON OF RANDOM REGRESSION ANIMAL AND SIRE MODELS OF PRODUCTION TRAITS IN THE FIRST THREE LACTATIONS OF IRANIAN HOLSTEINS

A. Mohammadi, S. Alijani

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
Corresponding author: Ali Mohammadi, E-mail: alimohamadi36@gmail.com
Original scientific paper

Abstract: This study was conducted to compare of random regression (RR) animal and sire models for estimation of the genetic parameters for production traits of Iranian Holstein dairy cows. For this purpose, the test day records were used belonged to first three lactations of cows and for, milk, fat and protein yields traits where, collected from 2003 to 2010, by the national breeding center of Iran. The genetic parameters were estimated using restricted maximum likelihood algorithm. To compare the model, different criterion -2logL value, AIC, BIC and RV were used for considered traits. Residual variances were considered homogeneous over the lactation period. Obtained results showed that additive genetic variance was highest in the beginning and end lactation and permanent environmental variance was highest in beginning of lactation than other lactation period. Heritabilities estimate for milk, fat and protein yields by RR animal and sire models were found to be lowest during early lactation (0.05, 0.04 and 0.07; 0.05, 0.19 and 0.13; 0.14, 0.19 and 0.15, for milk, fat and protein yields and in first, second and third lactation respectively). However, estimated heritabilities during lactation did not vary among different order Legendre polynomials, and also between RR animal and sire models. The variation in genetic correlations estimate in the RR animal and sire models was larger in the first lactation than in the second and third lactations. Thus, based on the results obtained, it can be inferred that the RR animal model is better for modeling yield traits in Iranian Holsteins.

Keyword: Dairy cow, random regression model, genetic parameter, test day

Introduction

The use of appropriate method for the genetic components evaluation of dairy cattle is an important program of dairy animal production. In conventional method, lactation yields are calculated based on the test day (TD) records. TD records are actually repeated observations measured along a trajectory days in milk (DIM) and the mean and covariance between measurements change gradually along the trajectory. Among the models that consider TD production, random regression model (RRM) has been widely observed to increase the accuracy of breeding value predictions (*Strabel et al., 2004*). Several studies have reported that heritability of daily milk yields varied with DIM. In addition, genetic correlations between repeated measurements usually tended to decrease as the time between them increased (*Pander et al., 1992*). The extension of test records to compute 305-day yields is unable to account for these changes in the covariance structure. Secondly, the assumption that 305-day yields across parities measure the same trait suffers from the same limitations. An appropriate model for the analysis of repeated measurements over time or age should account for the mean and covariance structure that changes with time or age and should be feasible in terms of estimating the required genetic parameters.

Schaeffer and Dekkers (1994) introduced the concept of the RRM for the analysis of TD records in dairy cattle as a means of accounting for the covariance structure of repeated records over time or age. Although, some investigations have been carried out in Iranian Holstein dairy cows in regard to the estimation of genetic parameters of milk yield traits by RR animal model (*Mohammadi et al., 2012a; Mohammadi et al., 2012b*) and RR sire model (*Bohlouli and Alijani, 2012*), but compare RR animal and sire models in first three lactation has not yet been evaluated. Moreover, in literature several approaches were used lower additive genetic (AG) effect order of fit Legendre polynomial (LP) model than for the permanent environmental (PE) (*Mohammadi et al., 2012c; Takma and Akbas, 2009; Lopez-Romero and Carabano, 2003*). It seems that might be sufficient to capture most of the genetic and permanent environmental variability observed in the shape of daily milk production, producing less oscillatory and less extreme values for both variances and genetic correlations (*Lopez-Romero and Carabano, 2003*). Therefore, the purpose of this study is estimation of genetic parameters and comparison of random regression animal and sire models of production traits, using of LP (lower order LP for the AG effect than for the PE effect) and -2LogL value, Akaike's information criterion (AIC), Bayesian information criterion (BIC) and residual variance (RV) of the first three lactations of Iranian Holsteins.

Materials and Methods

The TD milk yield records obtained from national breeding center of Iran, belonged to the first lactation dairy cow from 2004 to 2010. Age range by parity was 21 to 46 months, 32 to 65 months and 42 to 80 months for first, second and third lactation respectively. Edited data included the following: The TD data were excluded before 5th day and after the 305th day of lactation. In addition, irregular data for milk yield (<1.5 and >70 kg), fat percentage (<1.5 and >9 %), and protein percentage (<1 and >7 %), (then were converted to fat and protein yields) were excluded. Also, only cows with more than 5 TD records, and herds with more than 10 cows per herd in year of calving were kept. The sires having progeny fewer than 10 were eliminated. Four calving seasons (spring, summer, fall and winter) and 6 subclasses for age at calving for the first lactation (<26, 26 to 28, 28 to 30, 30 to 32, 32 to 33 and >33 months), 4 classes for the second lactation (<40, 40 to 43, 43 to 45, and >45), and 3 classes for the third lactation (<54, 54 to 58, and>58) were defined. This resulted to classes of cows calving age-season, which were included in the RRM as fixed regression part. RRM used to fit yield records was:

$$y_{tijklm} = HTD_i + Yc_j + MF_k + \sum_{n=1}^{p} AS_{mnl}\, x_n + \sum_{n=0}^{r} a_{mn}\, x_n + \sum_{n=0}^{r} pe_{mn}\, x_n + e_{tijklm}$$

Where y_{tijklm} is the t^{th} record (milk, fat and protein yields) of m^{th} cow in i^{th} subclass herd-test-date (*HTD*), j^{th} (j= 1 to 7) calving year (*YC*) and k^{th} (k= 2 and 3) milking times (MF); AS_{mnl} is the n^{th} fixed regression coefficient for m^{th} cow belong to l^{th}; a_{mn} and pe_{mn} are in RR animal model, regression coefficients n^{th} for AG and PE effects on m^{th} cow and are in RR sire model, random regression coefficients n^{th} sire for AG and PE effects on m^{th} cow, respectively; p is the order fitting of fixed regression coefficients; r (r= 2 to 6) orders number of LP; x_n is n^{th} LP, for t^{th} day and e_{tijklm} random residual effect associated with y_{tijklm}. Estimation of genetic parameters with restricted maximum likelihood (*REML*) methodology was done by REMLF90 (Misztal et al., 2002) program. For the standardized days in milk, the nth LP is given as (*Krikpatrick et al., 1990*);

$$\Phi_{(d_t^*)i} = \frac{1}{2^i}\sqrt{\frac{2i+1}{2}}\sum_{m=0}^{i/2}(-1)^m\binom{i}{m}\binom{2i-2m}{i}(d_t^*)^{i-2m}$$, where d^*_t, is the t^{th}

DIM; t, is time and i, is order LP. The matrices notation of the model can be written as, $\boldsymbol{y = Xb + Qa + Zpe + e}$; where $\boldsymbol{y}$ is the a vector of observations, $\boldsymbol{b}$ is the a vector of fixed effects, $\boldsymbol{a}$ and **pe** were vectors of AG and PE effects respectively, $\boldsymbol{e}$ is the vector of residual effects and $\boldsymbol{X}$, $\boldsymbol{Q}$ and $\boldsymbol{Z}$ are the incidence matrices. The (co)variance structure for random parts of the RR animal model was defined as:

$var\begin{bmatrix} a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} G \otimes A & 0 & 0 \\ 0 & I\sigma_p^2 & 0 \\ 0 & 0 & R \end{bmatrix}$, Where G is the genetic covariance matrix among RR coefficients, $\otimes$ is the Kronecker product function, A is the additive genetic relationship matrix coefficients among animals, σ_p^2 is the variance of the PE effects, I represents an identity matrix, and R is the diagonal matrices of residual variance. The (co)variance structure for random parts of the RR sire model was defined as:

$var\begin{bmatrix} s \\ pe \\ e \end{bmatrix} = \begin{bmatrix} G \otimes A & 0 & 0 \\ 0 & I\sigma_p^2 & 0 \\ 0 & 0 & R \end{bmatrix}$, Where G is sire genetic (co)variance matrix among RR coefficients and A is additive numerator relationship matrix between sires. For the RR animal model, heritability for i[th] day in the lactation was calculated as: $h_i^2 = \frac{\sigma_{a(t)}^2}{\sigma_{a(t)}^2 + \sigma_{pe(t)}^2 + \sigma_e^2}$, Where $\sigma_{a(t)}^2 = \mathbf{qGq}'$, $\sigma_{pe(t)}^2 = \mathbf{qPq}'$,where q is the vector of the associated LP; G and P are the (co)variance matrices for AG and PE, RR coefficients, respectively; and $\sigma_{a(t)}^2$, $\sigma_{pe(t)}^2$ and σ_e^2 are AG, PE and residual variances for t[th] DIM, respectively. For the RR sire model, heritability for t[th] DIM was calculated as: $h_t^2 = \frac{4\sigma_{s(t)}^2}{\sigma_{s(t)}^2 + \sigma_{pe(t)}^2 + \sigma_e^2}$

Diagonal of above (co)variance matrices were sire AG variances ($\sigma_{s(t)}^2$) and PE ($\sigma_{pe(t)}^2$) for 5[th] day to 305[th] DIM. Number of records of milk, fat and protein yields and other descriptive statistics are summarized in Table 1. AG correlation for 305-days production between LP were calculated as:

$r_{g(i,j)} = \frac{Cov_{g(i,j)}}{\sqrt{Var_{g(i,i)} * Var_{g(j,j)}}}$, Where $Cov_{g(i,j)}$, is genetic covariance between i and j day, $Var_{g(i,i)}$ and $Var_{g(j,j)}$ are AG variance i and j day, respectively. Goodness of fit for the models (different LP) was examined using likelihood based criteria as -2Logl, AIC, BIC and RV values. AIC and BIC are: AIC = -2Logl + 2k and BIC = -2Logl + k*log (N - r(x)), where k is the number of parameters estimated, N is the sample size and r(x) is the rank of the coefficient matrix for fixed effects in the model. The model giving the lowest -2Logl, AIC, BIC and RV values is chosen as the better approximating model. Residual variance was considered homogeneous along the lactations, since the use of homogeneous residual variance in the

literature is cited as a good assumption for use in data analysis of dairy cattle (*Costa et al., 2008*). Estimation of genetic parameters with REML methodology was done by REMLF90 program (*Misztal et al., 2002*).

Table 1. Descriptive statistics of data sets for milk, fat and protein yields

	First lactation		Second lactation		Third lactation	
Parameter	**Model**		**Model**		**Model**	
	Animal	**Sire**	**Animal**	**Sire**	**Animal**	**Sire**
Milk yield						
TD records	928,513		686,871		445,499	
Means ±SD (kg)	30.14±7.481		32.74±10.006		33.26±10.753	
Number of cows with record	108,873		81,575		53,131	
Number of total animals	225,832	1593	183,407	1410	132,322	1131
Number of HTD	17,820		3,752		3,530	
Number of herd- calving year	1,483		305		289	
Fat yield						
TD records	788,577		586,584		381,896	
Means ±SD (g)	1±0.326		1.09±0.413		1.11±0.441	
Number of cows with record	96,511		72,518		47,422	
Number of total animals	206,371	1526	162,427	1336	120,414	1063
Number of HTD	16,499		3,465		3,308	
Number of herd- calving year	1400		283		270	
Protein yield						
TD records	653,317		483,048		311,108	
Means ±SD (g)	0.94±0.231		1.03±0.295		1.05±0.313	
Number of cows with record	79,501		59,147		38,304	
Number of total animals	171,360	1424	137,950	1247	98,466	982
Number of HTD	14,127		2,938		2,648	
Number of herd- calving year	1,227		254		223	

Results and Discussion

The values of comparison criteria (-2Logl, AIC, BIC, RV) for first three lactations and for the different LP of milk, fat and protein yields traits by RR animal and sire models were given in Tables 2 to 7. The choice for the best RR (animal and sire models) has been commonly taken based on test results of different criterions and genetic parameters. Among the LP models, that a lower order polynomial for the AG component than for the PE effects, the better the results observed by *Mohammadi et al., (2012b,c); Lopez – Romero and Carabano, (2003); Takma and Akbas, (2009)*. Thus, based on the results from most of the comparison criteria, it can be inferred that the models with a better quality fit were those which used lower order polynomial for the AG than for the PE effects.

a. Model comparison

a.1. First lactation

Choice of best LP model partly depends on partly the criteria that were used. While RR animal model with LP (2,6) for milk, fat and protein yields, had the lowest -2Logl, AIC and BIC values. Therefore, according to these criteria, the RR animal with LP (2,6) was selected as the best model. Furthermore, LP (2,6) for milk yield and LP (5,6) for fat and protein yields had lowest RV values (Table 2). According to comparison criteria's values, RR sire model with LP (5,6) for milk and protein yields and LP (3,6) for fat yield had the lowest -2Logl, AIC and BIC values. However, LP (2,6) for milk yield and LP (3,6) for fat and protein yield had lowest RV values (Table 3). The values of the criteria's were decreased when the order of fit for the PE effects than AG was increased in the LP models. This results agreeing with the results presented by *Takma and Akbas (2009)*; *Lopez – Romero and Biasus et al. (2011)* for Holstein-Friesian; *Costa et al. (2008)* for Brazilian Holstein and *Carabano et al. (2003)*; *Bignardi et al., (2009); El Faro et al., (2008); Meyer, (2001); Brotherstone et al., (2000); Albuquerque et al., (2005)*. The RV value for the three traits and both models decreased as order PE effect increased.

Table 2. Comparison criteria's used in the first lactation by RR animal model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	5,745,809.76	5,745,837.76	5,745,893.19	12.53
	LP(2,5)	19	5,731,287.98	5,731,287.98	5,731,325.98	11.73
	LP(2,6)	**25**	**5,725,100.92**	**5,725,150.92**	**5,725,249.90**	**11.27**
	LP(3,4)	17	5,845,163.30	5,845,197.30	5,845,264.61	12.59
	LP(3,5)	22	5,845,163.42	5,831,062.42	5,831,150.53	11.71
	LP(3,6)	28	5,825,896.61	5,825,952.61	5,826,063.47	11.30
	LP(4,5)	26	5,949,498.45	5,949,550.45	5,949,653.39	11.82
	LP(5,6)	37	6,066,980.98	6,067,054.98	6,067,201.48	11.40
Fat	LP(2,4)	14	337,253.53	337,281.53	337,335.96	0.04820
	LP(2,5)	19	336,219.52	336,257.52	336,331.29	0.04729
	LP(2,6)	**25**	**335,652.92**	**335,602.92**	**335,800.11**	0.04669
	LP(3,4)	17	455,945.73	455,979.73	456,045.82	0.04812
	LP(3,5)	22	454,931.95	454,975.95	455,061.48	0.04724
	LP(3,6)	28	454,265.78	454,321.78	454,430.63	0.04668
	LP(4,5)	26	575,895.08	575,947.08	576,048.16	0.04723
	LP(5,6)	37	696,701.50	696,775.50	696,919.34	**0.04656**
Protein	LP(2,4)	14	-294,064.67	-294,036.67	-293,983.39	0.01582
	LP(2,5)	19	-296,005.54	-295,967.54	-295,895.23	0.01540
	LP(2,6)	**25**	**-297,002.48**	**-296,952.48**	**-296,854.36**	0.01508
	LP(3,4)	17	-215,687.16	-215,653.16	-215,588.46	0.01584
	LP(3,5)	22	-217,614.93	-217,570.93	-217,487.21	0.01538
	LP(3,6)	28	-218,500.41	-218,444.41	-218,337.85	0.01508
	LP(4,5)	26	-210,562.43	-210,510.43	-210,411.48	0.01541
	LP(5,6)	37	-21,849.19	-21,775.19	-21,634.38	**0.01507**

LP (i,j) is i and j order for AG and PE effects respectively

Table 3. Comparison criteria's used in the first lactation by RR sire model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	5484,199.66	5,484,227.66	5,484,283.09	12.53
	LP(2,5)	19	5469,699.11	5,469,737.11	5,469,812.34	11.72
	LP(2,6)	25	5463,526.73	5,463,576.73	5,463,675.71	**11.24**
	LP(3,4)	17	5451,945.11	5,451,979.11	5,452,046.42	12.57
	LP(3,5)	22	5437,851.36	5,437,895.36	5,437,982.47	11.69
	LP(3,6)	28	5432,759.56	5,432,815.56	5,432,926.42	11.26
	LP(4,5)	26	5424,443.13	5,424,495.13	5,424,598.07	11.81
	LP(5,6)	**37**	**5409,632.05**	**5,409,852.05**	**5,409,852.55**	11.39
Fat	LP(2,4)	14	97,856.22	97,880.22	97,938.65	0.04813
	LP(2,5)	19	96,475.70	96,513.70	96,587.57	0.04756
	LP(2,6)	25	95,434.10	95,484.10	95,581.29	0.0459
	LP(3,4)	17	97,639.89	97,673.89	97,739.98	0.04784
	LP(3,5)	22	97,469.80	97,513.80	97,599.33	0.04673
	LP(3,6)	**28**	**94,456.61**	**94,456.61**	**04,621.01**	**0.04653**
	LP(4,5)	26	95,640.84	95,692.84	95,793.92	0.04718
	LP(5,6)	37	96,184.02	96,258.02	96,401.86	0.04654
Protein	LP(2,4)	14	-491,247.93	-491,219.93	-491,166.65	0.01582
	LP(2,5)	19	-493,173.95	-493,135.95	-493,063.64	0.01540
	LP(2,6)	25	-494,162.09	-494,112.09	-494,016.95	0.01503
	LP(3,4)	17	-512,130.26	-512,096.26	-512,031.56	0.01583
	LP(3,5)	22	-514,043.99	-513,999.99	-513,916.27	0.01536
	LP(3,6)	28	-514,909.18	-514,853.18	-514,746.62	**0.01502**
	LP(4,5)	26	-516,223.87	-516,171.87	-516,072.92	0.01540
	LP(5,6)	**37**	**-516,885.57**	**-516,811.57**	**-516,670.76**	0.01505

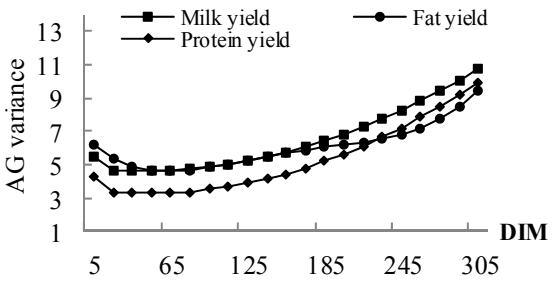
b. Variances and heritabilities

b.1. First lactation

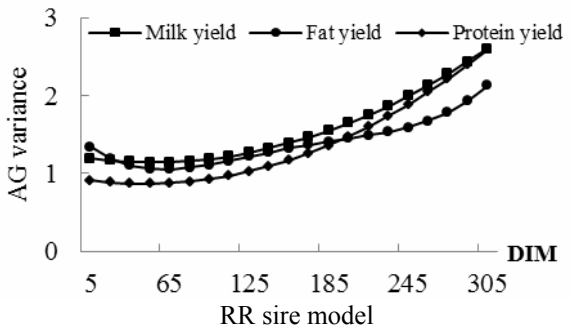
The AG and PE variances as a function of DIM for milk, fat and protein yields for RR animal and sire models for first lactation are shown in Figure 1. For all traits studied in each both RR animal and sire models, AG variance was observed higher at the end of lactation. On the other hand, the maximum PE variance was observed at the beginning of lactation, and after this period, PE variance decreased (5-50 DIM) and following by a small increase at the end of lactation. The trends in the AG and PE variances in this study for RR animal model and traits yields in first lactation are consistent with other studies (*Abdullahpour et al., 2010; El Faro et al., 2008; Bignardi et al., 2009; Strabel et al., 2005; Pool et al., 2000; De Melo et al., 2007; De Roos et al., 2004*). On the other hand, inconsistent the results achieved in this study, AG variances was increased as lactation progressed and was highest in middle lactation and subsequently

decreased at the end of lactation for Iranian Holsteins (*Shadparvar and Yazdanshenas, 2005*); and Turkish Holstein-Friesian (*Takma and Akbas, 2007*). Moreover, PE variance was highest in end lactation for Turkish Holstein-Friesian (*Takma and Akbas, 2007*). However, the trend in the AG and PE variances in this study for RR sire model was similar to the results obtained by *Bohlouli and Alijani (2012)*.

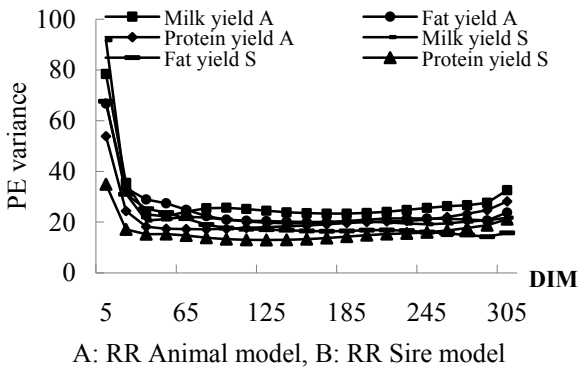
The minimum heritability for all traits in early lactation was observed (The heritabilities of RR animal model 0.05, 0.04 and 0.07 and heritabilities of RR sire model 0.08, 0.05, 0.05 for milk, fat and protein yields respectively). Generally, heritability for both models and all traits, increased sudden in during the lactation period. The trend of heritabilities of yield traits in this study for first lactation, were similar to results obtained in the Iranian Holsteins, by *Bohlouli and Alijani, (2012); Shadparvar and Yazdanshenas, (2005); Razmkabir. (2008)*. Also, agreeing with the results presented by *Biassus et al., (2011); Gengler et al., (2005); Olori et al., (1999) and Gengler et al., (1999)*. This increase in heritabilities estimate is associated not only with the increases on the values of AG components but also with the reductions in values of PE components between models. Because heritabilities is low in early lactation, is obtained PE at this stage of lactation high and given that AG variance was higher in late lactation. The small differences in heritabilities estimate between models do not indicate a preferred order of the LP.



RR animal model



RR sire model



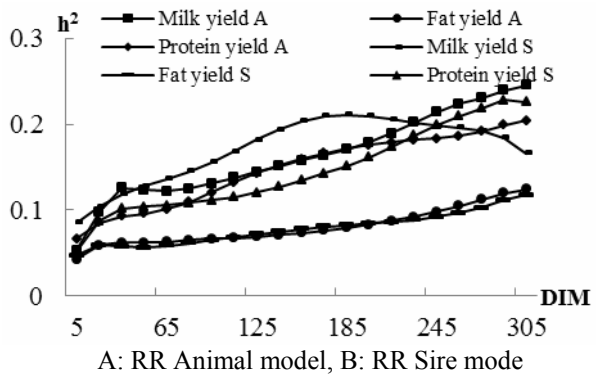


Figure 1. AG and PE variances in first lactation obtained for milk, fat (multiplied by 10^3) and protein yields (multiplied by 103) and Estimated heritability (h^2) for traits as a function of DIM

Table 4. Comparison criteria's used in the second lactation by RR animal model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	4,229,308.22	4,229,336.22	4,229,389.90	19.16
	LP(2,5)	19	4,078,380.18	4,078,418.18	4,078,491.04	19.02
	LP(2,6)	25	3,927,327.79	3,927,377.79	3,927,473.65	18.16
	LP(3,4)	17	3,997,675.53	3,997,709.53	3,997,774.72	19.03
	LP(3,5)	22	3,846,845.05	3,846,889.05	3,846,973.41	18.46
	LP(3,6)	28	3,695,816.86	3,695,872.86	3,695,980.23	**17.45**
	LP(4,5)	26	3,587,932.73	3,587,984.73	3,588,084.43	18.22
	LP(5,6)	**37**	**3,233,363.09**	**3,233,437.09**	**3,233,578.97**	17.71
Fat	LP(2,4)	14	511,006.34	511,034.34	511,087.06	0.07081
	LP(2,5)	19	509,667.76	509,705.76	509,777.31	0.06883
	LP(2,6)	**25**	**508,491.09**	**508,541.09**	**508,635.23**	0.06752
	LP(3,4)	17	606,574.43	606,608.43	606,672.45	0.07067
	LP(3,5)	22	605,039.63	605,083.63	605,166.48	0.06879
	LP(3,6)	28	603,826.87	603,882.87	603,988.31	**0.06748**
	LP(4,5)	26	645,428.45	645,480.45	645,578.36	0.06763
	LP(5,6)	37	716,087.18	716,161.18	716,300.51	0.06754
Protein	LP(2,4)	14	-6,793.21	-6,765.21	-6,713.67	0.02446
	LP(2,5)	19	-9,019.73	-8,981.73	-8,911.78	0.02354
	LP(2,6)	**25**	**-10,028.62**	**-9,978.62**	**-9,886.58**	**0.02286**
	LP(3,4)	17	65,633.83	65,667.83	65,730.41	0.02448
	LP(3,5)	22	63,256.46	63,300.46	63,381.45	0.02352
	LP(3,6)	28	62,196.44	62,252.44	62,355.52	0.02288
	LP(4,5)	26	66,436.65	66,488.65	66,584.36	0.02296
	LP(5,6)	37	67,524.55	67,586.55	67,734.76	0.02291

a.2. Second lactation

According to comparison criteria's values for second lactation, RR animal model with LP (5,6) for milk yield and LP (2,6) for fat and protein yields had the lowest for -2Logl, AIC and BIC values. However, LP (5,6) for milk yield, LP (3,6) for fat yield and LP (2,6) for protein yield had lowest RV values (Table 4). Furthermore, RR Sire model with LP (5,6) for milk yield and LP (3,6) for fat and protein yields had the lowest -2Logl, AIC and BIC values. Also, LP (2,6) for milk yield and LP (5,6) for fat yield and LP (3,6) protein yield had lowest RV values (Table 5).

Table 5. Comparison criteria's used in the second lactation by RR sire model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	4,411,819.78	4,411,847.78	4,411,901.46	19.36
	LP(2,5)	19	4,400,704.30	4,400,742.30	4,400,815.16	**18.02**
	LP(2,6)	25	4,394,670.29	4,394,720.29	4,394,816.15	17.07
	LP(3,4)	17	4,400,252.15	4,400,286.15	4,400,351.34	19.41
	LP(3,5)	22	4,388,902.53	4,388,946.53	4,389,030.89	17.99
	LP(3,6)	28	4,383,316.83	4,383,372.83	4,383,480.19	17.08
	LP(4,5)	26	4,382,730.14	4,382,782.14	4,382,881.84	18.16
	LP(5,6)	**37**	**4,371,164.33**	**4,371,238.33**	**4,371,380.21**	17.32
Fat	LP(2,4)	14	324,051.78	324,079.78	324,132.50	0.07078
	LP(2,5)	19	322,760.46	322,798.46	322,870.01	0.06878
	LP(2,6)	25	321,536.82	321,386.82	321,680.96	0.06859
	LP(3,4)	17	324,099.90	324,133.90	324,197.92	0.07071
	LP(3,5)	22	322,811.02	322,855.02	322,937.87	0.06872
	LP(3,6)	**28**	**321,228.29**	**321,284.29**	**321,389.73**	0.06737
	LP(4,5)	26	323,483.46	323,535.46	323,633.37	0.06875
	LP(5,6)	37	323,161.27	323,235.27	323,374.60	**0.06734**
Protein	LP(2,4)	14	-160,719.99	-160,691.99	-160,640.45	0.02445
	LP(2,5)	19	-162,932.71	-162,894.71	-162,824.77	0.02350
	LP(2,6)	25	-163,948.96	-163,898.96	-163,806.93	0.02287
	LP(3,4)	17	-167,528.72	-167,562.72	-167,432.14	0.02358
	LP(3,5)	22	-168,787.98	-168,743.98	-168,662.99	0.02349
	LP(3,6)	**28**	**-169,779.61**	**-169,723.61**	**-169,620.53**	**0.02282**
	LP(4,5)	26	-168,896.18	-168,844.18	-168,748.47	0.02352
	LP(5,6)	37	-169,562.56	-169,488.56	-169,352.35	0.02285

b.2. Second lactation

The trend of the AG variance estimates during the second lactation for RR animal and sire models and all traits were high at the beginning, then this trend decreased and subsequently increased at the end of lactation. However, the trend of the PE variance estimates during the second lactation was more similar to the first lactation (Figure 2). The AG and PE variances patterns observed in the study were comparable with those obtained by *Cobuci et al. (2011)* on Brasilian Holstein. The

minimum heritabilities for all traits in the second lactation were at the early lactation and trend of heritabilities was also similar to the results obtained of first lactation.

1

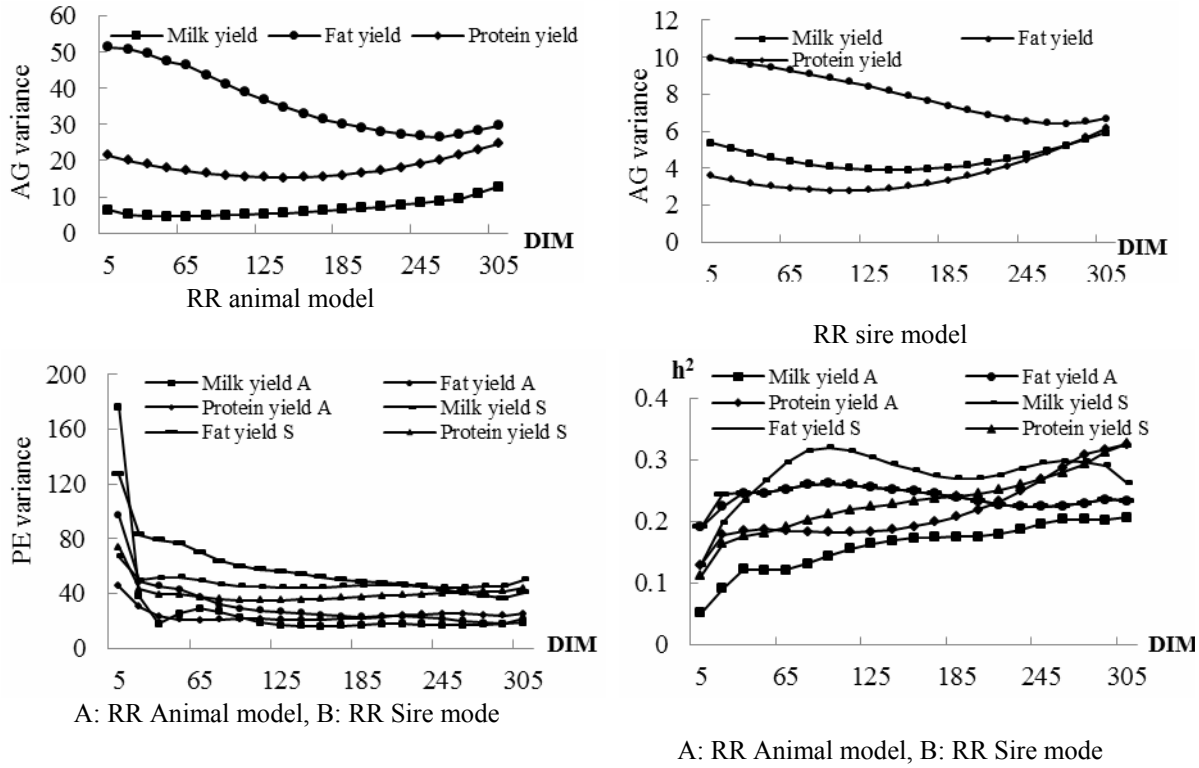


Figure 2. AG and PE variances in second lactation obtained for milk, fat (multiplied by 10^3) and protein yields (multiplied by 10^3) and Estimated heritability (h^2) for traits as a function of DIMa.3. Third lactation

According to results obtained of comparison criteria's values for third lactation, RR animal model with LP (2,6) for milk, fat and protein yields had the lowest for -2Logl, AIC and BIC values. However, LP (2,6) for milk yield, LP (3,6) for fat and protein yields had lowest RV values (Table 6). Moreover, RR sire model with LP (5,6) for milk and protein yields and LP (3,6) for fat yield had the lowest for -2Logl, AIC and BIC values. However, LP (2,6) for milk yield and LP (3,6) for fat and protein yields had lowest RV values (Table 7).

Table 6. Comparison criteria's used in the third lactation by RR animal model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	3,050,656.30	3,050,684.30	3,050,735.34	21.07
	LP(2,5)	19	3,042,083.66	3,042,121.66	3,042,190.92	19.43
	LP(2,6)	**25**	**3,037,826.83**	**3,037,876.83**	**3,037,967.96**	**18.34**
	LP(3,4)	17	3,116,815.18	3,116,849.18	3,116,911.15	21.09
	LP(3,5)	22	3,107,908.88	3,107,952.88	3,108,033.08	19.39
	LP(3,6)	28	3,103,942.97	3,103,998.97	3,104,101.04	18.37
	LP(4,5)	26	3,213,432.47	3,213,484.47	3,213,579.25	18.94
	LP(5,6)	37	3,241,645.56	3,241,719.56	3,241,928.44	18.63
Fat	LP(2,4)	14	377,214.95	377,242.95	377,293.04	0.07522
	LP(2,5)	19	376,045.34	376,083.34	376,155.19	0.07280
	LP(2,6)	**25**	**375,140.86**	**375,190.86**	**375,285.39**	0.07134
	LP(3,4)	17	443,975.77	444,009.77	444,074.05	0.07516
	LP(3,5)	22	442,788.17	442,832.17	442,915.36	0.07274
	LP(3,6)	28	441,831.03	441,887.03	441,992.91	**0.07123**
	LP(4,5)	26	455,416.16	455,468.16	455,566.48	0.07265
	LP(5,6)	37	576,442.18	576,516.18	576,656.09	0.07128
Protein	LP(2,4)	14	33,618.62	33,646.62	33,695.47	0.02606
	LP(2,5)	19	31,702.01	31,740.01	31,806.30	0.02488
	LP(2,6)	**25**	**30,889.61**	**30,939.61**	**31,026.84**	0.02406
	LP(3,4)	17	83,516.39	83,550.39	83,609.93	0.02605
	LP(3,5)	22	81,469.32	81,513.32	81,590.08	0.02483
	LP(3,6)	28	80,616.94	80,672.94	80,770.64	**0.02404**
	LP(4,5)	26	81,034.64	81,086.64	81,177.36	0.02495
	LP(5,6)	37	82,751.44	82,825.44	82,954.54	0.02407

Table 7. Comparison criteria's used in the third lactation by RR sire model and their levels

Trait	Model	Number of Parameters	-2Logl	AIC	BIC	RV
Milk	LP(2,4)	14	2,889,766.33	2,889,794.33	2,889,845.37	19.64
	LP(2,5)	19	2,896,038.46	2,896,080.46	2,896,145.72	19.38
	LP(2,6)	25	2,891,858.66	2,891,908.66	2,891,999.79	**18.28**
	LP(3,4)	17	2,897,117.58	2,897,151.58	2,897,213.55	21.09
	LP(3,5)	22	2,888,251.98	2,888,295.98	2,888,376.18	19.33
	LP(3,6)	28	2,887,742.47	2,887,798.47	2,887,900.54	18.37
	LP(4,5)	26	2,882,782.41	2,882,834.41	2,882,929.19	19.57
	LP(5,6)	**37**	**2,874,637.55**	**2,874,711.55**	**2,874,846.43**	18.58
Fat	LP(2,4)	14	245,627.43	245,655.43	245,705.52	0.07515
	LP(2,5)	19	244,507.21	244,545.21	244,613.19	0.07270
	LP(2,6)	25	243,622.79	243,672.79	243,762.24	0.07110
	LP(3,4)	17	245,594.39	245,628.39	245,689.22	0.07510
	LP(3,5)	22	244,444.14	244,488.14	244,566.86	0.07267
	LP(3,6)	**28**	**243,506.91**	**243,562.91**	**243,663.09**	**0.07103**
	LP(4,5)	26	244,861.89	244,913.89	245,006.92	0.07271
	LP(5,6)	37	244,584.23	244,658.23	244,790.62	0.07105
Protein	LP(2,4)	14	-73,910.45	-73,882.45	-73,833.60	0.02605
	LP(2,5)	19	-75,821.63	-75,783.63	-75,717.11	0.02482
	LP(2,6)	25	-76,592.40	-76,542.40	-76,455.17	0.02399
	LP(3,4)	17	-78,291.81	-78,257.81	-78,198.49	0.02604
	LP(3,5)	22	-80,310.36	-80,266.36	-80,189.60	0.02475
	LP(3,6)	28	-81,120.91	-81,064.91	-80,967.21	**0.02397**
	LP(4,5)	26	-80,921.91	-80,869.91	-80,779.19	0.02483
	LP(5,6)	**37**	**-81,559.19**	**-81,485.19**	**-81,559.19**	0.02402

b.3. Third lactation

The AG variances pattern of RR animal model in the third lactation was similar to those obtained in the second lactation. However, AG variances for milk and protein yields by RR sire model lowest was at beginning of lactation. Shape of heritability for all traits in third lactation and by RR animal model was similar to the obtained in the first and second lactations. Moreover, the patterns of heritability by RR sire model were the minimum at around 210 DIM.

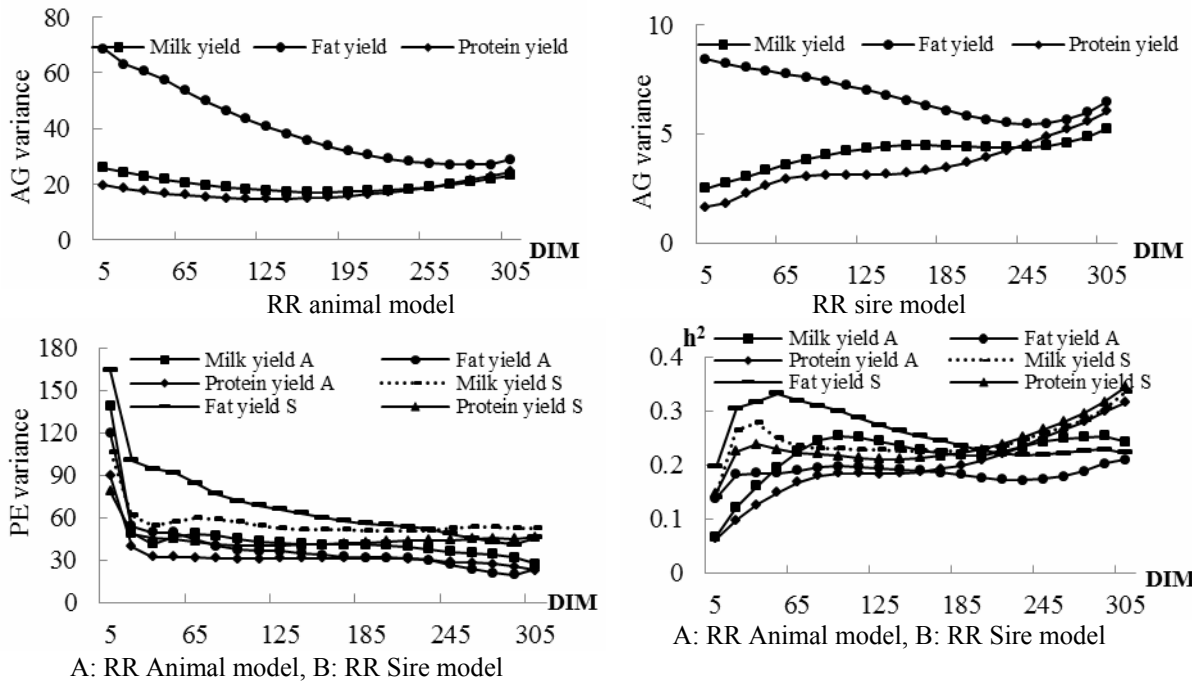


Figure 3. AG and PE variances in third lactation obtained for milk, fat (multiplied by 10^3) and protein yields (multiplied by 10^3) and Estimated heritability (h^2) for traits as a function of DIM

Genetic correlations

Generally, genetic correlations between TD yields for both RR animal and sire models was highest when periods closer to each other and the lowest was observed between distant TD. These results agree with results of *Biassus et al. (2011); Cobuci et al. (2011); Jakobsen et al. (2002) and Costa et al. (2008)*. The variation in genetic correlation estimates was larger in the first lactation than in the second and third lactations, although the trends within lactation were similar for both RR animal and sire models. These results are in agreement with previous studies which have reported the effect of parity on the estimation of genetic parameters in Holstein-Friesian (*Liu et al., 2000; Guo et al., 2002; Cobuci et al., 2011*)**.**

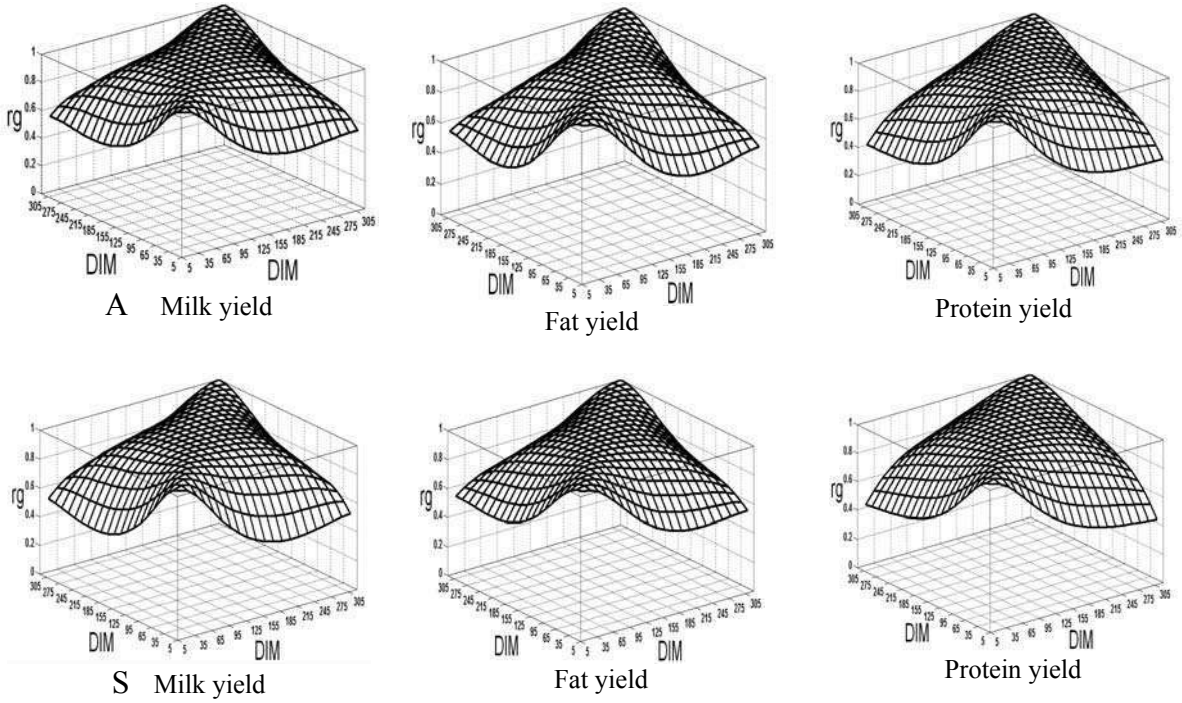


Figure 4. Genetic correlations in the first lactation obtained by RR animal model (A) and RR sire model (S) as a function of DIM. (Genetic correlations are presented only first lactation)

Conclusion

It is assumed that all mates are of similar genetic merit and this can result in bias in the predicted breeding values if there is preferential mating. According to in the RR animal model using of all animal records, therefore is for estimation of genetic parameters high accuracy. Thus, based on the results from the comparison of RR animal and sire models and comparison criteria, it can be inferred that the RR animal model which used lower order polynomial for the AG component than for the PE effects is better for modeling yield traits in Iranian Holsteins. Variations in heritability estimates across lactation were associated to different trends in genetic and PE variances. Trends of the heritability estimates during the second and the third lactation were more similar than those between the first and the second or the third lactation. Genetic correlations between individual TD records within traits and both RR animal and sire models and for different lactations were high for adjacent tests and decreased as the interval between tests increased.

Acknowledgment

The authors thank animal breeding center Karaj, Iran for providing the data.

Procena genetckih parametara i poređenje modela slučajne regresija grla i oca (random regression animal and sire models) za proizvodne osobine u prve tri laktacije goveda rase iranski holštajn

A. Mohammadi, S. Alijani

Rezime

Cilj ovog istraživanja je bio da se uporede modeli slučajne regresije (RR) životinje i oca u proceni genetičkih parametara za proizvodne osobine muznih krava rase iranski holštajn. U tu svrhu, dan testiranja korišćeni su podaci dnevnog testiranja u prve tri laktacije krava za osobine prinosa mleka, masti i proteina, koji su prikupljeni od 2003 do 2010 godine, od strane nacionalnog oplemenjivačkog centra Irana. Genetski parametri su ocenjivani koristeći algoritam ograničene maksimalne verodostojnosti. Da bi se uporedili modeli, korišćeni su različit kriterijumi - 2logL vrednost, AIC, BIC i RV u razmatranju osobina. Rezidualne varijanse su smatrane homogenim tokom perioda laktacije. Dobijeni rezultati su pokazali da je aditivna genetička varijansa bila je najveća u početku i na kraju laktacije i stalna varijansa životne sredine bila je veća u početku laktacije nego u drugim periodima laktacije. Procene heritabiliteta za prinosa mleka, masti i proteina, utvrđeni prema modelima slučajne regresije (RR) životinje i oca, utvrđeno je da su najniže tokom rane laktacije (0.05, 0.04 i 0.07, 0.05, 0.19 i 0.13, 0.14, 0.19 i 0.15, za prinose mleka, masti i proteina u prvoj, drugoj i trećoj laktaciji, respektivno). Međutim, procenjeni heritabikliteti tokom laktacije nisu varirali između Ležandra polinoma različitog redosleda a takođe između modelaslučajne regresije (RR) životinje i oca. Varijacija procenjenih genetskih korelacija u modelima slučajne regresije (RR) životinje i oca bila je veća u prvoj laktaciji nego u drugoj i trećoj laktaciji. Stoga, na osnovu dobijenih rezultata, može se zaključiti da je model slučajne regresije (RR Animal model) životinje bolji za modelovanje osobina prinosa goveda rase iranski holštajn.

References

ABDULLAHPOUR R., MORADI SHAHRBABAK M., NEJATI JAVAREMI A., VAEZ TORSHIZI R. (2010): Genetic analysis of daily milk, fat percentage and protein percentage of Iranian first lactation Holstein cattle. World Applied Science, 10, 1042-1046.

ALBUQUERQUE L.G., MEYER K. (2005): Estimates of covariance functions for growth of Nelore cattle applying a parametric correlation structure to model within-animal correlations. Livestock Production Science, 93, 213-222.

BIASSUS L.D.O., COBUCI J.A., COSTA C.N., RORATO P.R.N., NETO J.B., CARDOSO L.L. (2011): Genetic parameters for production traits in primiparous Holstein cows estimated by random regression models. Revista Brasileira de Zootecnia, 40, 85-94.

BIGNARDI A.B., EL FARO L., CARDOSO V.L., MACHADO P.F., ALBUQUERQUE L.G.D. (2009): Random regression models to estimate test-day milk yield genetic parameters Holstein cows in Southeastern Brazil. Livestock Science, 123, 1-7.

BOHLOULI M., ALIJANI S. (2012): Genotype by environment interaction for milk production traits in Iranian Holstein dairy cattle using random regression model. Livestock Research for Rural Develment, 24,7.

BROTHERSTONE S., WHITE I.M.S., MEYER K. (2000): Genetic modeling of daily milk yield using orthogonal polynomials and parametric curves. Journal of Animal Science, 70, 407-415.

COBUCI J.A., COSTA C.N., NETO J.B., FREITAS A.F. (2011): Genetic parameters for milk production by using random regression models with different alternatives of fixed regression modeling. Rev. Bras. Zootec. 40, 557-567.

COSTA N.C., MELO C.M.R.D., PAKCER L.U.A.F.D., TEIXEIRA N.M., COBUCI J.A. (2008): Genetic parameters for test day milk yield of first lactation Holstein cows estimated by random regression using Legendre polynomials. Revista Brasileira deZootecnia, 37, 602-608.

DE MELO C.M.R., PACKER I.U., COSTAAND C.N., MACHADO P.F. (2007): Genetic parameters for test day milk yields of first lactation Holstein cows by random regression models. Journal of Animal Consortium, 1, 325-334.

DE ROOS A.P.W., HARBERS A.G.F., DE JONG G. (2004): Random herd curves in a test-day model for milk, fat, and protein production of dairy cattle in the Netherlands. Journal of Dairy Science, 87, 2693-2701.

EL FARO L., CARDOSO V.L., ALBUQUERQUE L.G.D. (2008): Variance component estimates applying RRMs for test-day milk yield in Caracu heifers (Bos taurus Artiodactyla, Bovidae). Genetics and Molecular Biology, 31, 665-673.

GENGLER N., WIGGANS G.R., GILLON A. (2005): Adjustment for Heterogeneous Covariance due to Herd Milk Yield by Transformation of Test-Day Random Regressions. Journal of Dairy Science, 88, 2981-2990.
JAKOBSEN J.H., MADSEN P., JENSEN J., PEDERSEN J., CHRISTENSEN L.G., SORENSEN D.A. (2002): Genetic parameters for milk production and persistency for Danish Holsteins estimated in random regression models using REML. Journal of Dairy Science, 85, 1607-1616.
KIRKPATRICK M., LOFSVOLD D., BULMER M. (1990): Analysis of the inheritance, selection and evolution of growth trajectories. Genetics, 124, 979-993.
LIU Z., REINHARDT F., REENTS R. (2000): Estimating parameters of a random regression test day model for fist three lactation milk production traits using the covariance function approach. Interbull Bulletin, 25, 74-80.
LOPEZ-ROMERO P., CARABANO M.J. (2003): Comparing alternative random regression models to analyse first lactation daily milk yield data in Holstein–Friesian cattle. Livestock Production Science, 82, 81-96.
MEYER K. (2001): Estimating genetic covariance functions assuming a parametric correlation structure for environmental effects. Genetics Selection Evaluation, 33, 557-585.
MISZTAL I., TSURUTA S., STRABEL T., AUVRAY B., DRUET T., LEE D.H. (2002): BLUPF90 and related programs (BGF90). In: Proc 7th World Congress Genetics Applied Livestock Production, Montpellier, France CD-ROM communication. 28, 07
MOHAMMADI A., ALIJANI S., RAFAT A., BUHLOLI M., LATIFY M. (2012a): Estimation of genetic parameters for milk yield test day records by random regression model of Iranian Holstein dairy cattle. The fifth Iranian Congress on Animal Science, 29-30 August Isfahan University, Iran [In Persian].
MOHAMMADI A., ALIJANI S., RAFAT A., BUHLOLI M., LATIFY M. (2012b): Comparison of different polynomial functions in random regression models for estimation of genetic parameters to Test Day Milk Yields of Iranian Holstein dairy cattle. The fifth Iranian Congress on Animal Science, 29-30 August Isfahan University, Iran [In Persian].
MOHAMMADI A., ALIJANI S., RAFAT S.A., TAGHIZADEH A., BUHLOLI M. (2012c): Comparison of Fitting Performance of Polynomial Functions in Random Regression Model for Test Day Milk Yield in of Iranian Holstein Dairy Cattle. Research on Animal Productions. 6: 46-63 [In Persian].
OLORI V.E., HILL W.G., MC GUIRK B.J., BROTHERSTONE S. (1999): Estimating variance components for test day milk records by restricted maximum likelihood with a random regression animal model. Livestock Production Science, 61, 55–63.
PANDER B.L., HILL W.G., THOMPSON R. (1992): Genetic parameters of test day records of British Holstein heifers. Journal of Animal Production, 55, 11–21.

POOL M.H., JANSS L.L.G., MEUWISSEN T.H.E. (2000): Genetic parameters of Legendre polynomials for first parity lactation curves. Journal of Dairy Science, 83, 2640-2649.
SCHAEFFER L.R., DEKKERS J.C.M. (1994): Random regression in animal models for test-day production in dairy cattle. In: Proceedings of the 5th World Congress on Applied Livestock Production, Guelph, Canada, XVIII, pp, 443-446.
SHADPARVAR A.A., YAZDANSHENAS M.S. (2005): Genetic parameters of milk yield and milk fat percentage test day records of Iranian Holstein cows. Asian-Australian, Journal of Animal Science, 9, 1231-1236.
STRABEL T., PTAK E., SZYDA J., JAMROZIK J. (2004): Multiple-lactation random regression test-day model for Polish Black and White cattle. Interbull Bulletin, 32, 133-136.
STRABEL T., SZYDA J., PTAK E., JAMROZIK J. (2005): Comparison of Random Regression Test-Day Models for Polish Black and White Cattle. Journal of Dairy Science, 88, 3688-3699.
TAKMA C., AKBAS Y. (2009): Comparison of Fitting Performance of random regression model to Test Day Milk Yields in Holstein Friesians. Kafkas Universitesi Veteriner Fakultesi Dergisi, 15: 261-266.
TAKMA C., AKBAS Y. (2007): Estimates of genetic parameters for test day milk yields of a Holstein Friesian herd in Turkey with random regression models. Journal Archiv Tierzucht Dummerstorf, 50, 327-336.

Received 23 April 2014; accepted for publication 18 June 2014

Biotechnology in Animal Husbandry 30 (2), p 281-288, 2014 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.48
DOI: 10.2298/BAH1402281O

PATH COEFFICIENT MODEL FOR ASSESSMENT OF WEIGHT USING LINEAR TRAITS AT BIRTH AND AT WEANING IN NIGERIAN INDIGENOUS PIG

D. M Ogah[1]* N. D. Yusuf[2], M. M. Ari[1]

[1]Animal Science Department, Faculty of Agriculture, Nasarawa State University Keffi Shabu-Lafia campus
[2]Animal Science Department, College of Agriculture, PMB 33 Lafia, Nasarawa State, Nigeria.
Corresponding author: mosesdogah@yahoo.com
Original scientific paper

Abstract: Direct and indirect effects of some explanatory variables ((body length (BL), rump height (RH), rump length (RL) , rump width (RW) , shoulder width (SW), wither height (WH), heart girth (HG) and flank length (FL)) influence on live weight at birth and at weaning in Nigerian indigenous pigs, managed under semi intensive system were investigated using path analysis. Results of the analysis indicated that the correlation coefficient between body weight and body length at birth was highest (0.59) while at weaning, rump width had the highest correlation coefficient with body weight (0.60). The relationships were from low to high. The direct effect of linear traits to body weight at birth were highest with wither height and flank length similarly heart girth and rump length had better direct effect on body weight at weaning than other linear traits. The findings show that there is variability in relationship between body weight and linear traits with age, similarly both tissues and bone development play significant role in weight determination in the indigenous pigs. Thus selection for weight increase at adult phase can better be achieved at weaning, providing direction for selection towards increase weight in indigenous pigs.

Keywords: indigenous pig, path coefficient, selection, birth, weaning.

Introduction

Nigeria is estimated to have 4.4 million pigs and about 78% of these are found in the sub humid zones of Northern and southern guinea savannah (*Shaibu et al., 1997*). Most of the pigs are reared in the extensive system and their productivity have been reported to be low (*Okorie, 1978*). Efforts have been directed towards improving their productivity through selection and cross breeding.

Improvement of this animal through selection is important because of the inherent advantages associated with their adaptation.

Body weight is an important component of breed evaluation and plays a significant role in breeding value determination. Many factors are reported to influence body weight in most domestic animals, which include body length, chest girth etc. as reported by *Wu et al. (2008*) in rabbit, *Cankaya et al. (2008*) in calves, *Subalini et al. (2010*) in pigs. These traits have significant and positive correlation with body weight. The relationships between body size and shape of the animals, and different production traits, such as live weight, growth rate, carcass weight, milk yield, and nutritional requirements, have been investigated in different species by several authors (*Heinrichs et al., 1992; Wilson et al., 1997; Radović et al. 2007; Radović et al. 2009*). These relationships are considered an important way to describe growth and development of animals.

Systems of animal can be complex, it can be difficult to isolate causes and effect because each component potentially can influence others through a network of direct and indirect interactions (*Smith et al., 1997; Radović et al. 2009*). A mispecified model therefore can generate a serious bias in the estimation of the coefficient of each independent variable (*Jeonghoon, 2002*). To address this limitation, path analysis could be more suitable. It provide an effective means for finding of direct and indirect causal of association and permit a critical examination of the specific forces acting to produce a given correlation.

The aim of this study was to estimate body weight at birth and at weaning using biometric traits from direct and indirect associations using path analysis with a view to determine the most appropriate age to predict body weight and produce appropriate selection criterion for weight development in Nigerian indigenous pig.

Materials and Methods

The animals used in this experiment were 52 piglets from ten sows raised semi extensively by native farmers in Lafia, Nasarawa State, Nigeria. The data was generated between April and September 2009. The traits measured include body weight (BWT), body length (BL), rump height (RH), rump length (RL), rump width (RW), shoulder width (SW), wither height (WH), heart girth (HG) and flank length (FL) measured at birth period and the same traits measured at six period as described by (*Subalini et al., 2010*).

Statistical analysis

Descriptive statistics of the body weight and linear traits of pigs at birth and at weaning were computed. Pair wise correlation among body weight and linear traits were also determined. Standardized partial regression coefficient called

path coefficient (Beta weight) were calculated. The process gives direct comparison of values to reflect the relative importance of independent variables to explain variation in the dependent variable. The path coefficient from an explanatory variable (X) to a response variable (Y) as described by *Mendes et al. (2005)* is shown below

$$P_{yx1} - b_1 \frac{S_{x1}}{S_y}$$

Where
Pyx_1 = path coefficient for X_1 to Y (i=BL, RH, RL, RW, SW, WH, HG and FL)

b_1= partial regression coefficient
S_{X1}= standardized deviation of x_1
S_Y= standardized deviation of Y

The multiple linear regression model adopted was
$Y= a+b_1x_1 +b_2x_2 +b_3x_3+b_4x_4+b_5x_5+b_6x_6+b_7x_7+e$
Where
Y= endogenous variable (body weight)
a= intercept
b= regression coefficients
xi= exogenous variable,(BL, RH, RL, RW, SW, WH, HG and FL)
e= error term normally distributed with mean zero and variance

The significance of each path coefficient in the multiple linear regression model was tested by t-statistics using the following model.
The indirect effect of x_1 on y through x_j were calculated as follows
$IE_{yx\,i} = r_{xixj}p_{yxj}$
Where

IE_{yxi}= The direct effect of x_1 via x_j on y

$r_{xixj=}$ correlation coefficient between ith and jth independent variables.
p_{yxj}= path coefficient that indicates the direct effect of jth independent (exogenous) variable on the dependent (endogenous) variable.

Results and Discussion

The basic data of the indigenous pig at birth and at weaning are presented in Table1. The mean birth weight and weaning weight obtained here are higher than what *Ncube et al .(2003*) obtained for local pig genotype in Zimbabwe (0.97) and (4.17), but the birth weight in this study is comparable to that of local pigs of Mexico (1.32) as reported by *Mota et al. (2003*) with a superior weaning weight of (9.49). Other linear traits are similar to what was reported by (*Na-Lampang, 2010* and *Subalini et al., 2010*). The coefficient of variation for all the traits were generally low to moderate, this could be due to homogeneity of the population. Phenotypic correlations displaying the relationship between body measurements at birth and at weaning are given in Table 2. The highest correlation was predicted between rump height at birth and body length at weaning (0.814) while the lowest correlation was between body length at birth and rump height at weaning. There were positive relationship between the body measurements and live weight both at birth and at weaning. The relationship between the body measurement and weight at weaning were generally higher than that at birth. This similar finding was reported by *Cankaya et al. (2008*), who reported that there are variation in live weight determination and is common in animal research and that body measurements such as body length, hearth girth and chest width are reported as important indicators of the live weight in animal growth traits.

Table1. Descriptive statistics of body weight and linear traits of the indigenous pig at birth and at weaning

Trait	Age	Mean±se	Coefficient of Variation
Body weight	Birth	1.34±0.02	9.35
	Weaning	4.49±0.02	3.15
Body length	Birth	23.35±0.25	8.03
	Weaning	25.80±0.26	8.19
Rump height	birth	19.86±0.22	8.25
	Weaning	22.88±0.25	8.69
Rump length	Birth	10.13±0.18	12.91
	Weaning	13.25±0.20	11.92
Rump width	Birth	9.21±0.07	5.79
	Weaning	11.94±0.12	7.85
Shoulder width	Birth	12.25±0.19	11.52
	Weaning	14.25±0.28	10.38
Wither height	Birth	21.20±0.27	9.38
	Weaning	24.58±0.24	7.71
Heart girth	Birth	21.89±0.26	8.93
	Weaning	25.01±0.24	7.67
Flank length	Birth	10.59±0.14	8.93
	Weaning	13.56±0.12	9.42

Table 2. Correlation coefficient between traits at birth (above diagonal) and at weaning below diagonal

	WT	BL	RH	RL	RW	SW	WH	HG	FL
WT		0.59	0.16	0.43	0.58	0.34	0.18	23	0.39
BL	0.57		0.39	0.48	0.54	0.56	0.56	0.49	0.57
RH	0.32	0.52		0.1	-0.1	0.12	-0.2	-0.24	-0.26
RL	0.39	0.25	0.25		0.42	0.6	0.11	0.06	0.25
RW	0.6	0.5	0.11	0.39		0.6	0.55	0.56	0.74
SW	0.51	0.32	0.19	0.58	0.24		0.36	0.37	0.62
WH	0.36	0.6	0.25	0.1	0.37	0.23		0.94	0.87
HG	0.4	0.45	-0.26	-.03	0.41	0.13	0.77		0.92
FL	0.43	0.44	-0.29	-.10	0.39	0.35	0.6	0.82	

Bodyweight = WT, body length =BL, rump height = RH, rump length = RL, rump width = RW, shoulder width =SW, wither height = WH, hearth girth = HG, flank length = FL

The results of the path coefficient analysis of the independent variables at birth and at weaning are presented in Table 3 and 4. Path analysis permits the partitioning of correlation coefficient into component parts. The first component is the path coefficient (beta weight) that measures the direct effect of the predictor variables on the response variable. The second component estimates the effect of the predictor variable on the response variable through other predictor variable (indirect effect) (*Yakubu and Salako, 2009*). At birth both body length and rump width had moderate and positive correlation to body weight, but only body length had significant ($P<0.05$) direct effect on body weight. The indirect effect was 0.831 via flank length. Although wither height and flank length show high and positive direct effect on body weight, the estimate was outside the normal range.

Table 3. Analysis of direct and indirect effect of traits at birth

Traits	Correlation	Direct effect	Indirect effects							
	coeff.		BL	RH	RL	RW	SW	WH	HG	FL
BL	0.59	0.44		0.122	0.13	0.158	-0.2	0.683	0.966	0.831
RH	0.16	0.318	0.172		0.03	0.169	-0.7	0.154	-0.26	0.192
RL	0.43	0.272	0.213	0.03		0.123	-0.1	0.13	0.039	0.358
RW	0.58	0.296	0.239	0.191	0.11		-0.1	0.664	0.53	1.257
SW	0.34	-0.718	0.251	0.005	0.16	0.175		0.439	0.347	0.907
WH	0.18	1.217	0.25	0.255	0.03	0.162	-0.4		0.99	1.259
HG	0.23	-0.025	0.219	0.081	0.02	0.164	-0.3	1.138		1.338
FL	0.39	1.456	0.255	0.054	0.07	0.218	-0.1	1.052	0.894	

Bodyweight = WT, body length =BL, rump height = RH, rump length = RL, rump width = RW, shoulder width =SW, wither height = WH, hearth girth = HG, flank length = FL

Table 4. Analysis of direct and indirect effect of traits at weaning

Traits	Correlation	Direct effect	Indirect effects							
	coeff.		BL	RH	RL	RW	SW	WH	HG	FL
BL	0.57	-0.191		0.425	0	0.18	0.11	-0.21	0.445	0.059
RH	0.32	0.819	-0.099		0.004	0.012	0.06	-0.55	0.77	-0.166
RL	0.39	0.016	-0.047	0.208	0.139		0.19	-0.7	1.01	0.028
RW	0.6	0.358	-0.096	0.089	0.006	0.076		-0.43	0.419	0.049
SW	0.51	0.325	-0.062	0.152	0.009	0.084	-0.6		0.135	0.043
WH	0.36	-0.802	-0.114	0.205	0.002	0.134	0.07	0.793		0.074
HG	0.4	0.032	-0.086	0.557	0	0.145	0.04	0		0.102
FL	0.43	0.124	-0.083	0.519	-0.01	0.141	0.11	-0.48	0.85	

Bodyweight = WT, body length =BL, rump height = RH, rump length = RL, rump width = RW, shoulder width =SW, wither height = WH, hearth girth = HG, flank length = FL

At weaning the result of path analysis indicate that though body length and rump width had higher and significant correlation with body weight, they had least direct effect on the weight. To the contrary rump height had higher, positive and significant direct effect on weight, while wither height had higher negative (-0.802) effect. The variation in correlation and direct effect of the linear traits to body weight at older age explained what *Alvin et al. (1975)* and *Gurbuz et al. (1999*) asserted that using simple correlation coefficient between traits and explanatory variables may not explain the relationship in all aspect and may be inadequate in investigating the causal effect among the variables.

Conclusion

There are variation in body weight relationship with linear traits with age in the animals studied, this must be attributable to bone and muscle variation in the ages, suggesting the inappropriateness of early evaluation in weight determination.

Model za ocenu težine korišćenjem linearnih osobina na rođenju i zalučenju nigerijske autohtone rase svinja

D. M Ogah, N. D. Yusuf, M. M. Ari

Rezime

Direktni i indirektni efekti pojedinih varijabli (dužina tela (BL), visina krsta (RH), dužina krsta (RL), širina krsta (RV), širina plećke (SV), visina grebena

(WH), obim grudi (HG) i dužina slabine (FL) uticaj na živu mase na rođenju i odlučenju nigerijske autohtone rase svinja, u polu intenzivnom sistemu držanja, su ispitivani pomoću analize pravca/puta. Rezultati analize ukazuju da je koeficijent korelacije između telesne težine i dužine tela pri rođenju najviši (0,59), dok je na odbijanja, širina krsta imala najveći koeficijent korelacije sa telesnom težinom (0,60). Odnosi su bili od niskog do visokog. Direktan efekat linearnih osobina na telesnu težinu na rođenju su bili najviši sa visiniom grebena i dužinom slabine, slično tome, obim grudi i dužina krsta imali su bolji direktan uticaj na telesnu težinu na odlučenju od drugih linearnih osobina. Rezultati pokazuju da postoji varijabilnost u odnosu između telesne težine i linearnih osobina sa uzrastom, slično tome, razvoj tkiva i kostiju igra značajnu ulogu u određivanju težine u autohtonih svinja. Tako selekcija na povećanje težine u odrasloj fazi grla se može bolje postići pri odbijanju, pružajući pravac za selekciju na povećanje težine u autohtonih svinja.

References

ALVIN D. F., HAUSER L. (1975): The decomposition of effect in path analysis. American Sociological Review, 40, 37-47.

CANKAYA S., YAZGAN E.,.KAYAALP G.T, GOCMEZ Z., SERBESTER U. (2008): Canonical correlation analysis for estimation of relationship between some body measurement at birth and six month period in Holstein Friesian calves. Journal of Animal and Veterinary Advances, 7, 8, 953-958.

GURBUZ F., BASPINAR E., KESKIN S., MENDES M., TAKINDAL B. (1999) Path analysis technique 4. National Biostatistics meeting, 23-24

HEINRICHS, A. J.,. ROGERS G.W, COOPERJ.B. (1992): Predicting body weight and wither height in Holstein heifers using body measurements. Journal of Dairy Science, 75, 3576-3581.

JEONGHOON A. (2002). Beyond single equation regression analysis .Path analysis and multi- stage regression analysis. American Journal of Pharmaceutical Education, 66, 37-42.

MENDES M., KARABAYIR A., PALA A. (2005). Path analysis of the relationship between various body measurement s and live weight of American Bronze Turkey under three different lighting programs Tarim Bilimleri:Dergiai , 11, 2, 184-188.

MOTA D ., RAMIREZ-NECOECHEA R., ALONSO-SPILSBURY M., GARCIA-CONTRERAS C. (2003) Characterization of the productive performance in family pig farms located in Ayotzingo State of Mexico LRRD, 14,1.

NA- LAMPANG P. (2010). Morphological diversity of native pig in north east Thailand. In Proceedings of the 14th Asian-Australasian Association of Animal Production Pingtung Taiwan.

NCUBE M., DZAMA K., CHIMONYO M., KANEGONI A., HAMUDIKUWANDA D. (2003): Effect of boar genotype on reproductive performance of the local sows of Zimbabwe LRRD, 15, 2.
OKORIE J. U. (1978): A guide to livestock production in Nigeria. Mac Millian Ltd.London, 155.
RADOVIĆ Č., PETROVIĆ M., ŽIVKOVIĆ B., KOSOVAC O., RADOJKOVIĆ D., MIJATOVIĆ M., STOJANOVIĆ LJ. (2007): Effect of different fixed factors on pig carcass quality traits. Biotechnology in Animal Husbandry, 23, 1-2, 71-80.
RADOVIĆ ČEDOMIR, PETROVIĆ M., KOSOVAC O., STANIŠIĆ N., RADOJKOVIĆ D., MIJATOVIĆ M. (2009): The effect of different fixed factors on pig carcass quality and meat traits. Biotechnology in Animal Husbandry, 25, 3-4, 189-196.
SUBALINI E., SILVA G.L.L.P., DEMETAWEWA C.B.M. (2010): Phenotypic characterization and production performance of village pigs in Sri Lanka. Tropical Agricultural Research, 21, 2, 198-208.
SHAIBU B., AHIJU A., BAKSHI J. S. (1997): Nigerian National \agricultural Research Strategy Plan1996-2010. Department of Agricultural Science. Federal Ministry of Agriculture and Natural Resources.
SMITH F. A., BROWN J.H., VALONE T. J. (1997): Path analysis: A critical evaluation using long-term experimental data. The American Naturalist.
WILSON L.L., EGAN C.L., TEROSKY T.L. (1997): Body measurements and body weights of special-fed Holstein veal calves. Journal of Dairy Science, 80, 3077-3082.
WU Z., MA X., TIAN S., WU S., LI C., GUAN L., LI W., WANG H. (2008): Path analysis on body weight, body dimension and ear type of Saibei rabbit. In Proceedings of 9th World Rabbit Congress Italy.
YAKUBU A., SALAKO A. E. (2009): Path coefficient analysis of body weight and morphological traits of Nigeria indigenous chickens. Egyptian Journal of Poultry Science, 29, 111, 837-50.

Received 22 April 2014; accepted for publication 30 June 2014

Biotechnology in Animal Husbandry 30 (2), p 289-293, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.07'636.4
DOI: 10.2298/BAH1402289R

DETERMINATION OF PORK ADULTERATION IN MEATBALLS USING ENZYME LINKED IMMUNO SORBENT ASSAY (ELISA) TECHNIQUES CASE STUDY: SMALL MEDIUM ENTERPRISES MEATBALLS TRADERS AT JATINANGOR EDUCATION AREA, SUMEDANG DISTRICT, WEST JAVA, INDONESIA

L.B. Roostita, H. A. W. Lengkey

Faculty of Animal Husbandry, Universitas Padjadjaran
Jl. Raya Bandung Sumedang KM. 21, Jatinangor, Sumedang, West Java-Indonesia
roostita@gmail.com
Original scientific paper

Abstract: Adulteration of meatballs using pork begins to bloom in a row with the increase of beef price in the market. There is a risk that can occur around the education area of Jatinangor. Therefore the identification of meatballs adulteration with enzymes linked immuno sorbent assay (ELISA) approach need to be done in order to create certainty and security guarantee of the consumed products. Results showed that Small Medium Enterprises (SMEs) in Jatinangor who process meat (meatballs) was 21 meatball traders around Jatinangor. ELISA tests on 21 meatball samples taken from the SMEs merchant in Jatinangor showed no pork-adulterated meatballs on all of the samples tested.

Keywords: Pork, Adulteration, Meatballs, ELISA

Introduction

Jatinangor is one of education area in West Java that dominated by four large universities such as Universitas Padjadjaran, Institute Technology Bandung, Ikopin and IPDN. These conditions give rise to new demands for the fulfillment of food and snacks that are cheap and nutritious. One of the food that popular among college students is meatballs. Meatballs become favuorite because of sufficient nutritional value, taste and ease of preparation. However, the high price of meat and uncontrolled supply recently allegedly made it more difficult for producers to produce high quality meatballs with the appropriate raw materials.

To overcome the raw material problem, producers could substitute beef as main ingredient with other types of meat. However some producers who want to

reap greater profits misunderstand this clause. Pork used as substituent because it was cheaper and produces meatballs that have the closest characteristics of beef meatballs. This poses a serious threat to the security and halal processed meat products are consumed. The ingredients substitution practices break no laws but in many countries ingredients can also generate religious concern, such as pork contains ingredients are prohibited by Moslems (non-halal) (*Roostita, et al., 2009).*

Adulteration test on meat product were so difficult, especially heat processed product like meatballs, because heat process resulting denaturized proteins (*Hoffman*, *et al*., 1996). Therefore, antibodies to heat-stable soluble proteins, which retain their antigenicity after high temperature process, must be prepared (*Rencova*, *et al*., 2000). Enzymes linked immunosorbent assay (ELISA) approach allows the identification of different types of meat mixture in very low quantities or have undergone changes caused by processing. The test expected to provide a guarantee and assurance of the quality and safety of processed products (meatballs) generated by SMEs in the Jatinangor Education Area Sumedang, West Java-Indonesia.

Materials and Methods

The SMEs meatballs traders in Jatinangor Education Area (JEA) were identificated by survey method. Samples were taken from the SMEs meatball merchant at Educational Area Jatinangor, Sumedang District, West Java-Indonesia. Meatballs samples are prepared for enzyme linked immunosorbent assay test using Tepnel BioKits Pork Cooked Identification Test Kit Cat. No. 902012N (*Roostita, et al*., 2009).

Results and Discussions

Survey of SMEs meatballs traders in JEA showed there were 21 meatballs traders around JEA that frequently accessed by students. Only 13 (62%) of traders that produce their own meatballs, while 4 (19%) of traders purchased the meatballs from the traditional market and 4 (19%) others did not gave appropriate information.

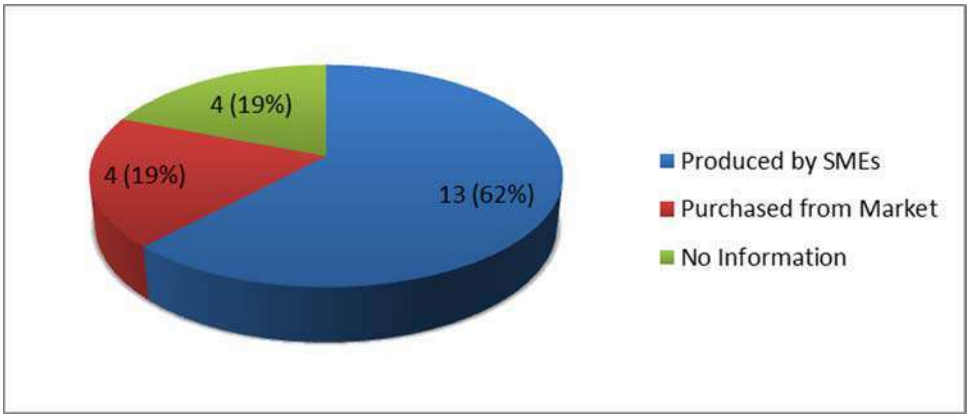


Figure 1. Survey of JEA SMEs Source of Meatball

Traders who produce their own meatballs were settled traders. It is because the traders want to maintain consumer safety and confidence in the quality of products. Meanwhile trader who purchased meatballs from the market or do not provide information are traders who used carts that are not settled. The ease in preparation becomes one of the considerations, in addition, the mobility of traders allow alternation buyers so the traders did not worries about losing customers. It is affect to the lack of attention to the quality of the meatballs sold.

- 1	- 2	- 3	- 4	- 5	- 6	- 7	- 8
- 9	- 10	- 11	- 12	- 13	- 14	- 15	- 16
- 17	- 18	- 19	- 20	- 21	+ 22	+ 23	+ 24

No Sample 1-21: Meatballs Samples, 22-24: Positive Control

Figure 2. Enzyme immunoassay result

Twenty-one samples were taken from the SMEs meatballs merchant to identify pork adulteration on sold meatballs and tested using ELISA. The ELISA kit was utilizing a biotin-avidin enhancement process. With increased concentrations of pork-specific protein in the extract, more of the protein will bind to antibody attached to the well. After allowing the reaction to proceed, unbound material is removed by washing. The amount of specific protein bound to the antibody coated well is determined by reaction firstly with biotinylated and also with a streptavidin-peroxidase conjugate. After incubation, access reagent is removed by washing. Finally, bound peroxidase activity is determined by adding a

fixed amount of Tetramethylbenzidine (TMB) substrate which develops a blue color (changing color to yellowish green on addition of acid stop reagent) in the presence if peroxidase. Color development is proportional to the original amount of specific pork protein in the samples extract (*Roostita, et al., 2009*).

The test on 21 samples taken from meatballs merchants in JEA showed no pork adulteration. This is because the meatball traders realize the importance of raw materials quality that related to halal meatballs. Merchants already have the knowledge of halal meatballs so that the traders do not worry about the meatballs sales. Halal is a sensitive issue because most of the residents of JEA were Muslim. If the trader commit an adulteration by mixing pork into the meatballs, although at certain times provide very high gain however this may threaten the business sustainability.

Conclusions

There were 21 meatballs traders around JEA that frequently accessed by students. Thirteen (62%) of traders produce their own meatballs, 4 (19%) of traders purchased the meatballs from the traditional market and 4 (19%) others did not gave appropriate information. The ELISA test on 21 samples taken from meatballs merchants in JEA showed no pork adulteration.

Acknowledgments

Authors would like to thank The Center for Research and Community Services of Universitas Padjadjaran for funding the research and Animal Disease and Veterinary Public Health Investigation Center for helping the ELISA Test. Later on, Lilis Suryaningsih, Wendry S. Putranto, Eka Wulandari and Gemilang Lara U. for the help during the research.

Određivanje svinjetine u ćuftama primenom enzim imuno sorbent test tehnike (ELISA): mala i srednja preduzeća, trgovci iz Jatinangor oblasti, Sumedang distrikt, zapadna Java, Indonezija

L.B. Roostita, H. A. W. Lengkey

Rezime

Upotreba (nedozvoljena) svinjetine u proizvodnji ćufti počinje da cveta sa povećanjem cene govedine na tržištu. Stoga identifikacija korišćenja nedozvoljene svinjetine u ćuftama korišćenjem enzim imuno sorbent testa (ELISA) je neophodna kako bi se osigurala sigurnost i bezbednost za konzumiranje proizvoda. Studija je uključila mala i srednja preduzeća (SME) u Jatinangor oblasti koja prerađuju meso (ćufte) kao i 21 preduzeće koje se bave trgovinom proizvoda od mesa u oblasti Jatinangor. ELISA testovi na 21 uzorku ćufti uzetih u malim i srednjim preduzećima (SME) u Jatinangor oblasti nisu pokazivali prisustvo svinjetine u analiziranim uzorcima.

References

ROOSTITA, L.B., LENGKEY, H. A. W., MUDJIARTININGSIH, S., ROSIANNIE, E., SURYANINGSIH, L., SETYADI, W.P., GHANI, A.R., GEMILANG, L. U. (2009): Qualitative Determination of Pork Species Content in Cooked Meats Using Enzyme Immunoassay Techniques: Case Beef Jerky And Shredded Meat Products In West Java – Indonesia. Biotechnology in Animal Husbandry 25 (5-6), p 895-899.

HOFFMAN K., FISHER K., MILLER E., KEMPER V.(1996): Experiments to Demonstrate the Effectiveness of Heat Treatments Applied to Canned Meats and Meat-and-Bone Meals. Fleishwirshaft, 76, 920-923.

RENCOVA E., SVOBODA I., NECIDOVA L. (2000): Identification by ELISA of Poultry, Horse, Kangaroo and Rat Muscle Specific Proteins in Heat-Processed Products. Vet. Med. Czech, 45, 12, 353-356.

Received 22 April 2014; accepted for publication 26 May 2014

Biotechnology in Animal Husbandry 30 (2), p 295-302, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.09'636.4
DOI: 10.2298/BAH1402295Z

SEROPREVALENCE OF ACTINOBACILLUS PLEUROPNEUMONIAE IN SWINE ORIGINATED FROM COMMERCIAL FARMS IN SERBIA

J. Žutić, D. Vojinović, V. Radosavljević

[1]Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia
Corresponding author: jadranka.zutic@gmail.com
Original scientific paper

Abstract: Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (*A.pleuropneumoniae)* is one of the most important respiratory disease of pigs and causes worldwide severe losses in pig farming. For *A.pleuropneumoniae* control and monitoring, the detection of ApxIV antibodies in the serum is the most frequently used serological method. The aim of this study was to investigate presence of antibodies against *A. pleuropneumoniae* in blood sera of gilts and sows using the ELISA test. Samples were taken from gilts and sows originating from four commercial swine farms in Serbia. For detection of ApxIV antibodies, commercial ELISA kit was used. A total of 453 blood sera samples of gilts (207) and sows (246) were examined. Antibodies against *A. pleuropneumoniae* were detected in 57 (12.58%) sera. Antibodies were present in 22 (10.62 %) sera of gilts and in 35(14.22%) sera of sows. Percentage of positive sera differed among the farms, ranging in gilts from 3.33-17.77 % and in sows from 8.95-22.64%. Serological methods is one of the most important procedures in the diagnosis of porcine pleuropneumonia particularly suitable for the control of animal health status in a large breeding.

Key words: *Actinobacillus pleuropneumoniae*, gilt, sow, antibodies, ELISA.

Introduction

The most significant problems in contemporary pig production are in connection with diseases of the respiratory system **(***Baker,2005; Hansen et al., 2010*). It is a characteristic of the current manner of production to set up agglomerations with concentrations of large numbers of animals within a small space. As a multifactorial disease, environmental conditions, population size, management strategies and pig-specific factors such as age and genetics also play significant roles in the outcome of respiratory disease (*Opriessing et al., 2011*).

Such conditions are especially favourable for respiratory pathogens and continuous presence of a high degree of virulence *in vivo*. As a result, there are increasingly frequent outbreaks of respiratory infections which are more difficult to control, with the maintenance conditions in large agglomerations exerting an extremely unfavorable effect (*Žutić et al., 2009*). The prevalence of respiratory disease is affected by the following: the presence of respiratory pathogenic organisms, the virulence of the pathogens present, the level of the pathogens in the house environment, the immunity of the pig and the time of exposure to the organisms, the presence of secondary opportunistic bacteria, the interactions between management, environment, the diseases and the pig (*The Pig Site Pig Health,2013*).

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae (A.pleuropneumoniae)* is one of the most important respiratory disease of pigs and is widely spread among pig-keeping countries (*Vidić et al., 2004; Bochev, 2007; Vaduva et al., 2011*). *A. pleuropneumoniae* is a small, gram-negative, encapsulated rod belonging to the family Pasteurellaceae.

There are two biotypes of *A.pleuropneumoniae,* differentiated on the basis of their requirement for factor V, (NAD) in biotype I (NAD-dependent) and biotype II (NAD-independent). There are 13 serotypes of biotype I and 2 serotypes of biotype II , based on surface polysaccharide antigens (*Maldonado et al., 2009; Bessone, 2010; Gotschalk, 2012*). The four major RTX toxins ApxI, ApxII, ApxIII and ApxIV are secreted by the different serotypes in various combinations (*Shin et al.,2010*). All serotypes can secrete ApxIV which can be produced only during infection, but not in vitro conditions. Presence of the Apx toxins is strongly correlated with virulence.

Growing pigs are most likely to be affected when they are 12-16 weeks old, but the disease can occur in all ages of swine (*Gotschalk and Taylor, 2006*). This disease is causing significant loss to farmers particularly when finisher pigs are involved. Pigs, asymptomatically infected with *A.pleuropneumoniae* in their upper respiratory tract, can transmit the infection. The main route of spread is by direct contact from pig to pig or by aerosol within short distances, although some authors reported that airborne transmission between closely located pig units is possible but rare (*Woeste and Grosse, 2007*). The clinical course of the disease can vary widely, ranging from the acute forms with severe clinical signs and a high mortality to the more chronic forms with few or even without any clinical symptoms (*Shi et al., 2012*). In the absence of treatment, the disease can progress very rapidly and death can occur within a few hours. Main clinical signs of the acute disease are anorexia, depression, fever, dyspnea and/or polypnea. Chronic infections are characterized by coughing and pleuritis in lungs (*Gotschalk et al., 2010*). The recent epidemiological studies indicate a very high rate of exposure reaching up to 100% of seropositivity of investigated farms (*Krejci and Newberry, 2011).* Confirmation by laboratory testing is essential for diagnosis, especially in monitoring schemes.

The health monitoring of herds is extremely important, firstly because of the need for the adequate strategy to be chosen for controlling the porcine pleuropneumonia and, at the same time, in order to prevent economic losses that this disease may cause. Identification of seropositive animals is an important measure to control and eliminate the disease from the swine farms. Also, serological tests showed seroconversion of each animal according to certain technological categories within the herd. Using ELISA, it is possible to detect ApxIV antibodies against all serotypes of the *A. pleuropneumoniae*, without cross-reaction with other bacterial species (*Dreyfus et al., 2004; Nussbaumer et al., 2008; Eamens et al., 2012*). The aim of the study was to investigate the presence of ApxIV antibodies against *A. pleuropneumoniae* in gilts and sows in four pig farms in Serbia.

Material and methods

For *A.pleuropneumoniae* monitoring purposes, the detection of ApxIV antibodies in the serum is currently the most frequently used serological method. ELISA test can be used to evaluate the *A. pleuropneumoniae* status of commercial herds especially for the diagnosis of latently infected, without clinical signs. For the investigations, samples were taken of the blood of 207 gilts and 246 sows originating from 4 commercial swine farms in Serbia. The capacity of each farm is about 700-1.000 sows. Investigations were carried out using the method of ELISA with the following diagnostic kits: Chekit APP-Apx IV: *A. pleuropneumoniae* antibody test Kit
(IDEXX APP-ApxIV Ab Test).

Results and Discussions

A total of 453 blood sera samples of gilts (207) and sows (246) were examined. Results of determination of antibodies against of *A. pleuropneumoniae* are given in Table 1.

The results of the investigations have shown that infection with *A.pleuropneumoniae* is present on all four examined farms. Antibodies against *A. pleuropneumoniae* were detected in 57 (12.58%) sera. Antibodies were present in 22 (10.62%) sera of gilts and in 35 (14.22%) sera of sows. Percentage of positive sera differed among the farms, ranging in gilts from 3.33-17.77% and in sows 8.95-22.64%**.** The results of this study show a significant decrease of seropositive animals. In previous study, antibodies against *A. pleuropneumoniae* were detected in 69.86% of sows and 76.72% of gilts (*Žutić J, et al.,2008)*.

Table 1. Results on presence of antibodies against *Actinobacillus pleuropneumoniae* in gilts and sows blood sera

Farms	Category	No. investigated	No. positive	% positive
1	gilts	49	7	14.28
	sows	55	9	16.36
2	gilts	53	5	9.43
	sows	61	8	13.11
3	gilts	60	2	3.33
	sows	67	6	8.95
4	gilts	45	8	17.77
	sows	63	12	22.64
Total gilts		207	22	10.62
Total sows		246	35	14.22
Total animals		453	57	12.58

It is probably result of intensive serological monitoring of gilts prior to fertilization and improving the living conditions of animals. A higher percentage of positive animals on the farm 4 are the result of old buildings and poor conditions.

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* is still a problem today in herds of many countries where the swine industry is important (*Gotschalk et al.,2010*).The recent epidemiological studies indicate a very high rate of exposure, reaching up to 100% of seropositivity of investigated farms. Of those, 90% in Northwest Germany, 96% in Belgium, 89% in Spain and 100% in Italy and Belarus (*Krejci and Newberry,2011*). In Belgium, seroprevalence for serovar 2 was 58% (range 0 to 100 %), 53% for serovar 3 (range 10 to 95%), and 35% for serovar 9 (range 5 to 100%) (*Maes et al., 2002*). In the first report of the presence of *A. pleuropneumoniae* in Turkey, 258 out of 384 blood samples (67 %) were positive (*Metiner,2007*). Shi (*Shi et all, 2012*) reports of 55.72% seropositive of the Tibetan pigs. The prevalence ranged from 42.68-71.11%. This agrees with Assavacheep (*Assavacheep et al., 2003*), who had reported that 60% pigs were seropositive to at least one serotype and 45% of the pigs were seropositive to more than one serotype**.**

The successful control of porcine pleuropneumonia depends on the efficiency of preventing intra- and inter-farm transmission of the infection. One of the ways to successfully control and eliminate the porcine pleuropneumonia is a timely and fast diagnostic procedure with the implementation of immunodiagnostic tests (*Žutić et al., 2008).*

Serological diagnostics of infections caused by *A.pleuropneumoniae* is essential measure for the identification of latently infected herds and the determination of multiplicity of serotypes within the herd. The serological control

of gilts is of particular importance to detect infected animal prior to fertilization, because the mothers can transmit the infection to their offspring at birth.

Conclusions

It is very important to identify the presence of individual agents in the etiopathogenesis of respiratory disease of pigs in a large agglomerations. Based on our results, ELISA is a reliable method for serological diagnosis of porcine pleuropneumonia. This method is specific and may be used for the routine surveillance of health status in pig herds.

Acknowledgements

This investigation was supported by a grant from the Ministry of Education and Science of the Republic of Serbia, Project No. 31079.

Seroprevalencija *Actinobacillus pleuropneumoniae* kod svinja poreklom sa komercijalnih farmi u Srbiji

J. Žutić, D. Vojinović, V.r Radosavljević

Rezime

Pleuropneumonija svinja jedna je od najznačajnijih respiratornih bolesti svinja koja širom sveta dovodi do gubitaka, posebno u intenzivnoj svinjarskoj proizvodnji. Uzročnik bolesti je *A.pleuropneumoniae*.Prema korištenju β-nikotinamid adenin dinukleotida (β-NAD) za rast, uzročnik je podeljen na biovar 1 (β-NAD ovisan) i biovar 2 (β-NAD neovisan). Svaki biovar je dalje podeljen na serotipove. Do sada je otkriveno 15 različitih serotipova, među kojima su ustanovljene znatne razlike u virulenciji ali isto tako i varijacije u virulenciji između sojeva istog serotipa.Glavne faktore virulencije predstavljaju toksini.Sojevi A.pleuropneumoniae stvaraju četiri tipa egzotoksina označenih ApxI, ApxII, ApxIII i ApxIV. Za razliku od ostalih, ApxIV toksin proizvode svi serotipovi i visoko je specifičan za *A.pleuropneumoniae*.Bolest se klinički manifestuje kašljem, teškim disanjem i visokom temperaturom, a patoanatomski hemoragično-nekrotičnim promenama na plućima.U brojnim je istraživanjima dokazano da ovaj patogen često učestvuje u interakcijama bilo sa bakterijskim ili virusnim patogenima kao što su Mycoplasma hyopneumoniae, PRRSV i PCV2.Glavni put širenja je direktni kontakt među svinjama ili aerosolom na kratkoj distanci kao i mogućnost prenosa uzročnika sa majki na prasad.U cilju praćenja pojave i kontrole pleuropneumonije, najčešće se koriste serološke metode koje otkrivaju prisustvo

antitela za ApxIV toksin *A.pleuropneumoniae*.Cilj je ovog rada bio da se ispitaju krvni serumi nazimica i krmača poreklom sa 4 svinjarske farme u Srbiji,na prisustvo specifičnih antitela za *A.pleuropneumoniae*. U ispitivanju je, za otkrivanje antitela korišten komercijalni ELISA kit (IDEXX APP-ApxIV Ab Test). Ukupno je serološki pregledano 453 uzorka krvnih seruma, i to 207 uzoraka od nazimica i 246 uzoraka od krmača. Antitela za *A.pleuropneumoniae* ustanovljena su u 57 (12,58%) od ukupno 453 pregledanih seruma životinja. Kod nazimica, antitela su ustanovljena u 22 (10,62%) a kod krmača u 35(14,22%) seruma.Procenat pozitivnih seruma životinja bio je različit među farmama i kretao se kod nazimica od 3,33-17,77 % a kod krmača 8,95-22,64%. U dijagnostici pleuropneumonije svinja, serološke metode predstavljaju jedan od najznačajnijih postupaka, posebno pogodnih za kontrolu zdravstvenog stanja životinja u velikim aglomeracijama, pri čemu je moguće otkriti i latentno inficirana grla.

References

ASSAVACHEEP P., PERSSON M., LUENGYOSLUECHAKUL S., WATANAPHANSAK S., LAOHASINNARONG D., PUNGKHUN P., WALLGREN P. (2003): Actinobacillus pleuropneumoniae in Thai pig herds. Prevalence of serum antibodies and relation to performance. J. Vet. Med. Ser. B. 50, 390-395.

BAKER R.B. (2005): Diseases of the respiratory system. Proceedings of the North Carolina Healthy Hogs Seminar.1-19.

BESSONE F.A., DUCOMMUN M, ZIELINSKI G.C., SARRADELL J.E. (2010): Serotype determination of a collection of Actinobacillus pleuropneumoniae isolates from Argentina. Proceedings of the 21st IPVS, Congress, Vancouver, Canada, pp. 594.

BOCHEV I. (2007): Porcine respiratory disease complex (PRDC): A review. I.Etiology, epidemiology, clinical forms and pathoanatomical features. Bulg. J.Vet.Med. 10,131-146

DREYFUS A., SCHALLER A., NIVOLLET S., SEGERS R.P., KOBISCH M., MIELI L., SOERENSEN V., HUSSY D., MISEREZ R., ZIMMERMANN W., INDERBITZIN F., FREY J. (2004): Use of recombinant ApxIV in serodiagnosis of Actinobacillus pleuropneumoniae infections, development and prevalidation of the ApxIV ELISA.Vet Microbiol. 99(3-4):227-238.

EAMENS G.J., GONSALVES J.R., WHITTINGTON A.M., TURNER B. (2012): Evaluation of serovar-independent ELISA antigens of A. pleuropneumoniae in pigs following vaccination or experimental challenge with respiratory pathogens and natural A. pleuropneumoniae 1 challenge. Aust.Vet.J, 90,490-498.

GOTSCHALK M., TAYLOR D. (2006): Actinobacillus pleuropneumoniae**.** Diseases of Swine. Blackwell Publishing, Ames, IA, 563-575.

GOTSCHALK M., LACOUTURE S., ANGEN O., KOKOTOVIC B. (2010): An atypical biotype I Actinobacillus pleuropneumoniae serotype 13 is present in North America IPVS. Congress.Vancouver. Canada, pp.596.

GOTSCHALK M. (2012): Actinobacillus pleuropneumonae: an old but still relevant swine pathogen in the XXI century. IPVS. Korea, pp.26-31.

HANSEN M.S., PORS S.E., JENSEN H.E., BILLE-HANSEN V., BISGAARD M., FLACHS E.M., NIELSEN O.L. (2010): An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark. J Comp Pathol. 143, 120-131.

KREJCI R., NEWBERRY J. (2011): Pleuropneumonia in pigs - its importance and prevention.Int.Pig. Topics, 26, 15-17.

MAES D.G., LAEVENS H., VERDONCK M., DE KRUIF A., CHIERS K., HAESEBROUCK F. (2002): Seroprevalence of Actinobacillus pleuropneumoniae serovars 2, 3 and 9 in slaughter pigs from Belgian fattening farms .Vet. Record, 151,206-210.

MALDONADO J., VALLS L., MARTINEZ E., RIERA P. (2009): Isolation rates, serovars, and toxin genotypes of nicotinamide adenine dinucleotide–independent Actinobacillus pleuropneumoniae among pigs suffering from pleuropneumonia in Spain. J. Vet. Diagn.Invest. 21,854–857.

METINER K.S. (2007): Presence and seroprevalence of Actinobacillus pleuropneumoniae in Pigs in Turkey. Acta. Vet. Brno, 76,237–244.

NUSSBAUMER I., MISEREZ R., HUSSY D., DOHERR M.G., FREY J., ZIMMERMAN W. (2008): The seroprevalence of Actinobacillus pleuropneumoniae in Swiss pig breeding herds-a study with the Apx IV ELISA. Schweiz. Arch. Tierheilkd, 150 (3), 103-109.

OPRIESSNIG T., GIMENEZ-LIROLA L.G., HALBUR P.G. (2011): Polymicrobial respiratory disease in pigs. Anim. Health. Res. Rev, 12, 133-48.

SHI X.C., ZHANG N.Z., ZHOU D.H., XU M.J., DANBA C., WU S.M.,GA G., MU Y., ZHU S.Q. (2012): First Report of A. pleuropneumoniae Prevalence in Tibetan Pigs in Tibet, China. J. Anim.Vet. Advan, 11, 4167-4170.

SHIN M., S.CHA S., W.LEE W., J.SUNG J., Y.ROH Y., H.YOO H.(2010): Characterization of apxIVA of Actinobacillus pleuropneumoniae Korean isolate. P.298.pp.604. Proceedings of the 21st IPVS, Congress, Vancouver, Canada.

THE PIG SITE (2013): Respiratory Diseases and Control Strategies. http://www.thepigsite.com/pighealth/article/353/respiratory-diseases-and-control-strategies.

VADUVA I., HERMAN V., CSUKA E., BIKSI I. (2011): Minimal Inhibitory Concentrations of Tiamulin against Actinobacillus pleuropneumoniae. Bull.UASVM, Vet. Med**,** 68, 303-305.

VIDIĆ B., GRGIĆ Ž., NOVAKOVIĆ Z., STOJANOVIĆ D. (2004): Selective media for the isolation of Actinobacillus pleuropneumoniae from the pig. Acta Veterinaria (Bgd) 54(5-6):395-401

WOESTE K., GROSSE E. (2007): Transmission of agents of the porcine respiratory disease complex (PRDC) between swine herds; a review. Part 2-Pathogen transmission via semen air and living/nonliving vectors. Deut. Tierarztl. Woch, 114, 364-373.
ŽUTIC J., MILOŠEVIC B., VOJINOVIC D. (2008): Investigation results on presence of antibodies against Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae in swine blood sera. Proceedings, pp.209-212. X Episootiology Days, Tara,Serbia.
ŽUTIĆ M., AŠANIN R., MILIĆ N., IVETIĆ V., VIDIĆ B, ŽUTIĆ J., AŠANIN J.(2008): Isolation and identification of Actinobacillus pleuropneumoniae in pigs lungs at farms and their sensitivity to antibiotics,Acta Vet.Bgd, 58, 499-507.
ŽUTIC M., IVETIC V., RADANOVIC O., ŽUTIC J., JAKIC-DIMIC D., SAVIC B., PAVLOVIC I., STANOJEVIC S. (2009): Investigations of representation of certain bacteria strains in lungs of pigs with pneumonia. Vet. Glasnik, 63, 3-15.

Received 20 March 2014; accepted for publication 4 June 2014

Biotechnology in Animal Husbandry 30 (2), p 303-311, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.5
DOI: 10.2298/BAH1402303H

HETEROSIS EFFECT IN HYBRID LAYING HENS

P. Hristakieva, M. Oblakova, M. Lalev, N. Mincheva

Agricultural institute - Stara Zagora,6000, Bulgaria
Corresponding author: pavlinahristakieva@abv.bg
Original scientific paper

Abstract: The new original egg laying lines T, P and N selected at the Institute of Agriculture – Stara Zagora were used. Hybrid T♂ × P♀, P♂ × T♀ crosses were obtained and used for paternal line. Thereafter, the following breeding schedule of paternal and maternal lines was applied: Group I – (P♂×T♀)♂ ×N♀; group II – (T♂×P♀)♂ ×N♀; group III – T♂×N♀; and group IV – P♂×N♀. The production traits of original and hybrid birds were recorded: live weight at the age of 8 and 18 weeks, age of sexual maturity in days, 150 days egg production, average egg weight – at 2-week intervals until end of lay; livability, heterosis effect. The live weights of hybrids at 8 and 18 weeks of age were statistically significantly lower compared to original lines. The values of heterosis for this parameter were negative for all four hybrid combinations. The earliest beginning of egg lay occurred in (T♂×P♀) ♂ ×N♀ (162.08 days of age) and P♂×N♀ (163.11 days of age). The relative (%) heterosis for age of sexual maturity of studied hybrid combinations had moderate to low negative values. Average egg weights of hybrids were higher and the values of heterosis – positive for all four groups varying from 0.97% to 1.63%. The average 150 days egg production was lower in purebred lines compared to hybrids. The highest average 150 days egg production was determined in P♂×N♀ hybrids – 142 eggs. The heterosis effect for egg production in hybrids was significant.

Key words: crossbreeding, laying hens, heterosis, egg production

Introduction

The production of hybrid birds is essential for attaining high productivity from modern egg laying hens. Several crossbreeding methods are used for production of hybrids: between breeds, between strains (two-line, three-line and four-line) and combined crossbreeding, which results in heterosis.

Heterosis is a complex biological event, usually seen in first-generation crosses (F_1) and is characterised by increased livability and productivity (*Belorechkov, 2004, Szwaczkowski et al., 2003).*

The effect of heterosis is generally higher for reproduction traits than for growth potential *(Fairfull, 1990)* and is influence by the maternal side and nutrition *(Liu et al., 1995)*.

Singh et al. (1992), Chaubal et al. (1994), Minvielle et al. (2000) and Khalil et al. (2004) reported a lower egg production of original purebred chicken lines compared to their crosses. A mild positive heterosis effect from 0 to 5% for egg weight in produced crosses has been observed by *Fairfull et al. (1987), Fairfull (1990)* and *Groen et al. (1998*). *Khalil et al. (1999)* and *Sabri et al. (2000)* detected a significant maternal effect on the live weight of offspring at an early age (0 – 8 weeks of age), and according to *Prado-Gonzalez et al. (2003*) the maternal influence was manifested from hatching to 4 weeks of age.

The monitoring and comparison of production traits of original lines and their crossbred hybrids aimed to establish the best heterosis effect on the commercial product.

Materials and Methods

The experiment was performed in the Poultry Breeding Unit at the Institute of Agriculture, Stara Zagora, in 2009-2012. The newly selected egg laying lines T, P and N were used for production of hybrid birds.

Line T has red feathering, brown eggshell and is characterized with optimal live body weight and low feed consumption per one egg produced.

Line P has red feathering and high egg weight.

Line N has white feathering, used as maternal line for production of feather-autosexing egg-laying hybrids. It has been selected for high egg production and good hatching traits.

Hybrid two-line birds for the paternal line were T♂ × P♀, P♂ × T♀ crosses 2009/2010. This reciprocal crossbreeding aimed to obtain the best two-line paternal combination which should be bred to the maternal N line to produce the three-line stock hybrids.

The last stage was the crossbreeding of paternal and maternal lines: Group I – (P♂×T♀) ♂ ×N♀; group II – (T♂×P♀)♂ ×N♀; group III – T♂×N♀; and group IV – P♂×N♀.

In 2011/2012, the following traits of layers from original and hybrid lines produced by the aforementioned schedule were monitored:

- Live weight – at 8 and 18 weeks of age (g)
- Age of sexual maturity in days – at attaining 50 percent production for each group.
- Egg production – daily over a 150-day period (number of eggs)
- Average egg weight – at 2-week intervals until the end of lay (g)

- Livability – in percentage as ratio between the number of birds at a specified age and the number of hatchlings (%)
- The heterosis effect was calculated according to the formula *(Fairfull, 1990)*:

$$H\% = [F_1-(P_1+P_2)/2] / [(P_1+P_2)/2] \times 100,$$

where: H% – heterosis (%)
F_1 – average values of traits of hybrid lines
$P_{1,2}$ – average values of traits of original lines

Original lines and hybrids were divided in groups, according to the crossing schedule. They were housed in separate 20 boxes at 10 hens with 1 cock in the same premise on deep litter and fed rations respective to their age ad libitum.

Data were processed with Excel 2003-ANOVA using the Descriptive Statistics and F-Test Two-Sample for Variances procedures *(Zhelyazkov and Tsvetanova, 2002)*.

Results and Discussion

Table 1 presents production traits of pure and hybrid chicken lines. With regard to the live body weight at 8 weeks of age, hybrids were statistically significantly lighter than chickens from the original lines.

Table 1. Production traits of pure and hybrid chicken lines

Parameters	Parental line			Crosses			
	T♂ x T ♀	P♂ x P♀	N♂ x N♀	(P♂ x T♀)♂xN♀ Group I	(T♂x P♀)♂xN♀ Group II	T♂ x N♀ Group III	P♂ x N♀ Group IV
Body weight(g) age 8 wks	538.8±4.97cd	521.40±4.79c b	577.17±4.47c	495.95±5.62a	491.12±6.36a	509.67±6.36b	492.31±6.69a
age 18 wks	1472.1±18.4c	1444.26±14.08a	1490.99±10.25cd	1421.80±9.01a	1392.28±9.01b	1431.69±8.86a	1396.91±9.23b
Age of sexual maturity (days)	173.68±1.09a	178.5±2.47b	168.76±1.83c	168.12±2.33c	162.08±2.40d	169.03±1.67ce	163.11±1.07d
Average egg weight (g)	60.25±1.85a	60.65±1.17a	60.86±1.78a	61.58±0.64a	61.32±0.53a	61.15±0.56a	61.57±0.91a
Egg production for 150 days	102.63±3.61a	100.76±6.53a	111.36±5.03b	132.03±4.61c	139.60±3.33c	125.61±4.21d	142.42±4.67c
Livability (%)	95.83±1.44a	98.05±0.88a	91.67±2.02b	97.74±0.48a	97.59±0.72a	96.69±1.03a	95.58±1.41a

* different letters within a row indicate statistically significant differences

The lowest body weight at 8 weeks of age was determined in birds from group II – 491.97 g, followed by birds from group IV (492.31 g), group I (495.95 g) and the heaviest hybrids were from group III (509.67 g). Purebred lines were significantly superior to hybrids as body weight was concerned. There were no considerable differences between the weights of paternal and maternal lines.

At 18 weeks of age, chickens from the pure lines N and T have attained the highest body weights – 1490.99 g and 1472.10 g, respectively, while birds from line P were the lightest (1444.26 g), but these average weights were statistically significantly higher than live weights of hybrid combinations. Within the hybrids, the lowest weight was that of group II (1392.28 g), which was significantly lower only vs group IV.

The earliest beginning of lay was observed in birds from group II - 162.08 days of age, followed by group IV (163 days of age), with insignificant differences. The sexual maturity was attained at a statistically significantly later age in groups I (168 days) and II (169 days).

Lines T and P, used as paternal lines in the breeding plan for production of hybrids, attained sexual maturity at 173.68 and 178.5 days of age ($p \leq 0.05$). The

beginning of lay of the maternal line N (168 days) was the lowest compared to paternal lines T and P. In general, hybrid forms were statistically significantly superior to purebred paternal lines and were comparable to the maternal line with regard to the age of sexual maturity. This is in agreement with the data of *Chaubal et al. (1994)* and *Kicka (1997*) about earlier onset of sexual maturity in crosses of purebred lines. In this study, similar to what was reported by *El-Salamony et al. (2002)* there were no statistically significant differences in age of sexual maturity among original lines and hybrid combinations. *Lumatauw et al. (2002)* established that the age of sexual maturity varied from 150 days (Paraoakan) to 177 days (Bolinao).

The average weight of eggs produced from studied pure line and hybrid hens over the control period varied within a narrow range - 60.27 g, 60.65 g and 60.85 g for lines T, P and N; 61.15 g, 61.32 g, 61.57 g and 60.58 g за for groups III, II, IV and I respectively. Although the differences were not statistically significant, the average hybrid egg weights were higher.

The egg production of hens is influenced by numerous factors, particularly by the genotype and the production system *(Gerzilov et al., 2012)*. The established average 150 days egg production was lower in purebred lines compared to hybrid combinations. The highest average 150 days egg production was determined in hens from hybrid group IV – 142 eggs, followed by 139 eggs laid for 150 days by group II, 132 eggs (group I) and 125 eggs (Group III). The purebred maternal line produced the highest number of eggs – 111, for 150 days. The high production of eggs by hybrid combinations compared to pure lines corresponds to data reported by other authors *(Yahaya et al., 2009)*.

The livability of all studied hens was within the reference range. The lowest livability was recorded in line N (91.67%; $p<0.001$). Hybrid combinations and other purebred lines did not differ substantially with regard to this trait.

Table 2 presents the percent values of heterosis effect on production traits of hybrids. Heterosis for live body weight at 8 and 18 weeks of age ranged from high to low negative percentages – from minus10.37 to minus 3.22 for all hybrid combinations. This is in agreement with the findings of *Williams et al. (2002)* about a negative heterosis effect for live weight, increasing with age in the offspring of two White Plymouth Rock lines with high and low live weights.

Table 2 Heterosis effect (%)

Traits	(P♂ x T♀)♂xN♀ Group I	(T♂ x P♀)♂xN♀ Group II	T♂ x N♀ Group III	P♂ x N♀ Group IV
Body weight (g) 8 weeks of age	-9.62	-10.00	-8.65	-10.37
18 weeks of age	-3.22	-5.22	-3.36	-5.02
Age of sexual maturity (days)	-3.24	-6.70	-1.29	-6.05
Average egg weight (g)	1.63	1.20	0.97	1.34
Egg production for 150 days	31.56	33.06	17.40	34.28
Livability (%)	2.68	-7.97	3.13	0.75

After breeding local hens with Lohmann Brown and Leghorn, a number of researchers found out that the heterosis percentage for age of sexual maturity varied from –25% to 11.5% (*Singh et al., 1983; Fairfull et al., 1987; Bordas et al., 1996; Gavora et al., 1996; Mohammed 1997; Williams et al., 2002)*. *Lumatauw et al. (2002)* reported that the heterosis for age of sexual maturity in the offspring of Paraoakan x Banaba was 6.24%. In our experiment, the heterosis for age of sexual maturity had moderate to low negative values. The highest negative heterosis percentage was observed for group II (6.7%) followed by group IV (6.05%). The lay in birds from those groups began at the earliest age, followed by birds from groups I and III.

The heterosis for average egg weight was positive in all four hybrid groups and varied from 0.97% to 1.63%. This supported the superiority of hybrids over original breeder lines with regard to egg weight.

As egg production was concerned, the effect of heterosis on hybrids was high. The calculated percentages were from 17.40% (group III) to 34.28% (group IV). The positive high heterosis values confirmed that the offspring of original egg laying lines produced more eggs than purebred birds. This was also related to the age of sexual maturity, which occurred earlier in hybrid hens than in original lines. *Abou El-Ghar et al. (2012)* concluded that this could be attributed to increased number of produced eggs together with the lower age of sexual maturity.

The calculated heterosis for the other controlled trait – livability percentage, exhibited negative value only for hybrids from group II – minus 7.97%, whereas it was positive for the other two two-line combinations: 0.75% for group IV and 3.13% for group III. The three-line combination from group I had a heterosis percentage for livability of 2.68%. The data confirmed that only birds from group II were with lower livability compared to original breeder lines.

Conclusion

The heterosis percentage for live body weights at 8 and 18 weeks of age for all four studied hybrid groups had high to low negative values.

The heterosis effect for average egg weight was positive and ranged within 0.97% to 1.63%. This confirmed the superiority of hybrids over original breeder lines.

The positive heterosis values for egg production confirmed the high efficacy of crossbreeding for production of commercial egg laying hybrid birds.

Heterozis efekat u meleza kokoši nosilja

P. Hristakieva, M. Oblakova, M. Lalev, N. Mincheva

Rezime

Nove originalne linije kokoši nosilja T, P i N stvorene u Institutu za poljoprivredu - Stara Zagora su korišćene u ovom istraživanju. Hibridni meleziT ♂ × R ♀ , R ♂ × T ♀ su dobijeni i korišćeni za očinske linije. Nakon toga, primenjen je sledeći raspored očinskih i majčinskih linija: grupa I - (R♂×T♀)♂ ×N♀; grupa II - (T♂×R♀)♂ ×N♀; grupa III - T T♂×N♀; i grupa IV - R♂×N♀. Proizvodne osobine originalnih i hibridnih grla su evidentirane: živa masa u uzrastu od 8 i 18 nedelja, starost seksualne zrelosti u danima, 150-dnevna proizvodnja jaja, prosečna težina jaja – u intervalima od 2 nedelje do kraja nošenja; preživljavanje, heterozis efekat.

Mase živih grla hibrida u uzrastu od 8 i 18 nedelja starosti bile su statistički značajno niže u odnosu na originalne linije. Vrednosti heterozisa za ovaj parametar su bile negativne za sva četiri kombinacije meleza. Najraniji početak nošenja jaja zabeležen je kod (T♂×R♀)♂ ×N♀ (162,08 dana starosti) i R♂×N♀ (163,11 dana starosti). Relativni (%) heterozis za uzrast seksualne zrelosti ispitivanih kombinacija meleza imao je umerene do niske negativne vrednosti. Prosečne težine jaja meleza bile su veće i vrednosti heterozisa - pozitivne za sve četiri grupe i variraju od 0,97% do 1,63%. Prosečna 150-dnevna proizvodnja jaja je niža kod čistokrvnih linija u odnosu na meleze. Najveća prosečna 150 dnevna proizvodnja jaja je utvrđena za R♂×N♀ - 142 jaja. Efekat heterozisa na proizvodnju jaja u meleza je bio značajan.

References

ABOU EL-GHAR, R. SH., HANAN H. GHANEM, HEDAIA M. SHALAN and O.M.ALY. (2012): Heterosis from Crossing Some Local Strains With Two Commercial Lines Of Laying Hens. Egypt. Poult. Sci. Vol (32) (III): 515-529

BELORECHKOV, D. (2004): Modern views on hybridisation in poultry breeding. Poultry v Breeding, 6 : 13 – 15.

BORDAS, A., MERAT, P., MINVIELE, F. (1996): Heterosis in egg- laying lines under divergent selection for residual feed consumption. Poult. Sci., 75:20- 24.

CHAUBAL, D.V., SOLANSKI, J.V; SHUKAI, R.K, RANK, D.N, MISHRA, R.K, KHAMAK. (1994): Performance of White Leghorn pullets under reciprocal recurrent selection for tw ogeneration. Indian J. Poult. Sci. 29 (3): 211- 217

EL-SALAMONY, A.I., ABOU-ASHOUR, A.M; EZZELDIN, Z.A. (2002): A study of heterosis in some egg production traits in Norfa layers. Egypt. J. Agric Res. 80(3): 1337-1352.

FAIRFULL R.W., GOWE, R. S., NAGAL, J. (1987): Dominance and epistatis in heterosis of White Leghorn strain crosses. Canadian J. of Anim. Sci. 67: 663-680

FAIRFULL, R. W. (1990): Heterosis. Pages 913–934 in Poultry Breeding and Genetics. R. D. Crawford, ed. Elsevier Science, Amsterdam.

GAVORA, J. S., FAIRFULL, R. W., BENKEL, B. F., CANTWELL, W. J., CHAMBERS, J. R., (1996): Prediction of heterosis from DNA fingerprints in chickens. Genetics 144: 777-784. (October, 1996).

GERZILOV, V., V. DATKOVA, S. MIHAYLOVA, BOZAKOVA, N. (2012): Effect of poultry housing systems on egg production. Bulg. J. Agric. Sci., 18: 953-957

GROEN, A.F; HEELSUM A.V; KIMANI, C.W., VAN DRUNCU, C.P. (1998): Heterosis and reciprocal effects for egg laying traits estimated from a full dialed cross with six commercial White Leghorn lines, using mixed model analysis. Arch Geflugelk 62 (3): 115-120.

KHALIL, M. K., HERMES I. M., AL- HOMIDAN, A. H. (1999): Estimation of heterotic components for growth and livability traits in a crossbreeding experiment of Saudi chickens with White Leghorn. Egypt. Poultry Sci., 19 (3): 491 – 507.

KHALIL, M.K., AL-HOMIDAN, A. H., HERMES H.I. (2004): Crossbreeding components in age at first egg and egg production for crossing Saudi chickens with White Leghorn. Livestock Research for Rural Development 16 (1). 10pp

KICKA, M.A.M; STINO, F.K.R, KAMAR, G.A.R. (1997): Genetic studies on some economical traits of chickens in subtropics. Egypt. J. Anim. Prod.11 (1): 41-46.

LIU, G., E.A.DUNNINGTON, P. B. SIEGEL. (1993): Maternal effects and heterosis for growth in reciprocal cross populations of chickens. J. Anim. Breed. Genet. 110:423–428.
LUMATAUW, S., ANGEL L. LAMBIO, CELICIO R. ARBOLEDA, CL.T.DAGAAS. (2002): Heterosis and Combining Abilities in Crosses among Three Genetic Groups of Philippine Native Chickens (*Gallus gallus domesticus*, Linn.). Philippine Journal of Veterinary and Animal SciencesVol 28, No 1(abstr.)
MINVIELLE, F. MONVOISIN, J. I. COSTA, J. MAEDA Y. (2000): Long term egg production and heterosis in quail lines after within line or reciprocal recurrent selection for high early egg production. British Poult. Sci. 41 : 150- 157.
MOHAMMED, K. A. H. (1997): Improvement of some Egyptian strains of chickens by crossing with egg- type commercial breeds. Second Hungarian Egyptian Poultry Conference, 16- 19 September 1997. Godollo, Hungary.
PRADO – GONZALES, E. A., RAMIREZ AVILA, L., SEGURA –PREISINGER, R.(2003): Internationalization of breeding programs – breeding egg type chickens for a global market, 6th World Congress on Genetics Applied to Livestock Production, 26, 135 – 142;
Anim. Genetics and Breeding Unit, Univ. of New England, Armidale, Australia.
SABRI, H. M., HENRY, R. W., ROBERT, H. H., CHARLES, J. W. (1999): Genetic parameters for egg and related characteristics of White Leghorne hens in a subtropical environment. Genetics and Molecular Biology, 22 (2): 183 – 186.
SINGH, P., SINGH, R. V., CHANDARY, R. P., VIKRAM SINGH (1983): Diallel crossing for estimation of G. C. A., S. C. A., heterosis and other genetic effects for various economic traits in White Leghorn. Indian Vet. J., 60:384- 389.
SINGH, P., CHAUDHARY, M.L., BRAH, G.S and SANDHU, J.S. (1992): Evaluation of two White Leghorn strains and their reciprocal crosses for part year productivity. Indian J. Of Poult. Sci. 27(3): 139-143.
SZWACZKOWSKI, T., CYWA - BENKO, K., WEZYK, ST. (2003): A note on in breeding effect on productive and reproductive traits in laying hens. Anim. Sci. papers and Reports, 21(2): 121 – 129
WILLIAMS, S.M., S.E. PRICE, SIEGE, P.B. (2002): Heterosis of Growth and Reproductive Traits in Fowl. Poultry Science 81:1109–1112
YAHAYA, K.H., O. O.ONI, G. N. AKPA, ADEYNIKA, I. A. (2009): Evaluation Of Layer Type Chickens Under Reciprocal Recurrent Selection. Bayern Journal of Pure and Applied Sciences, 2(1):177 - 182
ZHELYAZKOV, E., TSVETANOVA, Y. (2002): Manual of Genetics. Stara Zagora, 2002:175-195

Received 20 March 2014; accepted for publication 6 June 2014

Biotechnology in Animal Husbandry 30 (2), p 313-320, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.31'636.5
DOI: 10.2298/BAH1402313S

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

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

Institute for Animal Husbandry, Autoput 16, P.Box 23, 11080 Belgrade, Republic of Serbia
Corresponding author: zdskrbic@gmail.com
Original scientific paper

Abstract: High market demand for high-quality chicken paws, as well as their role in the assessment of the welfare of broiler chickens are the main reasons for the research in this area. In order to determine the effects of rearing system and the body weight of moderate growing broilers on the frequency and severity of incidence of footpad dermatitis, experimental research was conducted on a total of 300 Redbro chickens reared in free range and floor system in the production facility until the age of 84 days. Based on an individual weighing of broilers and visual evaluation of the incidence and severity of footpad lesions at the end of the experiment, the frequency of the individual scores for footpad dermatitis was determined, as well as the average score in each of the five weight groups. The effect of body weight on the incidence and severity of footpad dermatitis was not statistically confirmed, but the absence of the most difficult forms of footpad dermatitis in broiler group with the lowest body weight was recorded. Rearing broiler chickens in free range system manifested a positive effect in terms of increased frequency of broilers without lesions and less frequency of moderate and severe lesions in relation to rearing system in the production facility. Also, the effect of rearing system on the frequency of the most severe degree of dermatitis in broilers Redbro was statistically confirmed.

Key words: broiler, body weight, footpad dermatitis, rearing system

Introduction

Contact lesions on the plantar surface of the feet are usually defined as footpad dermatitis. These are inflammatory lesions that are ranked based on the size of the affected area and its depth. In severe cases they are ulcerative changes that are covered with scab formed from exudates, litter and fecal matter. The occurrence of footpad dermatitis is a problem of productivity, economic efficiency and welfare of broiler production. Before the 80-ies of the last century, there was

no interest in exploring footpad dermatitis due to the low market value of chicken paws. Today there is a huge demand for quality chicken paws that have become the third economically important chicken product (*Shepherd and Fairchild, 2010*). The pain caused by severe lesions and as a result, difficult movement, may affect the reduced feed intake and reduced weight gain of broilers. In addition, the occurrence of pain and limited ability to meet basic physiological needs, as well as the expression of the essential behaviours are reasons why footpad dermatitis is used as an indicator of welfare. Factors that have been identified as important for the development of contact dermatitis in broiler production conditions are temperature and humidity, the quality and type of litter, stocking density, photoperiod, the composition of the mixture for feeding, types and distribution of drinkers and season (*Ferrante et al., 2006; Bilgili et al., 2006; Škrbić et al., 2009; Škrbić et al., 2012; Meluzzi et al., 2008*). Some studies point to the importance of genotype in terms of susceptibility to the development of footpad dermatitis (*Ask, 2010*). Differences in the incidence of footpad dermatitis were determined depending on the genetic predisposition of hybrids to growth rate (*Kjaer et al., 2006*) and in broilers of moderate growth rate, depending on the rearing system (*Đukić Stojčić et al., 2013*). In male broilers, a higher frequency incidence and severity of footpad dermatitis cases were determined compared to female animals (*Bilgili et al., 2006)*, which may be associated with greater body weight and more intensive contact with the litter. However, there are conflicting data on the effect of gender on the occurrence of footpad dermatitis (*Kjaer et al., 2006*), as well as the data confirming the connection between gender and body mass of chickens and footpad dermatitis (*Nagaraj et al., 2007*).

The aim of the study was to investigate the effect of rearing system and body weight of broiler genotypes predisposed to moderate growth rate on the frequency and severity of occurrence of footpad dermatitis.

Materials and methods

The experiment was two-factorial (rearing system and weight, 2x2), with a total of 300 Redbro chickens. Day-old chicks were moved into the facility with a floor system and reared until age of 84 days. The stocking density was 12 birds/m^2. Feeding was *ad libitum* and complete diets based on corn/soybean, containing 22.2% CP and 3100 Kcal/kg; 19.4% CP and 3110 kcal/kg, or 17.3% CP and 3170 Kcal/kg. Light program after the initial 23 L:1 D for 7 days, included six hours of darkness per day. After 42 days, chickens in the rearing system with free range (n=150) were provided daily access to the range area of 10 m^2/bird. At the end of the experiment, at the age of chicks of 84 days, individually weighing was carried out, and visual evaluation of occurrence and severity of footpad lesions in all chickens in the experiment (*Thomas et al., 2004*). The absence of lesions was

scored as 1, moderately severe lesions - score 2 and the worst forms of lesions - score 3. For the purpose of data processing, based on measured body weight groups were formed: I (2.0-2.5 kg); II (2.5-3.0 kg); III (3.0-3.5 kg); IV (3.5-4.0 kg) and V (> 4.0 kg), and in each of them the frequency of individual footpad dermatitis scores, as well as the average score, were determined.

Data were analysed by ANOVA using StatSoft software (STATISTICA 8, 2007). Data expressed in percentages were previously transformed to *arc sin.*

Results and Discussion

Table 1. Effects of body weight and rearing system of broilers on frequency and average score of footpad dermatitis

Treatment	Frequency of score, %			Average score
	1	2	3	
Group of body weight				
I	90.62	9.38	0	1.09
II	91.99	6.28	1.73	1.07
III	89.91	8.09	2.0	1.10
IV	93.22	4.28	2.5	1.08
V	96.15	1.92	1.93	1.06
Rearing system				
poultry house	90.90	6.26	2.83	1.05
free range	93.86	5.72	0.43	1.13
Significance				
body weight	ns	ns	ns	ns
rearing system	ns	ns	p=0.052	ns

ns- no significance

Test results did not confirm the hypothesis of a negative effect of higher body weight of Redbro broilers on score frequency, and the incidence and severity of footpad lesions (Table 1). Most chickens without lesions were recorded in the groups with the highest body weights (IV and V). However, in these groups, a higher incidence of lesions of the most difficult level was determined. In the correlation analysis of differently scored footpad dermatitis case, and using a system of nine scores, *Allain et al. (2009)* have found a negative correlation between the score indicating the absence of lesions and those that are related to the most severe ones, as confirmed by our results. In contrast, in the groups with lower final weights, a lower percentage of chicks without the lesion was established but also increased incidence of moderate lesions compared to the groups IV and V. Only in the group with the lowest body weights, the incidence of the most severe

lesions was not recorded. In support to the absence of the effect of body weight on the incidence and severity of footpad lesions are also quite similar average scores in groups ranging from 1.06 to 1.10.

In terms of the effect of rearing system on the incidence of footpad dermatitis, the differences between the free range system and the rearing system in poultry house were not statistically confirmed. A slight positive effect of free range system may be concluded on the basis of a number of birds with the absence of footpad lesions, as well as lower incidence of moderate to severe lesions. Also, differences in the frequency of occurrence of footpad dermatitis of the most severe level between rearing systems were on the border of statistical significance (p=0.052).

Frequency of footpad dermatitis scores in groups of body weight, depending on the rearing system (Table 2), indicated some interaction effects of body weight and rearing systems. Free range rearing system showed a positive effect on the condition of the foot pads in broilers of greater body weight. The limit of the manifestation of this effect is the group III or the broilers of body weight 2.5-3.0 kg. In groups III, IV and V the increase in frequency of score 1 is registered in the free range system in relation to the rearing system in the poultry house. Also, the advantage of the free range system is reflected in the absence of the most severe forms of dermatitis, except in the group IV. The rearing system in the poultry house, the most serious cases of footpad dermatitis were registered in all groups of chickens except the first weight group.

Table 2. Frequency of footpad dermatitis scores in groups of body weight depending on the rearing system

Group	Rearing system	Frequency of score, %		
		1	2	3
I	free range	81.25	18.75	0
	poultry house	100	0	0
II	free range	94.34	5.66	0
	poultry house	89.65	6.9	3.45
III	free range	95.83	4.17	0
	poultry house	84.0	12.0	4.0
IV	free range	97.87	0	2.13
	poultry house	88.57	8.57	2.86
V	free range	100	0	0
	poultry house	92.3	3.85	3.85

Footpad dermatitis is the most common form of dermatitis in broiler production (*Haslam et al., 2007; Allain et al., 2009*) indicating their importance for the assessment of welfare and quality of products. The issue of ability to move of broilers is more pronounced with the intensification of their growth (*Kestin et al., 2001*) and in this sense the role of footpad dermatitis should also be considered. In

this regard, all the factors, genetic, growing conditions, diet, which affect the intensity of the growth may be brought in connection with the incidence of footpad dermatitis. The effect of gender on the incidence of footpad dermatitis has been considered in previous research as the effect of body weight leading to lower mobility and intensive contact with the foot litter. Higher frequency of footpad dermatitis in male chickens has been reported by *Bilgili et al. (2006)*, and in females by *Kjaer et al. (2006)*. Discrepancies of the results on the effect of gender and body weight, according to *Shepherd and Fairchild (2010)*, suggest a non-significant effect on the incidence of footpad dermatitis. The results of our research are in concordance with this statement. However, the total rejection of the effect of body weight on the incidence and severity of footpad dermatitis would not be entirely correct, because on the one hand generally unexpressed problem with footpad dermatitis in the flock, based on the average score of all groups and on the other hand, due to the complete absence of the most severe forms of footpad dermatitis only in the group with the lowest body weight.

Improved conditions of growing broilers, i.e. lower stocking density, the greater thickness of the litter, photoperiod similar to the natural, improve the status of broiler welfare, including reduced occurrence of footpad dermatitis (*Meluzzi et al., 2008; Škrbić et al., 2011*). In this sense, the positive effect of free range system could be seen relative to the rearing system in the production house. In the present study, the frequency footpad dermatitis of the most serious level was significantly reduced in the free range system. The full impact of the rearing system can be viewed on the interaction effect with the body weight. The positive effect of free range rearing system was manifested in broiler chickens in the group with the highest body weight. In contrast to our results, *Pagazaur and Warriss (2006)* have determined the most common incidence of footpad dermatitis, as well as the highest percentage of the most severe forms of footpad dermatitis in free range and organic rearing systems in relation to the three types of floor rearing systems in the poultry house. These results were likely influenced by different age of broilers in different rearing systems, considering that broilers in free range and organic system were grown up to a maximum age of 56 and 70 days, respectively, leading to deterioration of the condition of their foot pads. The problem with the comparison of the results of researches on this topic is in the rearing conditions that vary between farms, especially between the farm and the experimental conditions. Another reason is the use of different, non-standardized systems for evaluation of footpad dermatitis.

Conclusion

Generally, the condition of foot pads of Redbro broilers in the experiment was satisfactory in all weight groups and rearing systems. This may be related to

genetic predisposition of broilers for moderate growth and experimental conditions, which made it difficult and possibly unrealistic to minimize the effect of the studied factors. The effect of body weight on the incidence and severity of footpad dermatitis was not statistically confirmed, but the absence of the most severe forms of footpad dermatitis in broiler group with the lowest body weight was evident.

Statistically significant effect of rearing system was determined on the frequency of the most severe forms of dermatitis. Rearing of broilers in free range system increased the percentage of chickens without lesions and reduced the frequency of moderate and severe lesions in relation to rearing system in the poultry house.

Acknowledgment

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

Efekti sistema gajenja i telesne mase Redbro brojlera na učestalost i težinu footpad dermatitisa

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

Summary

Velika potražnja na tržištu za kvalitetnim pilećim nogama, kao i njihova uloga u proceni dobrobiti brojlera su osnovni razlozi za istraživanja u ovoj oblasti. U cilju utvrđivanja efekata sistema gajenja i telesne mase brojlera umerenog porasta na učestalost i ozbiljnost pojave footpad dermatitisa, sprovedeno je eksperimentalno istraživanje na ukupno 300 Redbro pilića gajenih u sistemu sa ispustom i podnom sistemu u objektu do uzrasta 84 dana. Na osnovu individualnog merenja telesne mase brojlera i vizuelne ocene pojave i težine lezija na nožnim jastučićima, na kraju ogleda, utvrđena je frekvencija pojedinačnih ocena za footpad dermatitis, kao i prosečna ocena u svakoj od pet težinskih grupa. Efekat telesne mase na učestalost i ozbiljnost footpad dermatitisa nije statistički potvrđen ali je utvrđeno odsustvo najtežih oblika footpad dermatitisa u grupi brojlera sa najmanjim telesnim masama. Gajenjem brojlera u sistemu sa ispustom ispoljen je pozitivan efekat u smislu veće frekvencije brojlera bez lezija i manje frekvencije umerenih i teških lezija u odnosu na sistem gajenja u objektu.Takođe je dobijena statistička potvrda efekta sistema gajenja na frekvenciju dermatitisa najtežeg stepena kod Redbro brojlera.

References

ALLAIN, V., L. MIRABITO, C. ARNOULD, M. COLAS, S. LE BOUQUIN, C. LUPO, V. MICHEL (2009): Skin lesions in broiler chickens measured at the slaughterhouse: relationships between lesions and between their prevalence and rearing factors. British Poultry Science 50, 407-417.

ASK, B. (2010): Genetic variation of contact dermatitis in broilers. Poultry Science 89, 866–875.

BILGILI S. F., ALLEY M. A., HESS J. B., NAGARAJ M. (2006): Influence of age and sex on footpad quality and yield in broiler chickens reared on low and high density diets. Journal of Applied Poultry Research 15, 433-441.

ĐUKIĆ STOJČIĆ M., MILOŠEVIĆ N., PERIĆ L., BJEDOV S. (2013): Plumage condition and foot pad lesions of medium growing broilers reared extensively in the poultry house and in traditional free range. Proceedings of 10th International Symposium "Modern Trends in Livestock Production", Belgrade, Serbia, 2-4 October 2013, 841-846.

FERRANTE V., LOLLI S., MARELLI S., VEZZOLI G., SIRRI F., CAVALCHINI L.G. (2006): Effect of light programmes, bird densities and litter types on broilers welfare. EPC 2006, Verona, XII European Poultry, Conference Verona, Italy. Word's Poultry Science Journal 62 (supplement):586.

HASLAM S.M., KNOWLES T.G., BROWN S.N., WILKINS L.J., KESTIN S.C., WARRISS P.D., NICOL C.J. (2007): Factors affecting the prevalence of foot pad dermatitis, hock burn and breast burn in broiler chicken. British Poultry Science 48, 264-275.

KESTIN S. C., GORDON S., SU G., SORENSEN P.(2001): Relationship in broiler between lameness, live weight, growth rate and age. The Veterinary Record 148, 195-197.

KJAER, J.B., G. SU, B.L. NIELSEN, P. SORENSEN (2006): Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poultry Science 85,1342–1348.

MELUZZI A., FABBRI C., FOLEGATTI E., SIRRI F. (2008): Effect of less intensive rearing conditions on litter characteristics, growth performance, carcase injuries and meat quality of broilers. British Poultry Science 49, 509-515.

NAGARAJ M., HESS J.B., BILGILI S.F. (2007): Evaluation of a feed-grade enzyme in broiler diets to reduce pododermatitis. Journal of Applied Poultry Research 16, 52-61.

PAGAZAURTUNDUA A., WARRISS P.D. (2006): Measurements of foot pad dermatitis in broiler chicken at processing plants. The Veterinary Record 158, 679-682.

SHEPHERD E.M., FAIRCHILD B.D. (2010): Footpad dermatitis in poultry. Poultry Science 89, 2043-2051.

ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M., PERIĆ L., MILOŠEVIĆ N. (2009): The Effect Of Stocking Density On Certain Broiler Welfare Parameters. Biotechnology in Animal Husbandry 25 (1-2), 11-21.
ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M., PETRIČEVIĆ V., ĐUKIĆ STOJČIĆ M., ŽIKIĆ D. (2011): The effect of stocking density on individual broiler welfare parameters 2. Different broiler stocking densities. Biotechnology in Animal Husbandry 27(1), 17-24.
ŠKRBIĆ Z., PAVLOVSKI Z., LUKIĆ M., PETRIČEVIĆ V., MILJKOVIĆ B., MARINKOV G. (2012): The effect of the diet on incidence of footpad lesions and productivity of broilers. Biotechnology in Animal Husbandry 28(2), 353-360.
THOMAS, D.G., V. RAVINDRAN, D.V. THOMAS, B.J. CAMDEN, Y.H. COTTAM, P.C.H. MOREL, C.J. COOK (2004): Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. New Zealand Veterinary Journal 52, 76-81.

Received 5 May 2014; accepted for publication 25 June 2014

Biotechnology in Animal Husbandry 30 (2), p 321-328, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 632.4
DOI: 10.2298/BAH1402321K

FUNGAL CONTAMINATION AND NATURAL OCCURRENCE OF T-2 TOXIN IN POULTRY FEED

V. Krnjaja, Z. Pavlovski, M. Lukić, Z. Škrbić, Lj. Stojanović, Z. Bijelić, V. Mandić

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
Corresponding author: vesnakrnjaja.izs@gmail.com
Original scientific paper

Abstract: In this study, a total of 41 poultry (chicken and laying hens) feed samples collected from different farms in Serbia in the beginning of 2014 were investigated for total fungal count, presence of potential toxigenic fungi and natural occurrence of T-2 toxin. The number of total fungi was determined using the plate count method whereas T-2 toxin was detected by enzyme-linked immune sorbent assay (ELISA) method.

Relative high percent of investigated poultry feed samples (43.90%) had the total fungal count 1 - 7 x 10^2 CFU g^{-1}, while in 29.27% of the samples that number was 1.4 - 14 x 10^4 CFU g^{-1}. In regard to potentially toxigenic fungi, species of *Fusarium* genus were isolated in most of poultry feed samples (58.54%), while species from genus *Alternaria* were isolated in least of samples (9.76%). The presence of T-2 toxin was detected in 75.61% of the samples, with concentration of 25.07 - 426.08 μg kg^{-1} (in average, 55.34 μg kg^{-1}). The statistical insignificant negative correlation (r = – 0.05) was obtained between total fungal count and concentrations of T-2 toxin.

In addition, a total fungal count and content of T-2 toxin in the samples were not above the maximum allowed levels, although the presence of species from genus *Fusarium* was found in 58.54% samples. These results indicated that the sanitary and hygienic conditions during the production of poultry feed in Serbia have been at satisfactory level.

Key words: poultry feed, total fungal count, T-2 toxin

Introduction

Trichothecenes are a large group of mycotoxins usually detectable in the various types of cereals, which are the main components of feed for poultry *(Riazipour et al., 2009)*. T-2 toxin belongs to the trichothecene mycotoxins produced by fungi of *Fusarium* genus, especially *F. sporotrichioides* and *F. poae*

(Maragos, 2006). Contamination of agricultural products with potential toxigenic fungi can cause the production of mycotoxins in undesirable concentrations *(Saleemi et al., 2010)*. It is always the risk to use contaminated food with mycotoxins or mouldy food. Even if the microbiological examination does not establish a high incidence of toxigenic moulds, food can be contaminated with mycotoxins, since the presence of the fungi is not always an indication of the presence of mycotoxins *(Krnjaja et al., 2007)*.

Intake of very low concentrations of mycotoxins cause mycotoxicosis that lead to the weakening of the immune system and deteriorating health of animals causing economic losses in the form of reduced production. Mycotoxin residues have a major impact on the production of meat and eggs. Therefore, their presence in animal feed may be a risk for human health. Poultry feed is often contaminated with mycotoxins, and this is the reason of frequent mycotoxicosis *(Oliveira et al., 2007)*. Mouth lesions and associated losses in productivity caused by T-2 toxin are a major concern to the poultry industry. T-2 toxin at concentrations as low as 400 μg kg^{-1} causes oral lesions by affecting the epithelial cells of oral mucous membranes *(Devegowda and Murthy, 2005)*.

The development of the toxigenic fungi causing contamination of animal feed with the mycotoxins is a serious problem that reduces the feed quality. The most important toxigenic fungal genera are *Aspergillus, Fusarium* and *Penicillium*. Almost all *Fusarium* species are capable of producing mycotoxins and differ from the genera *Aspergillus* and *Penicillium* in which only a few species are toxicogenics *(Nijs et al., 1996; Magnoli et al., 1998)*.

Moisture and temperature are two main factors for the development of fungal species and the production of mycotoxins in feed components. The most of toxigenic fungal genera have been isolated from maize grain as the main component of animal feed at 8, 12, 16 and 20% moisture levels stored at 25 and 35ºC *(Niaz et al., 2011)*.

In Serbia there are not sufficient data on the total fungal count, the presence of potentially toxigenic genera of fungi and their connection to the natural occurrence of T-2 toxin in poultry feed, especially when it comes to ready-made mixtures and not individual components. For this reason, the objective of this study was to determine the total fungal count and the natural occurrence of T-2 toxin, as well as their interdependence, and to determine the percentage of potentially toxigenic genera of fungi in poultry feed originating from Serbia.

Materials and Methods

During the first quarter of 2014, total of 41 samples of poultry (chicken and laying hens) feed were collected from different poultry farms in Serbia. Samples were taken from the production line using standard methods *(European*

Commission, 2006) and were kept in plastic bags and stored at 4ºC until analysis. The moisture content of poultry feed samples was determined using a moisture analyzer (Ohaus MB35, USA).

Quantitative determination of fungal colonies were done on solid medium (Sabouraud maltose agar) using the pour-plate method. First, 20 g of the sample was homogenized with 180 ml of normal saline (NaCl, 8.5 g/l) in the course of a few minutes on the orbital shaker (GFL 3015, Germany). Serial dilutions to 10^{-4} concentration were made and 1 ml of dilutions to 10^{-2}, 10^{-3} and 10^{-4} each, and applied on Sabouraud maltose agar in Petri plates (9 cm in diameter). The Petri plates kept in incubator (Memmert, Germany) at 25ºC for 5-7 days. Total fungal count was presented as colony-forming units (CFU) per gram of sample. Identification of fungal genera was done based on morphological characteristics according to fungal key of *Watanabe (1994)*.

The presence of T-2 was detected by ELISA assay according to the instructions Celery Techna ® ELISA kits on an ELISA reader (Biotek EL x 800TM, USA). The limit of detection was 25 μg kg^{-1} for T-2 toxin.

Correlation between individual values obtained for grain moisture content, total fungal count and concentration of T-2 toxin was determined using Pearson's correlation coefficient (Microsoft Office Excel 2007).

Results

By analyzing samples of poultry feed it was established that the number of fungi ranged from 0 to 14 x 10^4 CFU g^{-1}. Most of samples (43.90%) had a total fungal count from 1 to 7 x 10^2 CFU g^{-1}, whereas 29.27% of samples contained from 1.4 to 14 x 10^4 CFU g^{-1}. No fungi were detected in 7.32% of the samples (Table 1). Statistically insignificant positive correlation (r = 0.02) was determined between the moisture content and the total number of fungi, showing that with the increase of moisutre content also the total fungal count slightly increased. The moisture content of the poultry feed samples ranged from 8.04 to 12.67% with an average of 10.92%.

Table 1. Level of fungal contamination of investigated poultry feed samples

Fungal counts (CFU g^{-1*})	Number of samples	Frequency (%)
1.4-14 x 10^4	12/41	29.27
1.1-9 x 10^3	8/41	19.51
1-7 x 10^2	18/41	43.90
0	3/41	7.32

*Colony forming units per g of sample

Mycological survey of investigated poultry feed samples using Sabouraud maltose agar medium showed the presence of six fungal genera, *Alternaria*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. In most samples the species from genus *Fusarium* (58.54% positive samples) were isolated, followed by species from genera *Penicillium* (51.22% positive samples) and *Aspergillus* (46.34% positive samples), whereas species from genera *Alternaria*, *Mucor* and *Rhizopus* were present in 9.76, 10.07 and 14.63% of samples, respectively (Table 2).

Table 2. Fungal genera in investigated poultry feed samples

Fungal genera	Number of samples	Frequency (%)
Alternaria	4/41	9.76
Aspergillus	19/41	46.34
Fusarium	24/41	58.54
Mucor	7/41	10.07
Penicillium	21/41	51.22
Rhizopus	6/41	14.63

Mycotoxicological analysis showed the presence of 75.61% T-2 positive samples, and T-2 toxin was detected in 31 of the analysed samples of poultry feed. Concentrations of T-2 toxin were from 25.07 to 426.08 μg kg^{-1} with an average concentration for all investigated samples of 55.34 μg kg^{-1} (Table 3). Between the concentrations of T-2 toxin, and the moisture content and the concentration of T-2 toxin, and the total number of fungi, statistically insignificant negative correlations r = – 0.07 and r = – 0.05, respectively, were established.

Table 3. Concentration T-2 toxin in investigated poultry feed samples

Item	T-2 toxin
Sample size[a]	31/41
Incidence %	75.61
Range (μg kg^{-1})	25.07 - 426.08
Mean[b] (μg kg^{-1})	55.34

[a] Number of positive samples/Number of total samples
[b] Mean concentration in positive samples

Discussion

The assesment of total fungal count in animal feed is important criteria in the determination of hygienic quality and a necessary tool for assessing the potential risks and dangers of the increased presence of mycotoxins. According to the Regulation on quality of animal feed *(Official Gazette of the Republic of Serbia, 4/2010)*, mixtures and forage raw materials do not correspond to the

hygienic quality if they contain more than 200,000 spores in 1 g of mixture for older animals or 50,000 spores in feed for young animals. In Serbia the maximum allowed level of T-2 toxin is 500 μg kg^{-1} *(Official Gazette of the Republic of Serbia, 4/2010)*.

Identification of potentially toxigenic fungi in our research in most of the samples showed species from genera *Fusarium, Penicillium* and *Aspergillus*. The values for total fungal count and content of T-2 toxin in the investigated poultry feed samples have not exceeded maximum allowed limit confirmed by the Regulation (data not presented). In Serbia, similar results were reported by *Živković et al. (2005)*, *Bočarov-Stančić et al. (2011)*, *Janić-Hajnal et al. (2013)* and *Kapetanov et al. (2013)*. According to *Kapetanov et al. (2013),* the total number of *Fusarium* colonies was 15 x 10^4 and the concentrations of T-2 toxin was 480 μg kg^{-1} in investigated chicken feed. In the analysis of mycotoxins in samples of 40 different protein feed performed by *Bočarov-Stančić et al. (2011)*, in three samples of soybean and its processed products (meal and cake), only T-2 toxin was detected, and microbiological analysis identified the fungi of the genera *Aspergillus*, *Fusarium* and *Mucor*, of which species *F. solani* was the producer of T-2 toxin. The concentration of T-2 toxin did not exceed 375 μg kg^{-1}. According to the data presented by *Janić-Hajnal et al. (2013),* in 52% of samples of maize as the most important component of poultry feed, the sum of T-2 and HT-2 toxins has been detected. In a positive samples the concentration of T-2 toxin was from 25 to 200 μg kg^{-1}. Mycotoxicological analysis of 57 samples of poultry feed performed by *Živković et al. (2005)* detected T-2 toxin in 19 samples at a concentration of < 300 μg kg^{-1}, in 18 samples at a concentration of 500 μg kg^{-1} and in three samples at a concentration of 1000 μg kg^{-1}.

According to data from other countries with similar geographical and climatic conditions, in the 45 examined poultry feed mixtures from western Poland in 2010, *Cegielska-Radziejewska (2013)* have isolated as most common fungal species of genera *Aspergillus* and *Rhizopus* and total fungal count was from 5.5 x 10^1 to 7.0 x 10^3 CFU g^{-1} (average 7.0 x 10^2 CFU g^{-1}) and T-2 toxin was not detected. Mycotoxicological examination of 50 samples of poultry feed mixtures in Slovakia T-2 toxin was detected in 90% samples with an average concentration of 13 μg kg^{-1} (range 1-130 μg kg^{-1}) *(Labuda et al., 2005)*. Similarly, in Croatia, *Pleadin et al. (2012)* have not detected high concentrations of T-2 toxin (in average 18.2 μg kg^{-1}) in the investigated poultry feed.

Conclusion

For successful poultry production, it is necessary to ensure both healthy and high-quality fresh components that are included in the feed mixtures, and ready-made mixtures without contaminants that may cause adverse effects in the

production chain. The obtained results revealed the presence of contaminants such as potentially toxigenic fungi and the T-2 toxin, but the levels of these contaminants did not exceed allowed limits. Since the *Fusarium* species were isolated in most samples (58.54% positive samples) and the T-2 toxin was present in 75.61% of investigated samples, it is necessary to emphasize the need for continuous monitoring of the quality of animal feed as an important preventive measure to prevent conditions for increased production of mycotoxins.

Acknowledgment

The study was supported by the Ministry of Education, Science and Technological Development, The Republic of Serbia (projects TR-31023 and TR-31033).

Kontaminacija gljivama i prirodna pojava T-2 toksina u hrani za živinu

V. Krnjaja, Z. Pavlovski, M. Lukić, Z. Škrbić, Lj. Stojanović, Z. Bijelić, V. Mandić

Rezime

U radu su proučavani ukupan broj gljiva, prisustvo potencijalno toksigenih rodova gljiva i prirodna pojava T-2 toksina u 41 uzoraka hrane za živinu (piliće i nosilje) koji su sakupljeni iz različitih farmi u Srbiji početkom 2014. godine. Ukupan broj gljiva određen je primenom metode razređenja a T-2 toksin je detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Relativno visok procenat proučavanih uzoraka hrane za živinu (43,90%) imao je ukupan broj gljiva 1 - 7 x 10^2 CFU g^{-1}, dok je u 29,27% uzoraka ukupan broj bio 1,4 - 14 x 10^4 CFU g^{-1}. Od potencijalno toksigenih gljiva u ispitivanim uzorcima hrane za živinu u najvećem broju uzoraka (58.54%) izolovane su vrste iz roda *Fusarium*, dok su vrste iz roda *Alternaria* bile izolovane u najmanjem broju uzoraka (9.76%). Prisustvo T-2 toksina detektovano je u 75,61% ispitivanih uzoraka sa koncentracijom od 25,07 - 426,08 μg kg^{-1} (prosek 55.34 μg kg^{-1}). Statistički neznačajna korelacija (r = – 0.05) utvrđena je između ukupnog broja gljiva i koncentracija T-2 toksina.

Ukupan broj gljiva i sadržaj T-2 toksina u ispitivanim uzorcima nisu bili iznad maksimalno dozvoljenih količina iako je ustanovljeno prisustvo vrsta iz roda *Fusarium* u 58.54% uzoraka. Ovi rezultati ukazuju da su u Srbiji sanitarno-higijenski uslovi za proizvodnju hrane za živinu na zadovoljavajućem nivou.

References

BOČAROV-STANČIĆ A.S., SALMA N.M., PANTIĆ V.R., ADAMOVIĆ M.J., MILJKOVIĆ A.D., SUZIĆ S.V. (2011): Microbiological and mycotoxicological correctness of protein feed ingredients in Vojvodina. Jour. Nat. Sci., Matica srpska Novi Sad, 120, 211-218.

CEGIELSKA-RADZIEJEWSKA R., STUPER K., SZABLEWSKI T. (2013): Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. Annals of Agricultural and Environmental Medicine, 20, 1, 30-35.

DEVEGOWDA G., MURTHY T.N.K. (2005): Mycotoxins: effects on poultry performance and health. Pp. 25-56. *In*: Diaz D.E. (eds), The mycotoxin blue book. Nottingham University Press, Nottingham, United Kingdom.

EUROPEAN COMMISSION (2006): Commision regulation (EC) No. 401/2006. Official Journal of the European Union, L70, 12.

JANIĆ-HAJNAL E.P., KOS J.J., MASTILOVIĆ J.S. (2013): Presence of T-2 and HT-2 toxins in maize. Jour. Nat. Sci., Matica srpska Novi Sad, 124, 131-136.

KAPETANOV M.C., POTKONJAK D.V., STOJANOV I.M., ŽIVKOV-BALOŠ M.M., JAKŠIĆ S. (2013): Clinical and pathomorphological diagnostics of mycotoxicosis in parent poultry flock caused by T-2 trichothecene. Jour. Nat. Sci., Matica srpska Novi Sad, 124, 137-143.

KRNJAJA V., LEVIĆ J., TOMIĆ Z., STOJANOVIĆ LJ., TRENKOVSKI S., NEŠIĆ Z., MARINKOV G. (2007): The presence of potential toxigenic fungi in animal feed with particular rewiev on species of genera *Aspergillus* and *Fusarium*. Biotehnology in Animal Husbandry, 23, 102, 95-103.

LABUDA R., PARICH A., BERTHILLER F., TANČINOVÁ D. (2005): Incidence of trichothecenes and zearalenone in poultry feed mixtures from Slovakia. International of Food Microbiology, 105, 19-25.

MAGNOLI C., DALCERO A.M., CHIACCHIERA S.M., MIAZZO R., SAENZ M.A. (1998): Enumeration and identification of *Aspergillus* group and *Penicillium* species in poultry feeds from Argentina. Mycopathologia, 142, 27-32.

MARAGOS C.M. (2006): Measurment of T-2 and HT-2 toxins in eggs by high-performance liquid chromatography with fluorescence detection. Journal of Food Protection, 69, 11, 2773-2776.

NIAZ I., DAWAR S., SITARA U. (2011): Effect of different moisture and storage temperature on seed borne mycoflora of maize. Pak. J. Bot., 43, 5, 2639-2643.

NIJS M., ROMBOUTS F., NOTERMANS S. (1996): *Fusarium* moulds and their mycotoxins. J. Food Safety, 16, 15-58.

OFFICIAL GAZETTE OF THE REPUBLIC OF SERBIA (2010): Regulation on the quality of animal feed, No. 4.

OLIVEIRA G.R., RIBEIRO J.M., FRAGA M.E., CAVAGLIERI L.R., DIREITO G.M., KELLER K.M., DALCERO A.M., ROSA C.A. (2007): Mycobiota in

poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia, 162, 355-362.
PLEADIN J., PERŠI N., VULIĆ A., ZADRAVEC M. (2012): Survey of mycotoxin feed contamination in Croatia. Biotechnology in Animal Husbandry, 28, 2, 167-177.
RIAZIPOUR M., IMANI FOOLADI A.A., RAZZAGHI-ABYANEH M. (2009): Natural occurence of T-2 toxin in domestic and imported rice. Iranian J. Publ. Health, 38, 4, 111-116.
SALEEMI M.K., KHAN M.Z., KHAN A., JAVED I. (2010): Mycoflora of poultry feeds and mycotoxins producing potential of *Aspergillus* species. Pak. J. Bot., 42, 1, 427-434.
ŽIVKOVIĆ G.R., ŽIVANOV N.M., ŽIVKOVIĆ J.Z., MILOJEVIĆ M.J., TEODOSIN J.M., PEĆANAC S.L., MILIĆ D.V., BOČAROV-STANČIĆ A.S., ĐEKIĆ J.P. (2005): Mycotoxins and their impact on poultry production. Jour. Nat. Sci., Matica srpska Novi Sad, 108, 197-205.
WATANABE T. (1994): Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. Lewis Publishers, Boca Raton, Boston, London, Washington D.C. pp. 410.

Received 12 May 2014; accepted for publication 3 June 2014

Biotechnology in Animal Husbandry 30 (2), p 329-337, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.087.7'636.594
DOI: 10.2298/BAH1402329O

THE EFFECT OF ADDITION OF ORGANIC SELENIUM ON PHEASANT PRODUCTION CHARACTERISTICS

S. Obradović[1]*, B. Živković[2], B. Petrujkić[3], V. Živković[2], M. Živkov-Baloš[4], M. Radulović[1]

[1]State University of Novi Pazar, Department of Chemical-Technological Sciences, Novi Pazar
[2]Institute for Animal Husbandry, Belgrade-Zemun
[3]University in Belgrade, Faculty of Veterinary Medicine, Belgrade
[4]Scientific Institute of Veterinary Medicine, Novi Sad
*Corresponding author: obradovi@sbb.rs
Original scientific paper

Abstract: The effect of organic selenium as feed additive contained in the feed was investigated, applied in concentrations of 0.3 and 0.4 mg Se/kg mixture on production characteristics pheasant. The study was conducted on a total of 45 common pheasant individuals *Phasianus colchicus,* six weeks old, divided into three equal groups of 15 individuals for 60 days. The control pheasants group was fed with a standard mixture without addition of selenium during the experiment, while the mixture of group I contained 0.15 g/kg and pheasants group II had 0.20 g/kg additives with organic selenium. The results showed that different amounts of organic selenium presence in pheasants' feed had a positive effect on analyzed productivity indicators of pheasants' growth rate, both their final body weight and weight gain ($p<0.05$). Among examined groups of pheasant, gained differences in average feed conversion and total production index were not statistically significant ($p> 0.05$). The best production results were achieved by individual II group, which also had the highest selenium content in muscle tissue of the pectoral muscles, drumstick and thigh ($p <0.05$).

Key words: pheasant, selenium, production results

Introduction

Pheasants brought up in aviaries receive is gaining importance not only as a game species, but also as a good dietary foodstuff for human consumption. The basic preconditions for a successful breeding of pheasants are providing of housing conditions, health care and feed quality throughout the production process. Technological modifications of high-quality pheasants' meat obtaining involve usage of different biologically active substances (nutricines), organic growth

promoters and related minerals to improve production parameters and higher percentage of bioactive substances in meat obtaining (*Adams, 1999*).

Selenium has many physiological functions in the organism and actively participates in the regulation of the body's cells redox potential, as well in antioxidant defense against free radicals in the organism of mammals and birds. Therewith, selenium is an integral part of many proteins that affect the normal functioning of the thyroid, prostate, pancreas, reproductive organs, and the entire immune system. The largest depots of selenium are placed in liver, kidney cortex, pancreas, pituitary, while the lowest concentrations are in adipose tissue (*Rotruck et al., 1973; Surai, 2000, 2002*).

Previous researches in poultry nutrition have confirmed that better production performance, health improvement and obtaining of functional products, can be achieved by organic selenium usage, which is usually linked to methionine amino acid, so-called selenium amino acid preparations (*Cantor, 1997; Edens, 2001*). Selenium deficiency in the poultry organism causes many pathological changes and ailments as: pancreas atrophy, kidney damage, exudativa diathesis, reduced insemination and immunity.

Security level of selenium in the bird's nutrition depends on its chemical composition, with the different of inorganic forms of selenium as selenite and selanatas. Chelated forms of selenium are highly soluble, easily absorbed, and certain organs such as liver, pancreas, skeletal muscles, and kidneys showed a high degree of affinity on them and incorporate them into their proteins. Unused portion of absorbed selenium is excreted from the poultry organism through urine, feces and lungs (*Mihailović, 1996; Jacques, 2001*).

There is a relatively large amount of literature data processing needs and problems of providing selenium in poultry nutrition nowadays, but few of the available researches highlight the influence of organic selenium on production performances and meat quality of pheasants. The aim of this study was to determine the effects of various organic selenium aspects in the nutrition of pheasants, with special emphasis on basic production parameters, utilization of nutrients and quality of certain meat categories.

Materials and methods

The experiment was conducted at the pheasant farm LU Čačak (March, April 2013.) on 45 individuals of common pheasant *Phasianus colchicus*, average body mass K group 385 g, I group 389 g, II group 381 g, six weeks old, divided into three equal groups of 15 individuals each (females and males 8:7). During the experiment which lasted 60 days zoo-hygienic conditions, accommodation and nutrition were adapted to under floor heating system. For pheasants feeding pellet mixture of ordinary composition was used, but the control pheasant group did not

receive organic selenium with the feed (selenomethionine), and as a feed additive for other two groups Alkosel product was used (*Lallemand, Fra*) which composition includes organic selenium in concentration of 2000-2400 mg/kg. Mixtures are composed of FSH "Agroprodukt" – Knić (Serbia) in premix mixer.

Table 1. Ingredients and chemical composition of feed for pheasants (%)

Component, %	**K** (Control)	**I**	**II**
Maize	40.50	40.485	40.48
Feeding meal	3	3	3
Soybean pellet	24	24	24
Sunflower pellet (33% total protein)	4.3	4.3	4.3
Alfalfa meal	3	3	3
Feeding yeast	3.5	3.5	3.5
Soybean grits	12	12	12
Sunflower pellet (42% total protein)	5	5	5
Lysine	0.1	0.1	0.1
Methionine	0.2	0.2	0.2
Feeding chalk	1.6	1.6	1.6
Mono-Ca-phosphate	1.5	1.5	1.5
Iodized salt	0.3	0.3	0.3
Premix	1	1	1
Alkosel	-	0.015	0.020

The average chemical feed composition of all three groups, g/kg: Proteins 242.50; Cellulose 60.50; Ash 73.50; Calcium 10.20; Total phosphorus 8.20; ME MJ/kg 12.75; Lysine 13.50; Methionine + Cystine 9.00. **Addition of the mixture per kg**: Vitamin A (IU/kg) 15000;Vitamin D_3 (IU/kg) 3000; Vitamin E (mg/kg) 32; Biotin (mg/kg) 0.20; Vitamin C (mg/kg) 15; Folic acid (mg/kg) 1.20; Niacin (mg/kg) 30; Pantothenic acid (mg/kg) 15; Vitamin B_6 (mg/kg) 3.20; Vitamin B_2 (mg/kg) 7; Vitamin B_1 (mg/kg) 2.10; Vitamin B_{12} (mg/kg) 0.03; Choline chloride (mg/kg) 500; Fe (mg/kg) 40; Mn (mg/kg) 80; Cu (mg/kg) 8; Zn (mg/kg) 60; J (mg/kg) 0.80; Co (mg/kg) 0.45; Se (mg/kg) 0.30; Antioxidant (mg/kg) 100.
g kg^{-1}

According to the manufacturer's specifications recommended preparation dosage for poultry feeding amounts 100 g/ton, respectively 0.2 mg/kg of organically bounded selenium. During the experiment pheasants of group I were fed with the mixture supplemented with 150 g/t of this preparation, what was equivalent with selenium concentration of 0.3 mg/kg, while the feed of the group II of pheasants contained 200 g/t of this additive, respectively 0.4 mg/kg of selenium. Pheasants were had the ad libitum access to feed and water during the experiment.

Used feed was chemically analyzed at the beginning of the experiment by standard testing methods (*AOAC, 1990*); therewith the energy content and amino acids were obtained by calculations. Ingredients composition of the used pheasants' feed during the experiment is shown in Table 1. The chemical composition of the used premix is shown in the table below (Manufacturer premix FSH "Agroprodukt" – Knić)

Body weight control measurements were performed on decimal, technical balance every ten days, before feeding, and the amount of consumed feed was measured every day. Also, health status of tested individuals, mortality and all behavioral changes were permanently monitored during the experiment. At the end of the 60 days experiment seven individuals were sacrificed from each group (4 females and 3 males). After slaughter and measuring, the basic body parts were segregated (brisket and drumstick with thigh) and measured on decimal scale (accuracy ± 0.03 g), and liver samples were taken for determination of selenium content. Based on the obtained data the production index was calculated at the end of the experiment.

The chemical meat composition of brisket, drumstick and thighs were analyzed by usage of standard testing methods (*AOAC, 1990*), and the search included determination of water content, lard, protein and ash. Selenium content in muscle tissue and pheasants' organs was determined by atomic absorption spectrometry, hydride technique. Statistical data analysis was performed by analysis of variance usage with assessment of statistical significance with the t-test.

Results and Discussion

The chemical composition of the used pheasant's mixtures presented in Table 1 is in accordance with the recommended standards *NRC (1994), Blake and Hess, (2009)*.

Final average pheasants' body weights at all three groups (Table 2) were in the normal range for individuals of this age, with the most intensive growth recorded on 50 and 60 day of the experiment, what is consistent with the results of other researchers (*Strakova et al., 2004; Šperanda et al., 2005*). The best results with statistical significance in terms of body weight was achieved by individuals from group II, when their average body weight was higher by 2.12% compared with the body weight of pheasants from group K ($p < 0.05$), while body weight of the pheasants from group I was statistically significant ($p < 0.05$) and higher than group K for 1.71%. Accordingly, in comparison to the pheasants group K, group II had higher values of total body weight gain for 3.33%, what was statistically significant ($p < 0.05$), and individuals of group I for 2.70%, but this difference was not statistically significant. These are normal values for the investigated production parameters for pheasants growing (*Ristić, 2005; Blake and Hess, 2009*).

Based on the obtained production results (Table 2) it can be concluded that the presence of organic selenium in mixtures for pheasants feeding caused achieving of greater feed intake in groups I and II in relation to K group of pheasants, but these differences were not significant ($p > 0.05$)

Analyzing the feed conversion, evidently the best feed conversion was achieved by group II (with addition of 0.04 mg / kg of selenium in the feed) and it

was amounted 3.14, then group I (with addition of 0.03 mg / kg of selenium in the feed) with the amount of 3.17, and at last the group K with 3.21. Statistical analysis of feed conversion results showed no significant differences among examined groups of pheasant ($p > 0.05$). Gained results are consistent with a numerous researches in which it was found that selenium addition in poultry feed has a positive impact on weight gain, feed conversion ratio reducing and feeding efficiency improving (*Aravind et al., 2001; Arruda et al., 2004; Payne and Southern, 2005*).

The value of production index ranged from 54.90 (K group) to 57.24 (group II). The highest value of this indicator was in pheasants of group II, what is the result of a higher vitality percentage and better feed conversion of this group. Among examined groups of pheasant, gained differences in average weight of the pectoral muscle were not statistically significant ($p > 0.05$) and ranged from 221.50 g in group K to 229.42 g in the group II. During the experiment are not registered mortality in the tested groups

Similar results with no established statistical differences were obtained for drumsticks and thighs mass (Table 3), where the lowest mass had individuals from the group K (197.00 g), and the largest from the group II (205.16 g). Based on the data presented in Table 3, a substantial uniformity of the pheasants' chemical meat composition among groups was obviously, what showing the absence of significant differences ($p > 0.05$), what is consistent with the researches of *Cvrtila et al. (2007)*.

Analysis of the average selenium concentration in pectoral muscle (0.135 mg/g), drumsticks with thighs (0.110 mg/g) and liver (0.410 mg / g) of pheasants from group II showed that they were statistically significant ($p < 0.05$) compared with the K group (pectoral muscle 0.121 μg/g; drumstick with thigh 0.102 μg/g; liver 0.345 μg/g). The presence of organic selenium in pheasants feeding mixtures caused higher concentrations of selenium in the muscle tissue and liver of pheasants' group I, but no significant differences. Based on shown data (Table 3) it can be concluded that concentration of selenium in muscle tissue and liver of pheasant depends on the amount of selenium in feed. Similar results of increasing selenium in broilers had presented a numerous researchers (*Arruda et al., 2004; Payne and Southern, 2005; Bou et al., 2005*) who found that the concentration of selenium in muscle tissue and organs was increased by usage of organic selenium compared with the usage of inorganic forms of this mineral.

Table 2. Production indicators during the test

Groups /days	1	10	20	30	40	50	60
	BW- body weight of live pheasants (g)						
K	385.00	454.00	542.15	663.25	791.75	922.95	1056.45
I	389.00	455.00	543.80	667.25	800.40	936.30	1074.55*
Index, %	-	0.22	0.30	0.60	1.09	1.45	1.71
II	381.00	455.50	545.00	669.40	803.00	939.80	1078.80*
Index, %	-	0.33	0.53	0.93	1.42	1.83	2.12
Groups /days	The average total weight gain per period (g)						
	0-10	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**0 - 60**
K	69.00	88.15	121.10	128.50	131.20	133.50	671.45
I	70.00	88.80	123.45	133.15	135.90	138.25	689.55
Index, %	1.45	0.74	1.94	3.62	3.58	3.56	2.70
II	70.50	89.50	124.40	133.60	136.80	139.00	693.80*
Index, %	2.17	1.53	2.73	3.97	4.27	4.12	3.33
Groups /days	The average feed consumption per period (g)						
	0-10	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**0 - 60**
K	217.00	237.00	330.00	367.00	386.00	395.00	1932.00
I	218.00	240.00	337.00	375.00	391.00	403.00	1964.00
Index, %	0.46	1.27	2.12	2.18	1.30	2.03	1.66
II	219.00	239.00	336.00	372.00	390.00	402.00	1958.00
Index, %	0.92	0.84	1.82	1.36	1.04	1.77	1.35
Groups /days	The average feed conversion rate per period (g / g)						
	0-10	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**0 - 60**
K	3.14	2.69	2.73	2.86	2.94	2.96	3.21
I	3.11	2.70	2.73	2.82	2.88	2.92	3.17
Index, %	-0.97	0.52	0.18	-1.39	-2.21	-1.48	-1.15
II	3.11	2.67	2.70	2.78	2.85	2.89	3.14
Index, %	-1.23	-0.68	-0.88	-2.51	-3.10	-2.25	-2.04

Production index (60 days)		
K	**I**	**II**
54.90	56.50	57.24
Index, %	2.90	4.25

*p<0.05; ** p<0.01

Table 3. Chemical composition and selenium content in muscle and liver of pheasant

Parameters in muscle tissue	**Pectoral muscles**			**Thighs and drumsticks**		
	K	**I**	**II**	**K**	**I**	**II**
Average pH of meat	6.1	6.0	6.2	6.0	6.0	6.1
Mass, g	221.50	224.60	229.42	197.00	203.25	205.16
Moisture, %	72.63	72.38	72.49	73.04	73.00	73.12
Lard, %	1.08	1.10	1.11	4.25	4.26	4.23
Total proteins, %	25.14	25.15	25.11	21.52	21.48	21.51
Ash, %	1.18	1.20	1.20	1.12	1.11	1.09
Selenium, µg/g	0.121	0.129	0.135*	0.102	0.106	0.110*

The content of selenium in the liver			
	K	**I**	**II**
Liver weight, g	22.35	22.50	22.50
Selenium, µg/g	0.345	0.383	0.410*

*p<0.05; ** p<0.01

Previous studies indicated positive effects of selenium addition into feed overall metabolism of nutrients in the organism of animals and poultry. The effect of selenium is based on increasing the concentration of thyroid hormones T3 (triiodothyronine) and T4 (thyroxin), which serves to reduce the concentration of cholesterol in the blood and increase the absorption of glucose, while encouraging protein anabolism (*Iizuka et al., 2001; Gursu et al., 2003*). Organically bounded selenium in the form of selenium-methionine manifests a strong antioxidant effect on the poultry organism. It directly effects on the increase in enzyme glutathione peroxidase increasing in the liver and decreases lipid peroxidase concentration. The result is a high quality meat rich in selenium (*Kang et al., 2000*).

Conclusion

Based on the conducted research, it can be concluded that usage of organic selenium as pheasant mixtures' supplement had a positive effect on examined results of the productions and the selenium content in muscle and liver. The best production results were achieved in the group II which feed had the organic selenium used at a concentration of 0.4 mg/kg. Slightly lower values of production parameters were achieved by pheasants of group I, which mixture contained 0.3 mg/kg of selenium, while the lowest values of the examined parameters were established in the K group.

The results of this study show that pheasants except the status of hunting animals could have a great importance in the meat production of high quality with a significant content of antioxidant – selenium, which could contribute to the prevention of various diseases of the immune system and improve human health status. Also, from an economic point of view, by pheasants feeding mixtures containing selenium optimization, feed conversion could be rationalized and feed costs reduced.

Uticaj dodavanja organskog selena na proizvodne karakteristike fazana

S. Obradović, B. Živković, B. Petrujkić, V. Živković, M. Živkov-Baloš, M. Radulović

Rezime

Na osnovu sprovedenih istraživanja može se zaključiti da je upotreba organskog selena kao dodatka smešama za ishranu fazana imala povoljan učinak na ispitivane proizvodne rezultate i sadržaj selena u mišićnom tkivu i jetri. Najbolje

proizvodne rezultate je postigla II grupa fazana u čijoj hrani je upotrebljen organski selen u koncentraciji od 0,4 mg/kg. Nešto niže vrednosti proizvodnih pokazatelja su ostvarili fazani I grupe, čija je smeša sadržavala 0,3 mg/kg selena, dok su najniže vrednosti ispitivanih parametara ustanovljene u K grupi.

Rezultati ovog istraživanja pokazuju da fazani osim statusa lovne divljači mogu imati veliki značaj u proizvodnji kvalitetnog mesa sa značajnim sadržajem antioksidanta selena koji bi doprineo prevenciji raznih oboljenja imunog sistema i poboljšanja zdravlja ljudi. Takođe, sa ekonomske tačke gledišta optimatizacijom smeša za ishranu fazana koje sadrže selen može se racionalizovati konverzija hrane i ujedno smanjiti troškovi ishrane.

References

ADAMS C. A. (1999): Enzymes and Emulsifiers, In Nutricines, Food Components in Health and Nutrition, Nothingham University Press, Thrumpton, Notttingham, UK, 73-89.

AOAC-ASSOCIATIN OF OFFICAL ANALYTICAL CHEMISTS (1990): Official Methods of Analysis, Washington, DC: AOAC, p 246.

ARAVIND K.L., GOWDA C.V., MANJUNATH P.B., RAJENDRA Y.A., GANUPLE P.S. (2001): Indian Journal Poultry Science 36, 58-62.

ARRUDA J. S., RUTZ F., PAAN E. A. (2004): Infuence of replacing dietary inorganic with organic selenium (Sel-Plex) on performance of broilers, Nutritional Biotechnology in the Feed and Food Industry. Proceedings of the 20th Annual Symposium (Supply.1), May 22-26, 2006, Lexington, Kentucky, USA, 13.

BLAKE P. J., HESS B. J. (2009): Feeding Game Birds: Pheasant, Quail, and Partridge. 5M, New March 2009, ANR-1343. Alabama Cooperative Extension System (Alabama A&M University and Auburn University), 1-8.

BOU R., GUARDIOLA F., BARROETA A. C., CODONY R. (2005): Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat, Poltry Sience 7, 1129-1140.

CANTOR A. H. (1997): The role of selenium in poultry nutrition. Biotechnology in the Feed Industry. T. P. Lyons and K. A. Jacques, ed. Proc. Alltech 13th Annu. Symp. Nottingham University Press, UK, 155–164.

CVRTILA Ž., HADŽIOSMANOVIĆ M., KOZAČINSKI L., ZDOLEC N., FILIPOVIĆ I., SEVERIN K., MAŠEK T. (2007): Kemijski sastav mesa fazanskih kopuna, Meso, Zagreb, Vol. IX (2007) svibanj - lipanj br 3, 148-151.

EDENS F. W. (2001): Involvement of Sel-Plex in physiological stability and performance of broiler chickens, Biotechnology in the Feed Industry, Proceedings of Alltech's 17th Annual Symposium, T. P. Lyons and T. A. Jacques, ed. Nottingham University Press, Nottingham, UK, 349–376.

GURSU M.F., SAHIN N., KUCUK O. (2003): Effects of vitamin E and selenium on thyroid status, adrenocorticotropin hormone and blood serum metabolite and mineral concentrations of Japanese quails reared under heat stress (34 degrees C). J Trace Elem Exper Med, 16, 2-3, 95- 104.
IIZUKA Y., SAKURAI E., TANAKA Y. (2001): Effect of selenium on serum, hepatic and lipoprotein lipids concentration in rats fed on a high-cholesterol diet, J Pharm Soc Jap, 121, 1, 93-6.
JACQUES K. A. (2001): Selenium metabolism in animals: the relationship between dietary selenium form and physiological response, In: Science and Technology in the Feed Industry, Proceedings of Alltech's 17th Annual Symposium, (T,P, Lyons and K,A, Jacques, eds,), Nottingham University Press, Nottingham, UK, 319-348.
KANG B.P.S., MEHTA U., BANSAL M.P. (2000): Hyperlipidemia and Type-1-5'-monodeiodinase activity: Regulation by selenium supplementation, Ind. J Biochem Biophys, 37, 3, 183-187.
MIHAILOVIĆ M., MATEČIĆ D., BABIĆ M., PAVLOVIĆ O., RAKONIĆ S. (1996): Uticaj deficita selena na prirast pilića u brojlerskom tovu, Veterinarski Glasnik, 38, 1, 97-201.
NRC-NATIONAL RESEARCH COUNCIL (1994): Nutrient Requirements of Poultry Ninth Revised Edition, 1994. National Academies Press, 174.
PAYNE R. L., SOUTHERN L. L. (2005): Comparison of inorganic and organic selenium sources for broilers. Poultry Science, 84, 898-902.
RISTIĆ Z. (2005): Monografija „Fazan“, Prirodno-matematički fakultet, Novi Sad, 2005, 592.
ROTRUCK J,T., POPE A.L., GANTHER H.E., SWANSOWN A.B., HAFEMAN D.G., HOEKSTRA W.G. (1973): Selenium: Biochemical role as a component of glutathione peroxidase, Science, 179, 588-590.
STRAKOVA E., VITULA F., SUCHY P. VEČEREK V. (2004): Growth intensity and carcass characteristics of fattened pheasant poults. Krmiva 47 (2005), Zagreb, 2, 73-82.
SURAI P.F. (2000): Organic selenium: benefits to animals and humans, a biochemist's view, Biotechnology in the Feed Industry, Proceedings of Alltech's 16th Annual Symposium, (T,P, Lyons and K,A, Jacques, Eds,), Nottingham University Press, UK, 205-260.
SURAI P.F. (2002): Selenium in poultry nutrition: a new look at an old element, 1, Antioxidant properties, deficiency and toxicity, WPSA Journal, Vol 58, 333-347,
ŠPERANDA M., FLORIJANČIĆ T., BOŠKOVIĆ I., BOGUT I., BOGUT H., GUTZMIRTL H., SENČIĆ Đ., ANTUNOVIĆ Z., BODAKOŠ D. (2005): Utjecaj organskog selena na rast i biokemijske pokazatelje u serumu fazanskih pilića. Krmiva 47 (2005), Zagreb, 6, 295-301.

Received 13 April 2014; accepted for publication 5 June 2014

Biotechnology in Animal Husbandry 30 (2), p 339-347, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.146.2
DOI: 10.2298/BAH1402339L

THE EFFECT OF STARTER DOSAGE AND FERMENTATION TIME ON pH AND LACTIC ACID PRODUCTION

H. A.W. Lengkey, R. L. Balia

Universitas Padjadjaran, Bandung, Indonesia
Corresponding author: lengkeyhendronoto@gmail.com
Original scientific paper

Abstract: The aim of this research is to observed the effect of starter dosage and fermentation time on pH and lactic acid production. This study used Completely Randomized Design (CRD) factorial pattern 5 x 3. The first factor is starter dosage and the second factor is fermentation time. Kefir samples were prepared using of 5, 10, 15, 20 and 25% (v/v) kefir grains, with three fermentation time 8, 16 and 24 hours. Results indicated that kefir with 10% starter and 16 hours fermentation time give the best results in pH (3.95) and lactic acid production (1.476%).

Key words: fermentation time, pH, lactic acid production, starter dosage

Introduction

Kefir, a slightly acidic fermented milk, is produced by adding lactic acid bacteria and yeasts, in the form of grains, to milk. Kefir is an acid, viscous, slightly carbonated dairy beverage *(Garrote et al., 2001)*. Traditionally kefir grains have been used for centuries in many countries, for example, in Tibet, China, as the natural starter in the production of the unique self-carbonated dairy beverage *(Saloff-Coste, 1996)*. Kefir grains looks like a waxy cauliflower substance. It proliferates abundantly when given the right environmental conditions. Kefir is a group of organisms, mostly varieties of lactobacillus and several yeasts, that have evolved to live together in a structure of their own making. Kefir traditionally is used to culture milk which it makes more digestible by consuming the lactose and to some extent breaking down casein and other proteins in some cases making the milk easier for people to digest. The organisms in kefir stop the proliferation of unhealthy organisms in milk, preserving the milk form spoiling. The organisms that grow in kefir are friendly to the human gut ecosystem and highly competitive with organisms that proliferate in unhealthy intestines. In the production of kefir, special kefir grains which consists of yeast, lactobacilli and streptococci are used as

starter. Different types of yeast and lactic acid bacteria have been found in kefir. Kefir generally takes 12 to 48 hours to form. The exact amount of time will vary depending on environmental factors, the most important of which is temperature. Cold retards the fermentation process so kefir will form more slowly in a cold area, and can be stopped by placing the grains in milk in the refrigerator. Heat speeds the process, so kefir will form more quickly in a warm area and will be more likely to over-culture. Standard room temperature were recommended, whenever possible. Allowing the kefir grains to remain in milk, longer than 48 hours risks starving the kefir grains and potentially damaging them. Kefir is an acidic and mildly alcoholic fermented dairy product that is believed to have functional properties *(Farnworth, 1999; Farnworth and Mainville, 2003; Farnworth, 2006)*. The microorganisms contained within the kefir grains typically produce lactic acid and antibiotics, such products inhibit the proliferation of both spoilage and pathogenic microorganisms in kefir milk *(Farnworth, 2006)*. However, a stable and constant starter culture, which is necessary for manufacturing a quality kefir beverage, is difficult to sustain due to complex microbiological composition in kefir grains. Detecting and identifying the bacterial compositions of kefir grains and kefir products with rapid method is often important for quality control of this product. On the other hand, the complete description of kefir microflora gives a clue to specify the several bioactive materials produced and inparticular those involved in grain-forming mechanism. Kefir grains contains a complex microbial symbiotic mixture of lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc,* and *Streptococcus* spp.), and yeasts (*Kluyveromyces, Saccharomyces* and *Torula*) included in a polysaccharide–protein matrix *(Witthuhn et al., 2005; Farnworth, 2006)*. Yeasts and lactic acid bacteria co-exist in a symbiotic association and are responsible for lactic-alcoholic fermentation. Lactic acid bacteria (LAB) in different original kefir grains were first assessed. The bacterial genera that were identified included *Lactobacillus, Leuconostoc* and *Lactococcus* and the yeast genera included *Zygosaccharomyces, Candida* and *Saccharomyces*. The distribution frequencies of the microbes in the different grains were determined and most of the grains were dominated by two microbial species. No pediococci, acetic acid bacteria or propionibacteria were detected *(Witthuhn et al., 2004)*. Full cream milk, low fat milk, can be used for fermentation. Store the culture out of direct sunlight on a bench, for about 24 hours, giving it a gentle stir or shake up to two or three times during that period. According to *(Lengkey, et al, 2013)*, the important conditions for kefir is pH. The yeasts and fungi are able to tolerate more acidic conditions. The mild conditions used in food fermentation produce few of the deleterious changes to nutritional and sensory quality. Using 10% starter, will get the best result for kefir pH (3.95). The kefir produced from cow, goat, sheep and buffalo milk, had the following chemical characteristics such as pH about 4.0, alcohol from 0.55 to 2.0%, fat content depends on the type of milk used, and this fermented milk have an acid, prickly and slightly yeasty taste *(Irigoyen, et al, 2005)*. But *Yaman et*

al (2010), conclude that the type of milk has an influence on pH than the starter culture at 21 hours fermentation time for kefir that made from cows milk. According to *Simova et. al (2002),* the pH of kefir between 4.35 – 4.5 and the lactic acid are between 8.18 – 8.20.

Material and Methods

The objectives of this study were to determine the pH of the kefir and the lactic acid production from some dosage of kefir starter and the fermentation time. The pasteurized cow milk samples contain fat 3.43%, protein 4.72%, lactose 4.30%, titratable acidity number expressed as pH value of 6.70, and lactic acid content 0.13%. 2.50 L milk was pasteurized at 72^{o}C for 15 minutes, and then was cooled until 23^{o}C. The milk then divided into 20 beaker glass (125 ml in each beaker glass). The kefir grain as the starter, collected from homemade kefir, were evaluated in this study. In the laboratory, 10% (w/w) of each grain was propagated at 20^{0}C for 20 h with two to three weekly transfers in sterilized milk, and kept at 4 ^{0} C for short- and long-term storage. The grains were cultured in sterile 10% reconstituted skim milk at 20^{o}C for 20 h. The kefir grains were then filtered and stored at 4 ^{o}C. Kefir grains were recovered from the mother culture having reached the fermentative end-point. Ten grams of each kefir grains were suspended in 90 g of sterile saline buffer (0.85% NaCl) and homogenized with a Stomacher for 20 min. The pasteurized cow milk samples contain fat 3.43%, protein 4.72%, lactose 4.30%, titratable acidity number expressed as lactic acid content 0.13% and pH value of 6.70. The starter were used 5, 10, 15, 20 and 25% (v/v) was added into the milk. Each treatment was four times repeated. The starter was mixed carefully, so the starter was mixed into the milk; and fermented for 24 hours at 27^{o}C (room temperature). And then the kefir was washed with 1 L cooked cold water each. Acidity are denoted by the term pH. The pH may be measured electrometrically or by means of dyes that changes color at different pH values. After 24 hours fermentation, the starter was washed with cool cooked water, and then straining the liquid through a stainless-steel sieve, and then weighed. The lactic acid production, was done according to titration method Manns Acid Test: lactic acid production (%) = ml NaOH x 0.009/sample (g) x 100%.

Results and Discussions

The effect of kefir starter on Kefir pH

Table 1. Kefir pH from some dosage of kefir starter and fermentation time

Fermentation time	Treatments					Average
	D-1 (5%)	D-2(10%)	D-3(15%)	D-4(20%)	D-5(25%)	
F-1 (8 hours)	4.6	4.05	3.89	3.70	3.50	3.958
F-2 (16 hours)	4.5	3.94	3.75	3.60	3.40	3.838
F-3 (24 hours)	4.3	3.86	3.64	3.50	3.30	3.740
Total	13.4	11.85	11.28	10.80	10.30	
Average	4.46	3.95	3.76	3.60	3.43	

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

From Table 1, the pH of the kefir, will more acid when the percentage of the dosage of starter used more higher. The pH of the kefir with 5% starter is 4.46 and the kefir with 25% starter is 3.43. When the dosage of starter is higher, the acidity of the kefir will more acid. *Irigoyen et al. (2005)* in their study reported that kefir produced from cow, goat, sheep and buffalo milk had the following chemical characteristics such as pH about 4.0, and this fermented milk have an acid, prickly and slightly yeasty taste. Proper treatment of the kefir is an important step in producing cultured milk. A prolonged shelf life of cultured milk products, be achieved by subjecting the finished product to heat treatment. Due to the low pH existing in cultured milk, it will have a shelf life of several weeks at room temperature.

Table 2. Analysis of variance for Kefir pH

Treatments	Kefir pH	Significancy 0.05
Starter dosage		
D-5	3.43	a
D-4	3.60	b
D-3	3.76	c
D-2	3.95	d
D-1	4.46	e
Fermentation time		
F-1	3.958	a
F-2	3.838	b
F-3	3.740	c

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages

F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

From Table 2, the pH of kefir are between 3.43 to 4.46 for 25% starter dosage to 5% starter dosage; and for fermentation time between 8 – 24 hours, the pH are 3.958 to 3.740. The important conditions are pH, the yeasts and fungi are able to tolerate more acidic conditions. The mild conditions used in food fermentation produce few of the deleterious changes to nutritional and sensory quality *(Lengkey et al, 2013)*. The taste and texture of kefir depends on several factors including fermentation time of the kefir cultures, the room temperature and the ratio of kefir grains to milk. If the room temperature has changed, may need to adjust the fermentation time to allow the kefir to culture. If the kefir grains have multiplied, then may find the taste and texture of the kefir change. To remedy this problem, simply remove a portion of the kefir grains and either start a second batch of kefir or find them a good home. Using 10% starter, will get the best result for kefir pH. Kefir generally takes 12 to 48 hours to form. The exact amount of time will vary depending on environmental factors, the most important of which is temperature. Heat speeds the process so kefir will form more quickly in a warm area and will be more likely to over-culture. We recommend standard room temperature whenever possible. The room temperature when this experiment was done is 27^0 C and the fermentation time are between 8 – 24 hours. Allowing the kefir grains to remain in milk longer than 48 hours risks starving the kefir grains and potentially damaging them. According to *Yaman, et al (2010)*, the kefir made from cows milk with fermentation time for 21 h, has pH 4.41 and the type of milk has an influence on pH than the starter culture.

The effect on lactic acid production

From Table 3, the lactic acid production will increase as the starter dosages rise. As the starter dosages 5% (D-1), the lactic acid production 0.933 and when the starter dosages 25% (D-5), the lactic acid production is 2.00. According to the fermentation time, when the fermentation time 8 hours (L-1) the lactic acid production is 0.774 and the highest is from 24 hours fermentation time (L-3) 1.910.

Table 3. Lactic acid production from some dosage of kefir starter

Fermentation Time	Treatments					Average
	D -1 (5%)	D-2 (10%)	D-3 (15%)	D-4 (20%)	D-5 (25%)	
F-1 (8 hours)	0.37	0.52	0.82	1.00	1.26	0.774
F-2 (16 hours)	1.10	1.18	1.40	1.58	2.12	1.476
F-3 (24 hours)	1.24	1.53	1.80	2.36	2.62	1.910
Total	2.71	3.23	4.02	4.94	6.00	
Average	0.933	1.073	1.34	1.646	2.00	

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

During the fermentation time, the kefir with 25% starter dosage has the highest lactic acid level, and the lowest is 5% starter dosage and based on fermentation time, the highest lactic acid percentage are from 24 hr fermentation time and the lowest is 8 hr fermentation time. To find out the distinction of the treatment in lactic acid, we used Duncan multiple range test in Table 4.

Tabel 4. Analysis of Variance for Kefir Lactic Acid (%)

Treatments	Lactic Acid average .....%.....	Significancy 0.05
Starter dosage		
D-5	2.00	a
D-4	1.646	b
D-3	1.34	c
D-2	1.073	d
D-1	0.933	e
Fermentation time		
F-3	1.910	a
F-2	1.476	b
F-1	0.774	c

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

As the starter dosage increase, it also increased the lactic acid, because the microorganism in kefir starter will increase the lactic acid content in kefir. This result is in accordance with the opinion, that during the fermentation process, the starter dosage will influence the lactic acid production. The dosage starter indicated that the effect of bacteria on lactic acid production, the ability of the bacteria to break the lactose, that is why when the starter dosage increased, will produce lactic acid more faster and then the lactic acid in kefir more higher. Lactic acid in the kefir was expected for 4 %, and it will recommended in kefir production, with 18 hr fermentation time with 10% starter dosage.

The kefir that produced from kefir grain, will produced kefir with pH between 4.35 – 4.5 and the lactic acid were between 8.18 – 8.20 were determined by enzymatic methods as described by Boehringer Mannheim (*Simova et al, 2002*). Kefir is an acidic and mildly alcoholic fermented dairy product that is believed to have functional properties *(Farnworth, 1999; Farnworth and Mainville, 2003; Farnworth, 2006)*. The microorganisms contained within the kefir grains typically produce lactic acid and antibiotics, such products inhibit the proliferation of both spoilage and pathogenic microorganisms in kefir milk *(Farnworth, 2006)*.

Conclusion

In conclusion, this study showed that the starter dosage and fermentation time has an influence on the pH and lactic acid of the kefir. Although the longer the fermentation time and the more starter dosage will result lower pH and also higher percentage of lactic acid, and it may influence the quality of kefir, but the best results are for 10% dosage starter and 16 hours fermentation time, because of the result of kefir pH 3.95 and lactic acid 1.476%.

Uticaj doze startera i vremena fermentacije na pH i proizvodnju mlečne kiseline

H. A.W. Lengkey, R. L. Balia

Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj doze startera i vremena fermentacije na pH i produkciju mlečne kiseline. U ovom istraživanju korišćen je Completely Randomized Design (CRD) obrazac 5 x 3. Prvi faktor je doza startera i drugi faktor je vreme fermentacije. Kefir uzorci su pripremljeni korišćenjem 5, 10, 15, 20 i 25% (v/v) kefir zrna, sa tri vremena fermentacije 8, 16 i 24 časa. Rezultati

pokazuju da kefir sa 10% startera i 16 sati fermentacije daje najbolje rezultate što se tiče pH (3.95) i proizvodnje mlečne kiseline (1,476%).

References

FARNWORTH, E.R. (1999). Kefir: from folklore to regulatory approval. J. Nutra. Funct. Med. Foods 1, 57–68.

FARNWORTH, E.R. (2006). Kefir-a complex probiotic. Food Sci. Technol. Bull.: Funct. Foods 2, 1–17 /http://www.foodsciencecentral.com/fsc/bulletin-ff-freeS.

FARNWORTH, E.R., MAINVILLE, I. (2003). Kefir: a fermented milk product. In: Farnworth, E.R. (Ed.), Handbook of Fermented Functional Foods. CRC Press, Boca Raton, FL, 77–112.

GARROTE, G.L., ABRAHAM, A.G., De ANTONI, G.L. (2001). Chemical and microbiological characterisation of kefir grains. Journal of Dairy Research, 68, 639–652.

HSI-CHIA CHEN, SHENG-YAO WANG, MING-JU CHEN. (2008). Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. Food Microbiology, 25, 492–501

IRIGOYEN, A., ARANA, A., CASTEILLA, M., TORRE, P., and IBANEZ, F.C. (2005). Microbiological, physicochemical and sensory characteristics of kefir during storage. Food Chemistry, 90, 613-620.

LENGKEY, H.A.W., SIWI, J.A. and BALIA, R.L. (2013). The Effect of Various Starter Dosages on Kefir Quality. Scientific Papers Animal Sciences, 59, 18, 113-116.

McCUE, P.P., SHETTY, K. (2005). Phenolic antioxidant mobilization during yogurt production from soymilk using Kefir cultures. Process Biochemistry, 40, 1791–1797.

RODRIGUES, K.L., GAUDINO CAPUTO, L.R., TAVARES CARVALHO, J.C., EVANGELISTA, J., SCHNEEDORF, J.M. (2005). Antimicrobial and healing activity of kefir and kefiran extract. International Journal of Antimicrobial Agents, 25, 404–408.

SALOFF-COSTE, C.J. (1996). Kefir. Nutritional and health benefits of yoghurt and fermented milks. Dannone World Newsletter, 11, 1–7.

SIMOVA, E., D. BESHKOVA, A. ANGELOV, Ts HRISTOZOVA, G. FRENGOVA and Z. SPASOV. (2002). Lactic acid bacteria and yeasts in kefir grains and kefir made from them. Journal of Industrial Microbiology and Biotechnology, 28, 1–6.

WITTHUHN, R. C., SCHOEMAN, T., and BRITZ, T. J. (2004). Isolation and characterization of the microbial population of different South African kefir grains. International Journal of Dairy Technology, 57, 1, 33–37.

WITTHUHN, R. C., SCHOEMAN, T., and BRITZ, T. J. (2005). Characterisation of the microbial population at different stages of kefir production and kefir grain mass cultivation. International Dairy Journal, 15, 383–389.
YAMAN, H., M. ELMALI and KAMBER, U. (2010). Observation of Lactic Acid Bacteria and Yeast Populations During Fermentation and Cold Storage in Cow's, Ewe's and Goat's Milk Kefirs. Kafkas Üniversitesi Veteriner Fakültesi, 16, Suppl-A, 113-118.
ZHOU JIANZHONG, LIU XIAOLI, JIANG HANHU, DONG MINGSHENG. (2009). Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. Food Microbiology, 26, 770–775.

Received 20 May 2014; accepted for publication 16 June 2014

Biotechnology in Animal Husbandry 30 (2), p 349-356 , 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 639.211
DOI: 10.2298/BAH1402349K

DIFFERENCES BETWEEN WHITE AND RED MUSCLE FIBERS DIAMETER IN THREE SALMON FISH SPECIES

E. Karahmet[1], A.Vileš[1], A. Katica[2], N. Mlaćo[2], A. Toroman[1]

[1]Agricultural and Food Sciences, Faculty University of Sarajevo,Zmaja od Bosne 8, 71000 Sarajevo,BiH
[2]Veterinary Faculty, University of Sarajevo, Department for Histology and Embriology, Zmaja od Bosne 90, 71000 Sarajevo, BiH
Corresponding author: amela10katica@yahoo.com
Original scientific paper

Abstract: Because of skeletal muscle is the main contributor to body weight in most fish, it is probable that the species of the fish is limited by the growth of this tissue. Several aspects of both somatic size and skeletal muscle growth was investigated in this research work included a total of 20 brown trout (*Salmo truta m. fario Lineus*), 20 brook trout *(Salvelinus alpinus*) and 20 rainbow trout (*Oncorynchus mykkis Walbaum*), the average weight of 200 grams. Gathered data showed that rainbow trout has a faster increasing white muscles then other two fish species at same body weight. Main peak of diameter white muscles was 31-40 μm (30.55%) and 41-50 μm (22.15%) for rainbow trout. In mean time in the other two fish groups (brown trout and brook trout) was 21-30 μm (40.1% or 39.27%) and 31-40 μm (39.27% or 33.85%) of measured cross sectional areas. Distribution measured cross sectional areas of red muscles laniary goes down from the <20 μm to >71 μm.

Key words: muscle fibers, diameter, rainbow trout, brown trout and brook trout**.**

Introduction

The main edible part of the fish myotome is composed of white muscle fibers. The number of muscle fibers recruited to reach a particular girth varies between families and strains and is influenced by environmental factors including diet, exercise, light and temperature regimes *(Johnston, 2000)*. In most fish, two types of muscle fibers are found; red fibers form a thin lateral superficial sheet just under the skin, whereas white fibers make up the underlying mass of the myotome *(Rabah 2005)*.

The majority of studies on muscle growth have measured the average cross-sectional areas or diameters of fibers at various stages of the life cycle.

However, average fiber size is a relatively insensitive and unreliable indicator of hypertrophic growth because of the recruitment of new muscle fibers. For example, the average fiber diameter in the white myotomal muscle of the rainbow trout remained within the range 90–95 mm between 34 and 52 cm body length due to the addition of new fibers, but increased to 135–140 mm at 62 cm body length once recruitment had ceased *(Stickland, 1983)*.

Muscle growth can therefore be studied as the contribution of hyperplasia (increase in fiber number) and hypertrophy (increase in fiber size) to muscle growth by various forms of histological methods combined with morphometric analysis *(Rowlerson and Vegetti, 2001)*. Diameter of red and white muscles fibers have a sigmoid characters, red muscles fibers becomes constant diameter 30-40 μm at body length 25-35 cm, in same time diameter of white muscles fibers have diameter 80-120 μm for same length *(Greer 1970)*.

In rainbow trout, ratio of cross-sectional area of red and white muscle was 25:1 *(Stickland, 1983)*. White muscle fibers in the size class 10–20 μm represented 10% between 2.2 and 10 cm body length, falling to 1% at 52 cm and were absent in 63 cm fish due to the cessation of new fiber recruitment.

Several studies have demonstrated that recently recruited fibers in fish are relatively small in size and that these fibers increase in cross-sectional area through hypertrophic growth. Because hyperplasia is associated with small fibers and hypertrophy is correlated with fibers of greater dimensions, the size of individual fibers can therefore be used to assess muscle growth at different life stages.

Hyperplastic and hypertrophic growth patterns have been described for several species of fish representing different taxonomic families which display a broad range of maximum reported sizes *(Weatherley and Gill, 1980, 1981, 1988; Stickland, 1983)*.

Texture is one of the criteria of flesh quality. It is a sensory characteristic for the consumer and an important attribute for the mechanical processing of fillets. Very soft texture is frequently reported and the industry is requesting methods able to measure fish texture, and is also seeking answers to what causes fillet softness *(Kiesling et al., 2006)*.

Materials and Methods

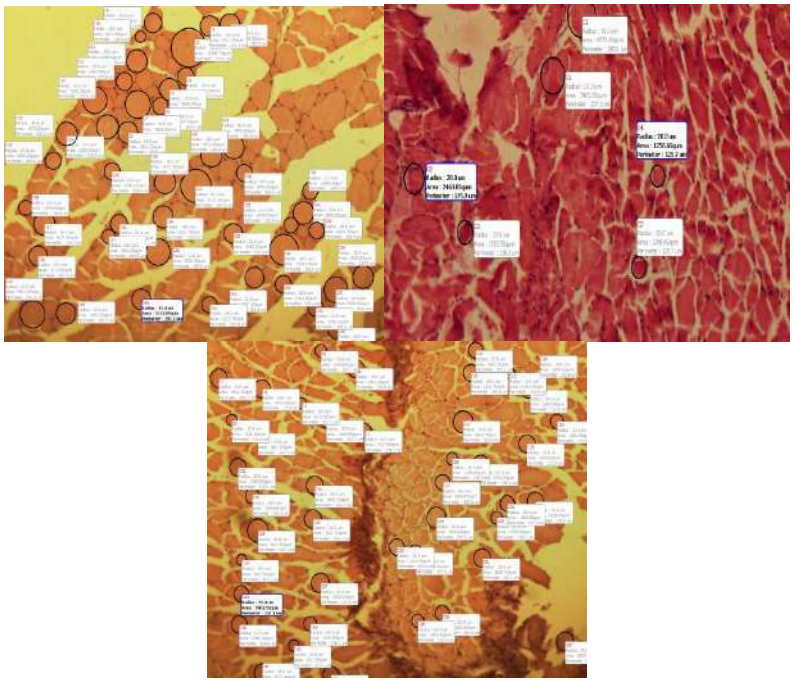
Sampling

Sixty fish in total were selected at random from a population from cages: *rainbow trout, brown trout and brook trout* ranging in length from 20-25 cm and average weight 200 g. None of the fish selected were maturing and all had been fed a commercial trout diet. They were anesthetized by solution of *p-aminobenzo etil ester, 50 mg*$^{-1}$ before slaughtering. Measured of body weight by *„Electronic Scale“BIRE K3052-P High Precision*.

Histological techniques and measurement of fiber size

Samples of white and red muscles were taken from epaxial myotome from left and right side (2x2x1 cm). Tissue blocks were orientated so fibers were cut at a right angle to their main axis during sectioning. After that the samples left in 10% formalin until histological investigation. Muscle sections were stained with haemotoxylin and eosin and mounted on microscope slides. Diameter of white and red fibers was measured by electro-microscope „MOTIC BA 200"enlarged 250x. All photos adjusted by software *„Motic images plus 2.0 ML"*.

The cross-sectional areas of individual fibers within each section were determined using an image analysis system consisting of a Leica RM 2145 computer with corresponding image analysis software and a video camera attached to a microscope. This setup enabled a field of muscle fibers viewed under the microscope to be projected onto the computer monitor screen.



Picture 1. ***White fibers Brown trout, Rainbow trout and Brook trout, Hematoxilin–eozin, 250X***

Results and Discussion

Relationships between the cross sectional areas of white muscle taken from three fish species showed significant differences between rainbow trout and the other two fish species. Main peak of diameter white muscles was 31-40 μm (30.55%) and 41-50 μm (22.15%) for rainbow trout. In mean time in the other two fish groups (brown trout and brook trout) was 21-30 μm (40.1% or 39.27%) and 31-40 μm (39.27% or 33.85%). Gathering data showed that rainbow trout has a faster increasing white muscles then other two fish species at same body weight (Table 1 and Figure 1).

Table. 1. Cross sectional area of white muscles three fish species %

Cross sectional area of white muscles three fish species %							
	< 20μm	od 21-30μm	od 31-40μm	od 41-50μm	od 51-60μm	od 61-70μm	> 70μm
Brook trout	14.97	39.27	33.85	9.04	1.80	0.51	0.51
Brown trout	17.25	40.11	26.16	11.43	3.49	0.58	0.97
Rainbow trout	11.30	19.65	30.55	22.15	6.65	7.25	2.45

As it easy to sea main peaks for brown trout and brook trout was between 21-30 μm, for Rainbow trout was between 31-40 μm and 41-50 μm. In this study, samples of rainbow trout were grown at higher rates than other two groups of fish.

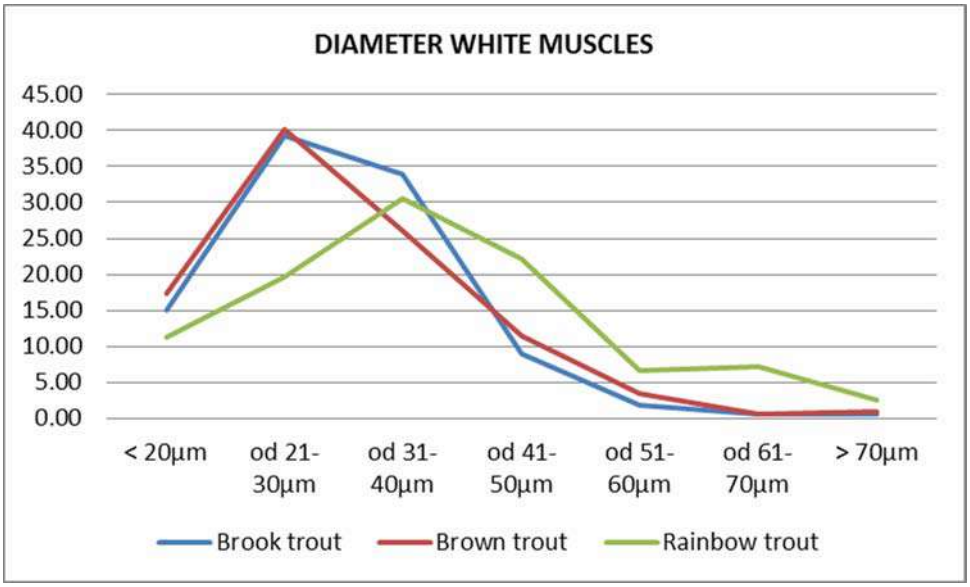


Figure 1. Cross sectional area of white muscles three fish species

Results in table 2 showed that there is a linear relationship between red muscle fiber cross sectional area and fish species in all three groups. Percentage of cross sectional areas were the highest for the lowest cross sections less than 20 µm, in all fish, between 49.05-59.40% than goes down to as diameter was bigger.

Table 2. Cross sectional area of red muscles three fish species %

Cross sectional area of red muscles three fish species %							
	< 20µm	od 21-30µm	od 31-40µm	od 41-50µm	od 51-60µm	od 61-70µm	> 70µm
Brook trout	49.05	27.83	16.03	3.77	1.88	1.41	0.00
Brown trout	59.40	33.66	5.94	0.99	0.00	0.00	0.00
Rainbow trout	51.43	29.67	14.76	2.09	1.48	0.57	0.00

Distribution measured cross sectional areas of red muscles laniary goes down from the <20 µm to >71 µm (Figure 1). Between 76.88% in brook trout to 78.10% in rainbow trout was measured cross sectional area of red muscles means that main distribution of red muscles were between these two measurements, and red muscles are actually not with bigger cross sections.

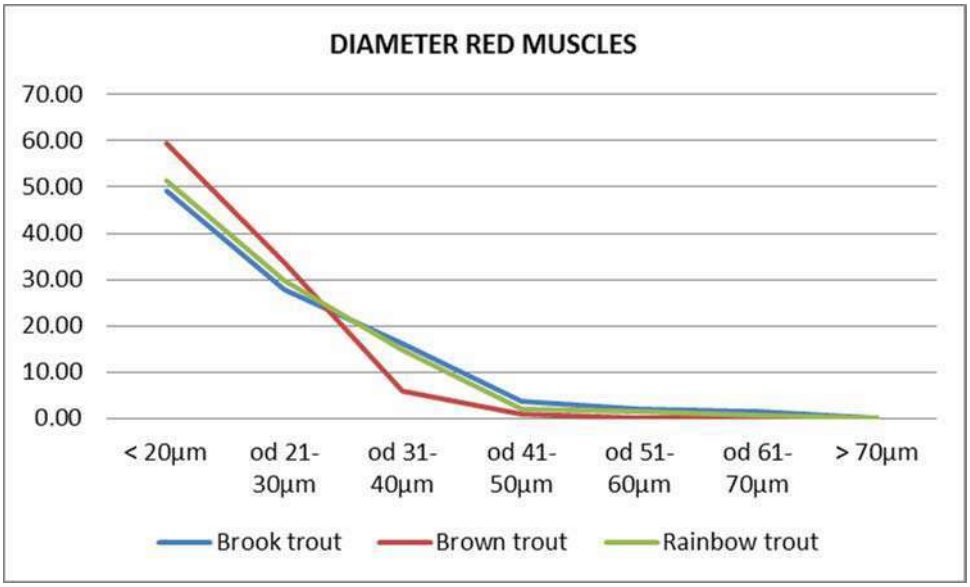


Figure 2. Cross sectional area of red muscles three fish species

The fiber cross section areas for white muscle in the present study varied deepens on fish species as it shown in analyzes from 31-40 µm (30.55%) and 41-50 µm (22.15%); this is in accordance with *Rabah (2005) and Johnston et al. (2004)*. The swimming muscle of salmon is divide into concentric layers of red muscle, they characterized by small fiber diameters, and white muscle that have larger fiber diameters (Figure 2). The red muscle fibers are relatively small and surrounded by a rich store of lipid droplets while the white fibers are relatively large and without lipid droplets.

Weatherley et al. (1988) and *Zimmerman et al. (1999)* proposed that beyond a critical fiber size the continuation of hypertrophic growth would result in impairing growth changes. According to their hypothesis the ultimate size to which a fish could grow would correlate with the attainment of this final critical size by all comprising fibers. They suggest that the critical fiber diameter at which surface area becomes limiting ranges from 120 to 270 in fish. In this study there were few diameters bigger than 60 µm it means that results show simiillar meassuring like in *Rabah (2005).*

The swimming muscle of salmon is divide into concentric layers of red muscle, they characterized by small fiber diameters, and white muscle that have larger fiber diameters. The red muscle fibers are relatively small and surrounded by a rich store of lipid droplets while the white fibers are relatively large and without lipid droplets.

The large range of fibre diameters seen in the white muscle of rainbow trout give it a characteristic mosaic appearance which was once assumed to be a mixture of small and larger fibres *Johnston et al. (2000)* and *Gjedrem (1997)*.

Conclusion

The growth of red and white muscle was investigated in three salmon fish species; brown trout, brook trout and rainbow trout, using fish from 20 to 25 cm in length. In the white muscle, fibre hyperplasia, initially, accounted for all groups of fish muscle growth to 50 μm but relative contribution was decreased in brown and brook trout. The results for the red muscle are more variable and hence more difficult to assess so it can say that cross sectional areas were less than 40 μm in all fish species.

Razlike u dijametru belih i crvenih mišićnih vlakana kod tri vrste salmonidnih riba

E. Karahmet, A. Vileš , A. Katica , N.Mlaćo, A. Toroman

Rezime

Zato što su skeletni mišići osnovni nosioci mase većine riba to je verovatno povezano sa vrstom i rastom ovog tkiva. Nekoliko aspekata veličine i mišićnog rasta je istraživano u ovom radu. Istraživanjima je ukupno obuhvaćeno 60 uzoraka ribe, po 20 uzoraka potočne pastrmke *(Salmo truta m. fario Lineus.),* potočne zlatovčice *(Salvelinus alpinus)* i kalifornijske pastrmke *(Oncorynchus mykkis Walbaum),* prosečne mase 200 grama.

Rezultati istraživanja su pokazali da kalifornijska pastrmka ima brži razvoj (hiperplaziju) belih mišićnih vlakana u odnosu na druge dvije grupe riba u odnosu na telesnu masu. Najveći broj izmerenih dijametara belih mišićnih vlakana kod kalifornijske pastrmke se nalazio od 31-40 μm (30,55%) i 41-50 μm (22,15%), dok su se u isto vrijeme izmereni dijametri belih mišićnih vlakana kod druge dve grupe nalazili u delu od 21-30 μm (40,1% or 39,27%) i 31-40 μm (39,27% ili 33,85%). Distribucija izmerenih dijametara crvenih mišićnih vlakana se linijarno kretala od <20 μm do >71 μm.

References

GJEDREM, T. (1997): Flesh quality improvement in fish through breeding. Aquac. Int. 5, 197– 206. Chapman and Hall.
GREER-WALKER M. (1970): Growth and Development of the Skeletal Muscle Fibres of the Cod (*Gadus Morhua* L.) Journal du Conseil, 33, 2, 228-244.
JOHNSTON I.A., MANTHRI S., ROBERTSON B., CAMPBELL P., MITCHELL D. And ALDERSON R. (2000): Familly and population differences in muscle fiber recruitment in farmed Atlantic salmon (*Salmo salar*) texture. Basic and Applied Myology, 10, 6, 291-296.
JOHNSTON I. A., MANTHRI S., BICKERDIKE R., DINGWALL A., LUIJKX R., CAMPBELL P., NICKELL D., ALDERSON R. (2004): Growth performance, muscle structure and flesh quality in out-of-season Atlantic salmon (*Salmo salar*) smolts reared under two different photoperiod regimes. Aquaculture, 237, 281-300.
KIESSLING A., RUOHONEN K., BJØRNEVIK M. (2006): Muscle fibre growth and quality in fish, Arch. Tierz., Dummerstorf 49, 137-146.
RABAH S. (2005): Light microscope study of *Oncorhynchus kisutch* muscle development. Egyptian Journal of Aquatic Research, 31, 1, 303-3013.
ROWLERSON A., VEGETTI A. (2001): Cellular mechanism of post-embryonic muscle growth in aquaculture species. In: Muscle development and growth. (Ed: Ian A. Johnston), Academic Press, London, 103-140.
STICKLAND N. C. (1983): Growth and development of muscle fibres in the rainbow trout (*Salmo gairdneri*). Journal of Anatomy, 137, 2, 323-333.
WEATERLAY A. H., GILL H. S., ROGERS S. C. (1980): Growth dynamics of mosaic muscle fibres in fingerling rainbow trout (*Salmo gairdneri*) in relation to somatic growth rate. Canadian Journal of Zoology, 58, 1535-1541.
WEATHERLEY A.H. and GILL H.S. (1981): Recovery growth following periods of restricted rations and starvation in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Biology, 18, 195-208.
WEATHERLEY A., GILL H., LOBO A.F. (1988): Recruitment and maximal diameter of axial muscle fibers in the teleosts and their relationship to somatic growth and ultimate size. Journal of Fish Biology, 33, 6, 851-859.
ZIMMERMAN A. M.A., LOWERY M. S. (1999): Hyperplastic development and hypertrophic growth of muscle fibers in the White Seabass (*Atractoscion nobilis*). Journal of Experimental Zoology, 284, 299-308.

Received 28 February 2014; accepted for publication 12 April 2014

Biotechnology in Animal Husbandry 30 (2), p 357-366, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.15
DOI: 10.2298/BAH1402357M

THE INFLUENCE OF GENOTYPE AND OSMOTIC STRESS ON GERMINATION AND SEEDLING OF MAIZE

V. Mandić[1], Z. Bijelić[1], V. Krnjaja[1], Z. Tomić[1], A. Simić[2], D. Ružić Muslić[1], M. Gogić[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: violeta_randjelovic@yahoo.com
Original scientific paper

Abstract: The aim of this research was to estimate the influence of different NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) on seed germination, and early seedling growth in two maize hybrid different maturity groups (ZP 560 – FAO 500 and ZP 666 – FAO 600). Germination was tested in sterile plastic vessels on filter paper moistened with different NaCl solutions, in the dark at 20 ± 1°C, in laboratory. Results of ANOVA indicated that hybrid ZP 666 had significantly higher root length (RL) (6.37 cm), shoot length (ShL) (2.06 cm), shoot fresh weight (ShFW) (43.86 mg), root dry weight (RDW) (7.56 mg), shoot dry weight (ShDW) (5.97 mg), seedling vigor index (SVI) (706.55) and dry matter stress tolerance index (DMSI) (55.85%) than hybrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg, 4.15 mg, 457.61 and 48.86%, respectively). Contrary, hybrid ZP 560 had significantly higher relative seedling water content (RSWC) (83.83%) and phytotoxicity of shoot (PhSh) (69.77%) than hybrid ZP 666 (81.16% and 62.51%, respectively). Generally, hybrid ZP 666 had better tolerance to salt stress than hybrid ZP 560. Germination energy (GE), germination (G), RL, ShL, root fresh weight (RFW), ShFW, RDW, ShDW, rate germination index (RGI), SVI, RSWC and DMSI were significantly decreased with the increase in osmotic stress induced by NaCl. Contrary, phytotoxicity of root (PhR) and PhSh significantly increased with the increase in osmotic stress.

Keywords: germination, early seedling growth, maize, osmotic stress

Introduction

In Serbia, approximately 6 million tons of maize grain is produced on 1.2 million ha. Most of the maize production (about 80%) is used to feed livestock. Also, silage maize is used to feed livestock, primarily dairy cows. However, maize production depends by many abiotic factors. Salinity is one of the abiotic factors

that limits the plant germination and early seedling growth *(Almansouri et al., 2001)* and plant growth and productivity of crops *(Flowers, 2004)*. Salinity land is soil contaminated with salts (ECe > 4 dS m^{-1} or 40 mM NaCl or osmotic potential < 0.117 MPa) *(Ashraf, 2009)*. In Serbia has 4.6% of saline and alkaline soils (solonetz and solonchak) of 5,112,000 ha agricultural land *(Ličina et al., 2011)*. High salt content in the soil reduces the ability of plants to absorb water, which results in growth crop reduction *(Munns 2002)*, causing disorders in ion absorption *(Karimi et al. 2005)*, increases absorption and accumulation of toxic ions *(Nawaz et al. 2010)*. Maize is moderately sensitive to salinity *(Maas and Hoffman, 1977; Ouda et al., 2008)*. Hovewer, maize hybrids differ in level of resistance to salt tolerance *(Radić et al., 2007; Carpici et al., 2009; Khodarahmpour, 2012)*. Many results showed that maize seed germination significantly reduced with increase in salinity levels *(Rahman et al., 2000; Blanco et al., 2007; Carpici et al., 2009; Bakht et al., 2011;* Khayatnezhad and Gholamin**,** *2011; Mirosavljević et al., 2013)*. The reasons for this were osmotic problems in the first phase of salt stress and Na^+ toxicity in the second phase *(Schubert et al., 2009)*.

The aim of this study was to estimate the effects of various NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) on germination and early seedling growth in two maize hybrids (ZP 560 and ZP 666).

Materials and Methods

The experiments were conducted under laboratory conditions in March 2014 (before sowing in the field) at the Institute for Animal Husbandry in Belgrade. Hundred seeds of each hybrids were placed in sterile plastic vessels (15 cm wide, 21 cm long and 4 cm high) on filter paper moistened with 10 ml different NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) prepared according to *Coons et al. (1990)*, and incubated at 20 ± 1°C in the dark for 14 days. The seeds were stored in paper bags in laboratory room. Seeds were sterilized in 1% NaOCl during 5 min and washed 3 times in sterile distilled water. The experimental design was arranged in a Randomized Complete Block Design (RCBD) with four replications.

According to *ISTA (2008)* germination energy (GE) and germination (G) were determined after 4 and 7 day after sowing, respectively. Root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW) were evaluated after 14 days. Root and shot dry weight were obtained after drying at 80 °C for 24 hours *(Mandić et al., 2012)*.

Also calculated rate germination index (RGI) according to *Islam et al. (2000)*, seedling vigor index (SVI) according to *ISTA (1999)*, relative seedling water content (RSWC) according to *Shalaby et al. (1993)*, phytotoxicity of shoot (PhSh) and root (PhR) according to *Chou (1976)* and dry matter stress tolerance index (DMSI) according to *Ashraf et al. (2006)*:

RGI = (No. of seeds germinated at 3 day/No of seeds germinated at 7 day) × 100

SVI = (Root length + Shoot length) x Germination percentage,

RSWC = ((Fresh weight - Dry weight)/ Fresh weight) × 100,

PhSh = ((Shoot length of control - Shoot length of treatment)/Shoot length of control) x 100

PhR = ((Root length of control - Root length of treatment)/Root length of control) × 100

DMSI = (Dry matter of stressed plant/Dry matter of control plants) x 100.

The data were statistically analyzed by ANOVA using program Statistica version 10. Duncan's Multiple Range Test was used to compare differences among treatment means at 5% level.

Results and Discussion

Results of ANOVA indicated that hybrid had highly significant effect on RL, ShL, ShFW, RDW and ShDW (Table 1). Hybrid ZP 666 had significantly higher RL (6.37 cm), ShL (2.06 cm)**,** ShFW (43.86 mg), RDW **(**7.56 mg) and ShDW (5.97 mg) than hybrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg and 4.15 mg respectively). Hybrids did not differ for GE, G and RFW although the values for these parameters were higher in ZP 666, except for GE. Hybrid ZP 666 has a longer root system, and proved that genotypes with longer root growth are resistant ability for drought *(Leishman and Westoby, 1994)*.

The osmotic stress had significant effect on germination and early seedling growth. GE, G, RL, ShL, RFW, ShFW, RDW and ShDW were significantly decreased with increasing osmotic stress. The highest values of GE (34.88%), G (97.25%), RL (12.21 cm), ShL (4.65 cm)**,** RFW (82.31 mg), ShFW (135.60 mg), RDW **(**10.66 mg) and ShDW (12.46 mg) were recorded at 0 MPa and the lowest at -1.5 MPa (0.25%, 13.00%, 1.28 cm, 0.24 cm,16.90 mg, 5.09mg, 3.21mg and1.47 mg, respectively). However for RL, ShL, RFW, ShFW parameters there was no significant difference between -1.2 and -1.5 MPa. With regard to EG and G treatment with -0.3 MPa can be the germination sensitivity threshold in the studied maize hybrids. At the higher osmotic stress levels of -0.3 MPa, EG and G were significantly decreased. Root and shoot growth were significantly reduced by increasing osmotic stress. RL, ShL, RFW, ShFW, RDW, ShDW were significantly reduced already at -0.3 MPa. Reduction of RL under osmotic stress occurs due to the growth inhibition and loss of turgidity. *Mirosavljević et al. (2013)* reported that osmotic stress had significant effects on mean germination time, time to 50%, RL, ShL, root and shoot weight, except on final germination. *Khodarahmpour (2012)* concluded that G, germination rate, RL, ShL, seedling length and SVI were decreased by increase in osmotic potential. *Demir and Arif (2003)* concluded that the root growth was more sensitive than shoot growth under salinity conditions. *Sozharajan and Natarajan (2014)* reported that salt stress decreased germination,

biomass and growth of maize seedlings due to ion toxicity, decrease osmotic potential and oxidative stress. High absorption of Na^+ and Cl^- ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination *(Taiz and Zeiger, 2002)*. *Shonjani (2002)* stated that germination rate, germination speed and RL of maize significantly decreased with increasing salt concentrations. *Cicek and Cakirlar (2002)* and *Idikut (2013)* stated that RL and ShL of maize were decreased with increasing of salt concentration level. Salt concentration decreased significantly G, germination index, RDW and ShDW of maize seedling *(Carpıcı et al., 2009)*. Many results showed that increased salt concentration reduces germination rate, germination speed, RL and ShL in several species *(Cicek and Cakirlar, 2002; Shonjani, 2002; Dağüstü, 2003; Dumlupınar et al., 2007; Taslak et al., 2007; Xiao-Xia et al., 2008; Turkyimaz et al., 2011).*

The interaction of hybrid and osmotic stress had significant effect on RL, ShL, ShFW and ShDW.

Table 1 The effects of hybrid and different osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW) of maize

Parameters	Hybrid (A)	Osmotic stress, MPa (B)						Means
		0	-0.3	-0.6	-0.9	-1.2	-1.5	
GE, %	ZP 560	32.75	28.50	32.50	5.00	2.50	0	16.88
	ZP 666	37,00	32,50	17,00	7,50	0	0.5	15.75
	Means	34.88^{a}	30.50^{ab}	24.75^{b}	6.25^{c}	1.25^{c}	0.25^{c}	16.32
G, %	ZP 560	97,00	94,00	85,50	67,50	32,50	3,00	63.25
	ZP 666	97,50	94,00	80,50	54,00	43,00	23,00	65.33
	Means	97.25^{a}	94.00^{ab}	83.00^{b}	60.75^{c}	37.75^{d}	13.00^{e}	64.29
RL, cm	ZP 560	9.36	6,34	4,32	2,62	1,51	0,93	4.18^{b}
	ZP 666	15,06	9,32	6,16	3,60	2,46	1,62	6.37^{a}
	Means	12.21^{a}	7.83^{b}	5.24^{c}	3.11^{d}	1.98^{e}	1.28^{e}	5.28
ShL, cm	ZP 560	3,80	1,62	0,65	0,37	0,25	0,14	1.14^{b}
	ZP 666	5,50	2,27	2,91	0,73	0,60	0,35	2.06^{a}
	Means	4.65^{a}	1.95^{b}	1.78^{b}	0.55^{c}	0.43^{c}	0.24^{c}	1.60
RFW, mg	ZP 560	76,35	67,80	49,45	41,12	25,42	14,58	45.79
	ZP 666	88,28	55.85	47.15	37.62	27.30	19.22	45.90
	Means	82.31^{a}	61.82^{b}	48.30^{c}	39.38^{c}	26.36^{d}	16.90^{d}	45.84
ShFW, mg	ZP 560	11327	48.02	5.40	16.25	7.90	4.12	32.50^{b}
	ZP 666	157.92	49.82	25.20	10.30	13.88	6.05	43.86^{a}
	Means	135.60^{a}	48.92^{b}	15.30^{c}	13.28^{c}	10.89^{c}	5.09^{c}	38.18
RDW, mg	ZP 560	10.58	8.58	6.45	6.68	4.38	2.60	6.54^{b}
	ZP 666	10.74	9.15	9.39	6.83	5.46	3.82	7.56^{a}
	Means	10.66^{a}	8.86^{b}	7.92^{bc}	6.75^{c}	4.92^{d}	3.21^{e}	7.05
ShDW, mg	ZP 560	11.36	6.52	1.40	1.70	2.88	1.16	4.15^{b}
	ZP 666	13.55	7.43	6.37	4.35	2.36	1.78	5.97^{a}
	Means	12.46^{a}	6.93^{b}	3.89^{c}	3.02^{cd}	2.62^{d}	1.47^{e}	5.06
Factor	GE	G	RL	ShL	RFW	***ShFW***	***RDW***	***ShDW***
A	ns	ns	**	**	ns	**	*	**
B	**	**	**	**	**	**	**	**
AB	ns	ns	**	**	ns	**	ns	**

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

Results of ANOVA indicated that hybrid had highly significant effect on SVI, RSWC, PhSh and DMSI (Table 2). Hybrid ZP 666 had significantly higher SVI (706.55) and DMSI (55.85%) than hybrid ZP 560 (457.61 and 48.86%, respectively). Contrary, hybrid ZP 560 had significantly higher RSWC (83.83%) and PhSh (69.77%) than hybrid ZP 666 (81.16% and 62.51%, respectively). Hybrids did not differ for RGI and PhR. Information on phytotoxicity is important parameters that are used to identify the phytotoxicity tolerance of the genotypes. Hybrid ZP 666 had relatively low phytotoxicity of shot that indicates, it was better in tolerating higher NaCl concentration. The result of this study is in agreement with researches *Kinfemichael (2011), Asmare (2013)* and *Mordi (2013)* who reported the presence of genetic variability between cultivars for tolerance and phytotoxicity effect of NaCl.

Table 2. The effects of hybrid and different osmotic stress on rate germination index (RGI), seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh), dry matter stress tolerance index (DMSI)

Parameters	Hybrid (A)	Osmotic stress, MPa (B)						Means
		0	-0.3	-0.6	-0.9	-1.2	-1.5	
RGI, %	ZP 560	10,77	2,37	1.63	0	1.63	0	2.73
	ZP 666	9.75	5.56	2.76	1.58	0	0	3.28
	Means	10.26^{a}	3.97^{b}	2.20^{bc}	0.79^{c}	0.81^{c}	0^{c}	3.00
SVI	ZP 560	1279,30	753,11	438,20	213,82	57,95	3,30	457.61^{b}
	ZP 666	2005,17	1087,79	729,69	233,20	137,83	45,60	706.55^{a}
	Means	1642.24^{a}	920.45^{b}	583.94^{c}	223.50^{d}	97.89^{de}	24.45^{e}	582.08
RSWC, %	ZP 560	88.37	86.91	84.55	85.17	78.08	79.90	83.83^{a}
	ZP 666	90.07	84.06	78.03	76.58	80.39	77.85	81.16^{b}
	Means	89.22^{a}	85.48^{a}	81.29^{b}	80.88^{b}	79.24^{b}	78.87^{b}	82.38
PhR, %	ZP 560	0	33.00	54.65	74.92	83.57	89.59	55.45
	ZP 666	0	37.77	58.99	75.99	83.72	89.22	57.61
	Means	0^{a}	35.38^{b}	56.82^{c}	73.96^{d}	83.64^{e}	89.40^{f}	56.53
PhSh, %	ZP 560	0	56.48	83.26	89.84	92.98	96.07	69.77^{a}
	ZP 666	0	58.75	46.75	86.76	89.20	93.58	62.51^{b}
	Means	0^{a}	57.61^{b}	65.00^{b}	88.30^{c}	91.09^{c}	94.82^{c}	66.14
DMSI, %	ZP 560	100.00	67.94	36.89	38.06	32.83	17.47	48.86^{b}
	ZP 666	100.00	68.64	65.15	46.13	32.06	23.17	55.85^{a}
	Means	100.00^{a}	68.29^{b}	51.02^{c}	42.10^{d}	32.44^{e}	20.32^{f}	52.4
Factor	RGI	SVI	RSWC	PhR	PhSh	DMSI		
A	ns	**	*	ns	**	**		
B	**	**	**	**	**	**		
AB	ns	**	*	ns	**	**		

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

The osmotic stress had significant effect on RGI, SVI, RSWC, PhR, PhSh and DMSI. RGI, SVI, RSWC and DMSI were significantly decreased with increasing osmotic stress. The highest value of RGI (10.26%), SVI (1642.24), RSWC (89.22%) and DMSI (100%) observed at 0 MPa and the lowest on -1.5 MPa (0%, 24.45, 78.87% and 20.32%, respectively). Contrary, PhR and PhSh were significantly increased with increasing osmotic stress and the lowest values recorded at 0 MPa (0% and 0%, respectively) and the highest at -1.5 MPa (89.40% and 94.82%, respectively). However for all studied parameters, except PhR and DMSI, between salinity levels -1.2 MPa and -1.5 MPa significant differences was not found. Similar, *Asmare (2013)* reported that the increase in NaCl concentrations significantly decreased seedling, shoot, and root vigor indices, and significantly increased PhR and PhSh of haricot bean. *Idikut (2013)* stated that SVI of maize were decreased with increasing of salt concentration level.

The interaction of salinity and genotypes had significant effect on SVI, RSWC, PhSh and DMSI.

Conclusion

The results showed that hybrids significantly differ for RL, ShL, ShFW, RDW, ShDW, SVI, RSWC, PhSh and DMSI. Hybrids did not differ for GE, G, RGI and RFW. Hybrid ZP 666 had better tolerance to salt stress than hybrid ZP 560. Hybrid ZP 666 had higher values for root and shoot parameters, SVI and DMSI and lower for PhSh than hybrid ZP 560. These genetic differences are important for selecting hybrids for cultivation in salt-affected areas. The osmotic stress induced by NaCl had a negative effect on germination and early seedling growth of maize seeds. Root and shoot growth were significantly reduced already at -0.3 MPa. Other examined parameters, except PhSh and PhR, significantly decreased with increasing osmotic stress. PhSh and PhR were significantly increased with increasing osmotic stress. Treatment with -0.3 MPa can be the germination sensitivity threshold in the studied maize hybrids.

Uticaj genotipa i osmotskog stresa na klijavost i klijance kukuruza

V. Mandić, Z. Bijelić, V. Krnjaja, Z. Tomić, A. Simić, Dragana Ružić Muslić, M. Gogić

Rezime

Cilj istraživanja bio je da se odredi uticaj različitog NaCl osmotskog rastvora (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) na klijavost semena i rani porast klijanaca dva hibrida kukuruza različite grupe zrenja (ZP 560 – FAO 500 and ZP 666 – FAO 600). Klijavost je testirana u laboratorijskim uslovima u sterilnim plastičnim kutijama na filter papiru natopljenom sa različitim NaCl osmotskim rastvorom, u mraku na 20 ± 1°C. Rezultati ANOVA pokazali su da je hibrid ZP 666 imao značajno veću dužinu korena (DK) (6.37 cm), dužinu stabla (DS) (2.06 cm)**,** masu svežeg stabla (MSvS) (43.86 mg)**,** masu suvog korena (MSuK) (7.56 mg), masu suvog stabla (MSuS) (5.97 mg), vigor indeks klijanca (VI) (706.55) i indeks tolerancije suve materije na stres (ITSM) (55.85%) nego hibrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg, 4.15 mg, 457.61 i 48.86%). Suprotno, hibrid ZP 560 imao je značajno veći relativni sadržaj vode u klijancima (RSVK) (83.83%) i fitotoksičnost stabla (FS) (69.77%) nego hibrid ZP 666 (81.16% and 62.51%, respectively). Generalno hibrid hibrid ZP 666 imao je bolju toleranciju na stres soli nego hibrid ZP 560. Energija klijanja (EK), klijavost (K), DK, DS, masa svežeg korena (MSvK), MSvS, MSuK, MSuS**,** indeks klijanja (IK), VI, RSVK i ITSM su signifikantno smanjeni sa povećanjem osmotskog stresa indukovanog sa NaCl. Suprotno, fitotoksičnost stabla i fitotoksičnost korena su se značajno povećali sa povećanjem osmotskog stresa.

References

ALMANSOURI M., KINET J.M., LUTTS S. (2001): Effect of salt and osmotic stresses on germination in durum wheat (*Triticum aestivum*). Plant Soil, 231, 243-254.

ASHRAF M. (2009): Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechology Advances, 27, 84-93.

ASHRAF M.Y., AKHTAR K., HUSSAIN F., IQBAL J. (2006): Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance. Pakistan Journal of Botany, 38, 1589-1597.

ASMARE H.A. (2013): Impact of salinity on tolerance, vigor, and seedling relative water content of haricot bean (*Phaseolus vulgaris* L.) cultivars. Journal of Plant Sciences, 1, 3, 22-27.
BAKHT J., SHAFI M., JAMAL Y., SHER H. (2011): Response of maize (*Zea mays* L.) to seed priming with NaCl and salinity stress. Spanish Journal of Agricultural Research, 9, 1, 252-261.
BLANCO F.F., FOLEGATTI M.V., GHEYI H.R., FERNANDES P.D. (2007). Emergence and growth of corn and soybean under saline stress. Scientia Agricola (Piracicaba, Braz.), 64, 5, 451-459.
CARPICI E.B., CELIK N., BAYRAM G. (2009): Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. African Journal of Biotechnology, 8, 4918-4922.
CHOU C.H., LIN H.J. (1976): Autointoxication mechanism of *Oriza sativa* L. Phytotoxic effects of decomposing rice residues in soil. Journal of Chemical Ecology, 2, 353-367.
CICEK N., CAKIRLAR H. (2002): The effect of salinity on some physiological parameters in two maize cultivars. Bulgarian Journal of Plant Physiology, 28, 66-74.
COONS M.J., KUEHL R.O., SIMONS N.R. (1990): Tolerance of ten lettuce cultivars to high temperature combined with NaCI during germination. Journal of the American Society for Horticultural Science, 115, 6, 1004-1007.
DAĞÜSTÜ N. (2003): In vivo evolution of winter wheat (Triticum aestivum L.) cultivars for resistance to NaCl stress at seedling stage. V National Field Crops Congress, 13-17 October, Diyarbakır, Turkey, Plant Growth Techniques, 2, 327-331.
DEMIR M., ARIF I. (2003): Effects of different soil salinity levels on germination and seedling growth of safflower (*Carthamus Tinctoriusl*). Turkish Journal of Agriculture and Forestry, 27, 221-227.
DUMLUPINAR Z., KARA R., DOKUYUCU T., AKKAYA A. (2007): The effects of electrical currents and salt concentration on germination and seedling characters of some durum wheat genotypes growing South Anatolian region. KSU Journal of Science and Engineering, 10, 2, 100-110.
FLOWERS T.J. (2004): Improving crop salt tolerance. Journal of Experimental Botany, 396, 55, 307-319.
IDIKUT L. (2013): The effects of light, temperature and salinity on seed germination of three maize forms greener. Journal of Agricultural Sciences, 3, 4, 246-253.
INTERNATIONAL SEED TESTING ASSOCIATION (ISTA) (2008): International rules for seed testing. Zurich: ISTA.
INTERNATIONAL SEED TESTING ASSOCIATION (ISTA) (1999): International Rules for Seed Testing. Seed Science and Technology, 24.

ISLAM M.SH., JAHAN Q.S.A, BUNNARITH K., VIANGKUM S., MERCA S.D. (2000): Evaluation of seed health of some rice varieties under different conditions. Botanical Bulletin- Academia Sinica 41, 293-297.
KARIMI G., GHORBANLI M., HEIDARI H., NEJAD K.R.A., ASSAREH M.H. (2005): The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrate*. Biology Plant, 4, 301-304.
KHAYATNEZHAD M., GHOLAMIN R. (2011): Effects of salt stress levels on five maize (*Zea mays* L.) cultivars at germination stage. African Journal of Biotechnology, 10, 60, 12909-12915.
KHODARAHMPOUR Z. (2012): Evaluation of salinity effects on germination and early growth of maize (*Zea mays* L.) hybrids. African Journal of Agricultural Research, 7, 12, 1926-1930.
KINFEMICHAEL G.A. (2011): The response of some haricot bean (*Phaseolus vulgaris*) varieties for salt stress during germination and seedling stage. Current Research Journal of Biological Sciences, 3, 4, 282-288.
LEISHMAN M.R., WESTOBY M. (1994): The role of seed size in seedling establishment in dry soil conditions -experimental evidence from semi-arid species. Journal of Ecology, 82, 2, 249-258.
LIČINA V., NEŠIĆ LJ., BELIĆ M., HADŽIĆ V., SEKULIĆ P., VASIN J., NINKOV J. (2011): The soils of Serbia and their degradation. Ratarstvo i povrtarstvo/Field and Vegetable Crops Research, 48, 285-290.
MAAS E.V., HOFFMAN G.J. (1977): Crop salt resistance - current assessment. Journal of the Irrigation and Drainage Division, ASCE, 103, IR2, 115-134.
MANDIĆ V., KRNJAJA V., TOMIĆ Z., BIJELIĆ Z., ŽUJOVIĆ M., SIMIĆ A., PRODANOVIĆ S. (2012): Genotype, seed age and pH impacts on germination of alfalfa. Romanian Biotechnological Letters, 17, 2, 7205-7211.
MIROSAVLJEVIĆ M., ČANAK P., ĆIRIĆ M., NASTASIĆ A., ĐUKIĆ D., RAJKOVIĆ M. (2013): Maize germination parameters and early seedlings growth under different levels of salt stress. Ratarstvo i povrtarstvo/Field and Vegetable Crops Research, 50, 1, 49-53.
MORDI P., ZAVAREH M. (2013): Effects of salinity on germination and early seedling growth of chickpea (*Cicer arietinum* L.) cultivars. International Journal of Farming and Allied Sciences, 2, 3, 70-74.
MUNNS R. (2002): Comparative physiology of salt and water stress. Plant Cell and Environment, *25,* 2, 239-250.
NAWAZ K., HUSSAIN K., MAJEED A., KHAN F., AFGHAN S., ALI, K. (2010): Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. African Journal of Biotechnology, 9, 5475-5480.
OUDA S.A.E., MOHAMED S.G., KHALIL F.A. (2008): Modeling the Effect of Different Stress Conditions on Maize Productivity Using Yield-Stress Model. International Journal of Natural and Engineering Sciences, 2, 1, 57-62.

RADIĆ V., BEATOVIĆ D., MRĐA J. (2007): Salt tolerance of corn genotypes (*Zea mays L.*). Journal of Agricultural Sciences, 52, 2, 115-120.
RAHMAN M., KAYANI S.A., GUL S. (2000): Combined Effects of Temperature and Salinity Stress on Corn Sunahry Cv. Pakistan Journal of Biological Science, 3, 9, 1459-1463.
SCHUBERT S., NEUBERT A., SCHIERHOLT A., SÜMER A., ZÖRB C. (2009): Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods. Plant Science 177, 196-202.
SHALABY E.E., EPSTEIN E., QUALSET O.C. (1993): Variation in salt tolerance among some wheat and triticale genotypes. Journal of Agronomy and Crop Science, 171, 298-304.
SHONJANI S. (2002): Salt sensitivity of rice, maize, sugar beet, and cotton during germination and early vegetative growth. Faculty of Agricultural and Nutritional Sciences, Home Economics and Environmental Management. Institute of Plant Nutrition Justus Liebig University Giessen, Inaugural Dissertation, 1-139.
SOZHARAJAN R., NATARAJAN S. (2014): Germination and seedling growth of *Zea mays* L. under different levels of sodium chloride stress. International Letters of Natural Sciences, 7, 5-15.
TAIZ L., ZEIGER E. (2002): Plant Physiology, Third Edition. Sinauer Associates, Sunderland, MA,, 690.
TASLAK İ., ÇAĞLAR Ö., BULUT S., ATKEN S. (2007): Determination of salt tolerance of same barley genotypes. I. Germination experiment. VII Field Crops Congress, 25-27 July, Erzurum, Turkey, 1, 188-192.
TURKYIMAZ B., YILDIZ AKTAS L., GUVEN A. (2011): Salinity induced differences in growth and nutrient accumulation in five barley cultivars. Turkish Journal of Field Crop, 16, 1, 84-92.
XIAO-XIA Q., ZHEN-YING H., BASKIN J.M., BASKIN C.C. (2008): Effect of temperature, light and salinity of seed germination and radicle growth of the geographically widespread halophyte shrub *Halocnemum strobilaceum*. Annals of Botany, 101, 2, 293-299.

Received 16 April 2014; accepted for publication 20 June 2014

Biotechnology in Animal Husbandry 30 (2), p 367-376, 2014
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.09
DOI: 10.2298/BAH1402367D

THE MAIN INDICATORS OF BIOSECURITY AND PRESENCE OF HOUSE MOUSE (*Mus musculus* L.) IN ANIMAL HUSBANDRY FACILITIES

S. Đedović[1], J. Bojkovski[2], G. Jokić[1], T. Šćepović[3], M. Vukša[1]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Zemun, Serbia
[2]University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia
[3]Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia
Corresponding autor: Suzana.Djedovic@pesting.org.rs
Original scientific paper

Abstract: Analysis of biosecurity indicators at critical control points intend to prevent undesirable infections in technological chains of production. Product quality is the basis for defining a biosecurity plan under the HACCP concept. General and specific biosecurity measures developed to prevent introductions of infective materials have been at the focus of attention in Serbia in recent years. The house mouse (*Mus musculus* L.) is usually accused for transferring pathogens into objects. The possibility of internal infections can be reduced by removing food sources and discovering their hiding places. The adaptability of *Mus musculus* to various conditions has affected the search for alternatives of their control. The objective of our research was to analyse the most important indicators of biosecurity and presence of *Mus musculus,* the 'cause-and-consequence' characteristics and mice control by environmentally safe substances in facilities with different technological processes. Method of questionnaire was used to define written biosecurity plan, isolation of objects, control of movement and for traffic visitors. Hygiene evaluation, i.e. mechanized cleaning, sanitary washing, facility disinfection, ventilation and facility sanitation, was performed visually. The biosecurity and wellbeing of animals were evaluated by the parameters: animal hygienic conditions of rearing, forage stocks, animal biosecurity and removal of animal carcasses. Longworth traps were used for mice trapping and determination of critical control points. The efficacy of sodium selenite was found in our study to range from 71.4% to 88.8% and it provided a good alternative for *Mus musculus* control in different production units because it does not interfere with technological production processes within facilities or cause animal resistance. Biosecurity measures need to be implemented using clear instructions in order to reduce biorisks and increase product safety.

Key words: HACCP**,** biosecurity indicators, efficacy, environmentally safe substances, *Mus musculus*

Introduction

The use of biosecurity plans *(Pritchard et al., 2005)* raises the level of biological safety of food, quality and volume of production and prevents unwanted situations *(Uhlenhoop, 2007; Bojkovski et al., 2010)*. It is not possible to protect all production elements (*Pinto and Urcelay, 2003*), those exposed to the highest risk should be considered first at critical points (*Anon. 2006*). Biosecurity and rodent control basically mean the prevention of pathogens from penetrating facilities from their external sources *(Klimpel et al., 2007; Fuehrer et al., 2012)*. The use of protective chemicals requires additional control and quality attestation to confirm that food had been produced under HACCP principles *(Pešić-Mikulec and Jovanović, 2003)*. The control of *Mus musculus* using environmentally safe ingredients increases the safety and quality of products when four biorisk stages have been covered (i.e. identification, characterization, exposure, monitoring and database).

There is an increasing need to evaluate the indicators of hygienic conditions *(Hristov et al., 2009)* and presence of *Mus musculus* in various facilities. Due to rodents' fast adaptation to various habitats and grave consequence that they may bring, indicators of their presence (active holes, feces and odour) should be closely monitored throughout the year *(Čamprag, 1983; Ružić, 1983)*. Favourable conditions in production facilities may rapidly increase their populations.

Control measures are normally used when rodents become abundant and damage considerable *(Đedović et al., 2012; Vukša et al., 2012)*. Frequent application of anticoagulants causes detrimental effects and triggers resistance to them *(Jokić et al., 2013)*. Environmentally safe products make a good alternative and rodent mortality can be achieved with one-off treatment.

Our research indicated a considerable significance of biosecurity plans, based on clearly defined indicators of hygienic conditions and presence of *Mus musculus,* and alternative environmentally safe substances for preserving product safety and quality.

Materials and Methods

Localities

Experiments were conducted in 6 agricultural facilities that use different production technologies (marked A, B, C, D, E, F). The facility A and B are storehouses for agricultural products, C and E include two production units (pig farms and storehouses), while D and F incorporate cattle farms and storehouses.

Manufacturers

The active ingredient sodium selenite was manufactured by Alfa Aesar A Jonson Matthey, Paris, France. The product Ekocel C was manufactured by Ciklonizacija, Novi Sad, Serbia. The active ingredient cellulose was manufactured by Natrocell Tecnologies Ltd., Great Britain. The product Natromouse was made by Pinus Plus d.o.o., Slovenia.

Methods

Several methods *(Sundrum et al.,1994; Bartussek et al., 2000; Bracke et al., 2001; Blockuis, 2008)* adapted to domestic conditions, were used for defining and evaluating the parameters and indicators of animal wellbeing and biosecurity. The evaluation scale used in this study *(Hristov and Reljić 2009, Hristov et al, 2009, Hristov and Stanković 2009)* included the following ratings: 5 - excellent (4.50-5.00), 4 - very good (3.50-4.49), 3 - good (2.50-3.49), 2 - satisfactory (2.00-2.49) and 1 - insufficient (0-1.99). *SWOT* analysis was applied to derive complete data for isolation from the biosecurity aspect: S-strength, W-weakness, O-opportunity, T-treatment.

The number of active holes, feces and special odour were indicators of the presence of *Mus musculus* in the facilities and these indicators were evaluated on a scale: weak, medium, strong *(Čamprag, 1983; Ružić, 1983)*.

The mice were trapped using Longworth traps during 400 nights per locality in order to locate critical points.

Experimental methods. Trials were set up according to the method PP 1/114(2) *(EPPO, 2004)*. Baits were laid in boxes for mice in portions of 10 g at 1-2 m distance. Each box was labelled with an ordinal number and product name. According to HACCP standard, a duplicate label was put up also on the wall above each box to be clearly visible and carrying a warning sign *(Bokelman, 1996)*.

The amount of bait eaten was measured daily for the duration of 15 days and fresh baits were laid daily. The abundance of rodents was determined based on the total amount of bait eaten and the ratio of the lowest and highest amounts of eaten bait per day, devided by the daily requirement of mice. Product efficacy was calculated according to Abbott's formula *(Abbott, 1925)*.

Results and Discussion

Table 1 shows the ratings for evaluation biosecurity indicators in 6 facilities considering the existance of formulated plans; the ratings were insufficient in 3 facilities, i.e. C, E and F (1.00) and good in the objects A, B and D (3.00, 3.30 and 2.75, respectively).

Table 1. Evaluation of biosecurity indicators in facilities of different types and capacities

	Evaluation of biosecurity plans*					
Indicators	**A**	**B**	**C**	**D**	**E**	**F**
Facility size (m^2)	800	1000	1200	1700	800	1200
Written biosecurity plan	3.00	3.30	1.00	2.75	1.00	1.00
Isolation of entire facility and its individual production units	3.50	3.80	1.15	2.30	1.10	1.75
Traffic control	3.20	4.00	1.80	3.00	1.20	1.50
Spatial conditions of rearing	-	-	1.00	2.90	1.00	1.75
Animal hygienic conditions of rearing	-	-	1.00	2.60	0.50	1.25
Forage stocks	-	-	0.90	2.60	0.45	0.50
Animal biosecurity	-	-	0.70	2.10	0.40	1.20
Removal of animal carcasses	-	-	1.00	2.50	1.50	2.00
Mechanized cleaning	3.20	3.80	1.20	2.80	1.30	2.50
Sanitary washing	3.40	3.60	1.30	2.30	1.45	2.50
Facility disinfection	3.00	3.60	1.00	1.30	1.00	2.00
Ventilation	3.00	3.00	1.00	2.00	1.20	2.30
Control of rodent populations	2.80	3.20	0.50	1.50	0.70	1.30
Facility sanitation	1.99	4.00	1.50	2.10	1.20	2.30
Average rating per facility	3.01	3.58	1.07	2.41	1.00	1.70
Total rating			2.13			

* Facility interior parameters evaluated

The average indicator ratings were negative in the facilities C and E (1.07 and 1.00, respectively). The surrounding topography on their localities supported rodent intrusion and the number of trapped animals was 95 and 28 (Table 3). Rodent population control also received negative ratings (0.50 and 0.70), which affects various technological processes and the quality of products.

The facility B was the only one given the rating 'very good' for traffic control (4.00) and for hygienic procedures in object (3.80, 3.60, 3.60). Traffic control received better ratings in the facilities A and D (3.20 and 3.00) than C, E and F (1.80, 1.20 and 1.50). Even though visits were limited and entry was not allowed into some critical areas (A and D), a lack of strict and consistent regime was a serious problem. An even greater problem, however, was the dysfunctioning state of disinfection barriers and points, and dress change upon entry.

Animal hygienic conditions of rearing were given the rating 'insufficient' in the facilities C, E and F (1.00, 0.50 and 1.25, respectively), and 'good' in the facility D (2.60). Forage stocks and animal biosecurity received negative ratings on the farms C, E and F (0.90 and 0.70, 0.45 and 0.40, and 0.50 and 1.20,

respectively), while forage stocks were 'good' on the farm D (2.60) and its animal biosecurity 'satisfactory' (2.10).

Hygiene within facility – mechanized cleaning, sanitary washing and disinfection - were very good in the facility B (3.66), good in A (3.20), satisfactory in D and F (2.13 and 2.33, respectively) and insufficient in C and E (1.16 and 1.15, respectively). Cleaning and washing are not necessarily thorough, and a good waste management is central *(Gibbens et al., 2005)* for providing good hygiene, which makes disinfectants more effective in reducing rodent populations (Table 3, facilities A and B, 2 and 5 animals, respectively). Sanitary procedures have been frequently disregarded, especially on the pig farms C and E.

Regular sanitation leads to success, as in the facility B (4.00). In all other facilities, the ratings were insufficient or satisfactory, which increase the possibility of introducing infective materials *(Stanković et al., 2011)*. Sanitation was the most important biosecurity measure for Spanish farmers in a similar study *(Casal et al., 2007)*, while all other indicators received mostly moderate ratings.

The total average rating of indicators was satisfactory (2.13), while facility isolation from potential sources of infection was an important protective measure *(Stanković and Hristov, 2009)*.

Table 2. **Active ingredients and their properties in control of *Mus musculus***

	Active ingredient	
	Sodium selenite	Cellulose
Molecular formula a.i.	$(C_6H_{10}O_5)n$	Na_2O_3Se
(%) a. i. in bait	0.1	45
Mode of activity	exchange of S-H group of functional cells with S-S bonds	metabolic disorder (water retention)
Mortality time	4-8 days	8-10 days
Bait acceptibility	good	weak
Bait alatability	weak	weak
Human hazard	weak	weak
Animal hazard	weak	weak
Control of *Mus musculus*	good	weak

The most important characteristics of these environmentally safe products and their active ingredients are given in Table 2, and it shows their different modes of activity and mortality times. Neither product poses a risk to humans or animals and they can be applied repeatedly over the year. Differences were evidenced in bait acceptibility and sodium selenite was better in that respect in all examined facilities. Palatability should be improved, particularly of the cellulose product, in order to significantly enhance bait uptake.

Table 3. The number of *Mus musculus* animals trapped, indicators of their presence and efficacy of active ingredient in six facilities

Facility	*Mus musculus*		Efikasnost (%)	
	No. of trapped animals	Indicator of presence*	Sodium selenite	Cellulose
A	2	*	83.3	•
B	5	*	81.8	•
C	95	***	88.8	70.6
D	11	**	81.1	66.7
E	28	***	71.4	60.6
F	30	***	75.0	•

* Classification of parameters (*Čamprag, 1983; Ružić, 1983*): weak*, medium** high***
• Active ingredient efficacy below 20 %.

Consistent implementation of the HACCP standard and formulated biosecurity plans were preconditions for having a low abundance of rodents (2 and 5 animals) and product efficacy of 83.3% and 81.8% in the facilities A and B. The facility B required repairs of its loft and closure of holes in walls, which should reduce mice numbers. The cellulose product had an efficacy below 20% in the facilities A, B and F.

Both products showed the highest effectiveness in the facility C (88.8% and 70.6%), and the high numbers of trapped animals and parameters of their presence indicated that control of *Mus musculus* had not been practiced for a long time. Active holes in walls inside and outside facility revealed the IV and V categories of presence (*Ružić, 1983*).

The facility D had good physical barriers. The trapped rodents (11) were caught at critical points near entrances. The indicators of rodent presence were estimated as medium. The efficacy of sodium selenite was 81.1%, while cellulose was weaker 66.7%.

In the facilities E and F, the indicators of presence of *Mus musculus* were high, the number of animals trapped was 28 and 30, respectively, with a growing tendency due to favourable conditions for their hiding and reproduction. Alternative food sources were available. The efficacy of sodium selenite in those facilities was 71.4% and 75.0%, respectively, while cellulose had 60.6% efficacy in the facility E, which requires a general repairs and regular preventive measures. The object F, farm of Holstein Friesian cattle breed with tether housing system required thorough cleaning of pads from mud deposits. Places for silage were not properly isolated and interior temperature was appropriate for reproduction of *Mus musculus*.

Conclusion

The present data demonstrate the biosecurity status of facilities.

The following conclusions may be inferred from the presented data:

- The objective of introducing a biosecurity plan in an agricultural production facility is to raise the level of biological security of food, its quality and production volume. The average rating of good (3.01) and very good (3.58) in the facilities A and B are indicative of well-implemented HACCP measures. The farms C, D, E and F received lower ratings and need to improve their animal biosecurity.
- The responsibility for production processes lies with the facility staff and they should especially rely on clearly formulated instructions and databases that are able to predict certain risks.
- Presence indicators of *Mus musculus* should follow over the year, especially in those objects with outdated constructions.
- All potential threats should be evidenced and adequate protection formulated. Different qualities of production require different levels of protection and corresponding control.
- The abundance of *Mus musculus* and critical control points should be determined by trapping. Environmentally safe products should be given preference as they have no effect on various segments of the environment and rodent resistance. Palatability should be improved, especially of the cellulose-based product.

Acknowledgment

This study was a part of Project III 46008 „Development of an integrated management system for harmful organisms on plants, aiming to overcome resistance and improve food quality and safety (2011-2014)“, which is financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Najznačajniji indikatori biosigurnosti i prisustva domaćeg miša (*Mus musculus* l.) u objektima stočarske proizvodnje

S. Đedović, J. Bojkovski, G. Jokić, T. Šćepović, M. Vukša

Rezime

Analiza indikatora biosigurnosti na kritičnim kontrolnim tačkama je namenjena sprečavanju neželjenih infekcija u tehnološkom lancu proizvodnje. Kvalitet proizvoda je osnov definisanja plana biosigurnosti po HACCP konceptu. Opšte i posebne mere biosigurnosti kojim se sprečava unošenje infektivnog materijala su teme prezentacija poslednjih godina u našoj zemlji. Za prenosioce patogena u objekte često se smatra domaći miš (*Mus musculus* L.). Uklanjanjem izvora hrane i otkrivanjem skloništa smanjiće se mogućnost infekcija unutar objekta. Sposobnost prilagođavanja različitim uslovima uticala je na pronalaženje alternativa kontrole *Mus musculus*. Cilj naših istraživanja su analize najznačajnijih indikatora biosigurnosti i prisustva *Mus musculus,* uzročno-posledične karakteristike i kontrola ekološkim materijama u objektima različitih tehnoloških procesa proizvodnje. Postavke pisanog plana biosigurnosti, izolacija, kontrola kretanja i promet posetioca utvrđivana je metodom upitnika. Ocene higijenskih uslova: mehaničko čišćenje, sanitarno pranje, dezinfekcija objekta, ventilacija i sanitacija objekta prikazane su vizuelnom metodom. Metodama biosigurnosti i dobrobiti životinja ocenjeni su parametri: higijenski uslovi odgoja životinja, zaliha hrane za životinje, biosigurnost životinja, uklanjanje uginulih leševa.Izlovljavanje jedinki *Mus musculus* i utvrđivanje kritičnih kontrolnih tačaka vršeno je klopkama tipa Longworth. .

Istraživanjima smo utvrdili da je efikasnost preparata na bazi natrijum selenita od 70,6% do 100% i da je dobra alternativa u kontroli *Mus musculus* u objektima različitih proizvodnih jedinica, jer ne ostavlja posledice na tehnološke procese proizvodnje i pojavu rezistentnosti. Potrebno je sprovoditi biosigurnosne mere po jasno definisanim uputstvima kako bi se smanjio biorizik i povećala bezbednost proizvoda.

References

ABBOTT W.S. (1925): A method of computing the effectiveness of an insecticide. J.Econ, Entomol, 18, 265-267.

ANON (2006): Farm and Home Biosecurity. University of Arkansas, http:/www.aragriculture. org/biosecurity/prodocer/farm_plan/introduction.htm.

BARTUSSEK H., LEE,B CH., HELD S (2000): Animal need index cattle, ANI 35 L/2000-cattle. Federal Research institute for Agriculture in Alpine Regions BAL, Gumpenstein. Austria. www.gum penstein.at/ publikationen/ignreport/ani35lc.pdf.

BLOCKUIS J.H. (2008): International cooperation in animal welfare: the Welfare Quality project. Acta Veterinaria Scandinavica 50, 1, S10.

BOKELMAN B. (1996): Quality Assurance and HACCP with Special Attention to Long Life Products.

BOJKOVSKI J.,STANKOVIĆ B., PETRUJKIĆ T., SAVIĆ B., DJOKOVIĆ R, PANTIĆ I., TURAJČAININ D. (2010): Review of investigations on influence of environmental chemical contaminants on hereditary base and reproductive capacities of landrace breed boars from pig farms,centar for reproduction and artificial insemination and biosecurity measures in Serbia. Lucrări Stiinłifice Medicină Veterinară, xliii, 2, 25-33.

BRACKE M.B.M., METZ M.H.J., DIJKHUIZEN A.A., SPRUIJT M.B. (2001): Development of a decision support system for assessing farm animal welfare in relation to husbandry systems: strategy and prototype. Journal of Agricultural and Environmental Ethics, 14, 321-337.

CASAL J., DE MANUEL A., MATEU E., MARTIN M. (2007): Biosecurity measures on swine farms in Spain: Perceptions by farmers and their relationship to current on-farm measures. Preventive Veterinary Medicine, 82, 1-2, 15, 138-150.

ČAMPRAG D. (1983): Kritični brojevi za štetočine. Priručnik izveštajne i prognozne službe zaštite poljoprivrednih kultura, 87-95.

ĐEDOVIĆ S., VUKŠA M , PETROVIĆ M., BOJKOVSKI J., PAVLOVIĆ I., JOKIĆ G., STOJNIĆ B. (2012): Control of Brown rat (*Rattus norvegicus*) on diarz farm in Serbia. Biotechnology in Animal Hausbandry, 28.3, 623-633.

FUEHRER H-P., BAUMANN T.A., RIEDL J., TREIBER M., IGEL P., SWOBODA P., JOACHIM A., NOEDL H. (2012): Endoparasites of rodents from the Chittagong Hill tracts in southeastern Bangladesh. Wiener Klinische Wochenschrift, 124, 27-30.

GIBBENS J.C., SHARPE C.E., WILESMITH J.W., MANSLEY L.M., MICHALOPOULOU E., RYAN J.B.M., HUDSON M. (2001): Descriptive epidemiology of the 2001 food-and-mouth disease epidemic in Great Britain: the first five months. Veterinary Record, 149, 729-743.

GILBERT M., MITCHELL A., BOURN D., MAWDSLEY J., CLITON-HADLEY R., WINT W. (2005): Cattle movements and bovine tuberculosis in Great Britan. Nature, 435, 491-496.

HRISTOV S., STANKOVIĆ B., PETRUJKIĆ T. (2009): Standardi dobrobiti i biosigurnosti na farmama goveda i svinja-uslovi smeštaja i držanja goveda i svinja. Veterinarski glasnik, 63, 5-6, 369-379.

HRISTOV S., RELJIĆ R. (2009): Ocena uslova smeštaja sa osvrtom na dobrobit krava. Zbornik naučnih radova Instituta PKB Agroekonomik, 15, 3-4, 79-88.

HRISTOV S., STANKOVIĆ B., DOKMANOVIĆ M. (2009): Standardi zaštite dobrobiti životinja na farmama goveda i svinja. Zbornik naučnih radova Instituta PKB Agroekonomik, 16, 3-4, 117-124.

HRISTOV S., STANKOVIĆ B.(2009): Welfare and biosecurity indicators evalution in dairy production. Biotechnology in Animal Husbandry 25, 5-6, 623-630.

KLIMPEL S., FORSTER M., SCHMAHL G. (2007): Parasites of two abundant sympatric rodent species in relation to host phylogeny and ecology. Parasitology Research, 100, 867-875.

JOKIĆ G., SĆEPOVIĆ T., ESTHER A., KATARANOVSKI D,, ĐEDOVIĆ S., VUKŠA P., VUKŠA M. (2013): First findings of house mouse (*Mus musculus* L) resistance to bromadiolone in Serbia. In: The 9th European Vertebrate Pest Management Conference (O., Huitu, and H., Henttonen, eds.), pp. 60.
PEŠIĆ-MIKULEC D., JOVANOVIĆ L. (2003): Principi HACCP koncepta u proizvodnji hrane. Ecologica (10) 39-40, 23-28.
RUŽIĆ A., (1983): Polifagne štetočine svih poljoprivrednih kultura (*Mus Musculus*). Priručnik izveštajne i prognozne službe zaštite poljoprivrednih kultura, 166-167.
OEPP/EPPO (2004) Field Tests against Synanthropic Rodents (Mus musculus, Rattus norvegicus, R.rattus), PP 1/114 (2). In: EPPO Standards PP1. Efficacy Evaluation of Plant Protection Products Miscellaneous, vol 5, 2nd edn. European and Mediterranean Plant Protection Organization, Paris, pp 36-47.
STANKOVIĆ B., HRISTOV S.(2009): Najčešći propusti u obezbeđenju biosigurnosti na farmama goveda i svinja. Zbornik naučnih radova Instituta PKB Agroekonomik sa XXIII savetovanja agronoma, veterinara i tehnologa, 15. 3-4, 103-110.
STANKOVIĆ B., HRISTOV S., ZLATANOVIĆ Z. (2010): Planovi biosigurnosti na farmama goveda i svinja. Zbornik naučnih radova Instituta PKB Agroekonomik sa XXIV savetovanja agronoma, veterinara i tehnologa, 16, 3-4, 125-132.
STANKOVIĆ B., HRISTOV S., BOJKOVSKI J., ZLATANOVIĆ Z., MAKSIMOVIĆ N., TODOROVIĆ-JOKSIMOVIĆ M., DAVIDOVIĆ V. (2011): The possibility of dairy farms isolation assessment-biosecurity aspect. Biotechnology in Animal Husbandry, 27 4, 1425-1431.
SUNDRUM A., ANDERSSON R., POSTLER G. (1994): Animal needs index 200- a guide fof the assessment of housing systems, Köllen-Verlag, Bonn, Germany.
PINTO C.J., URCELAY V.S. (2003): Biosecurity practices on intensive pig production systems in Chile. Preventive Veterinary Medicine, 59, 3, June 12, 2003, 139-145.
PRITCHARD G., DENNIS I., WADDILOVE J. (2005): Biosecurity: reducing disease risks to pig breeding herds. In Practice, 27, 230-237.
UHLENHOOP E. (2007): Biosecurity planning for livestock farms. U: Nikolić M.: Plan biosigurnosti na stočarskim farmama. Tematski zbornik „Dobrobit životinja i biosigurnost na farmama“, 1. međunarodna konferencija o dobrobiti i biosigurnosti na farmama u Srbiji, Poljoprivredni fakultet, Beograd, 227-238.
VUKŠA M., JOKIĆ G., ĐEDOVIĆ S., VUKŠA P., STOJNIĆ B. (2012): The justification for application and development trends of non-conventional rodenticides in protection of alfalfa from rodents. Biotechnology in Animal Husbandry, 28, 4, 659-673.

Received 21 March 2014; accepted for publication 25 April 2014

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

ERRATUM ET ADDENDUM / ISPRAVKA I DOPUNA

Journal Biotechnology in Animal Husbandry

U Broju 1, vol. 30 (2014), strane125 do 136, greškom je ponovo objavljen rad pod naslovom VARIABILITY IN SIZE AND SHAPE IN MUSCOVU DUCK WITH AGE: PRINCIPAL COMPONENT ANALYSIS, autora D.M. Ogah, M. Kabir, umesto rada pod naslovom PATH COEFFICIENT MODEL FOR ASSESSMENT OF WEIGHT USING LINEAR TRAITS AT BIRTH AND AT WEANING IN NIGERIAN INDIGENOUS PIG, autora D. M Ogah, N. D. Yusuf, M. M. Ari, koji objavljujemo u ovom broju časopisa.

ERRATUM ET ADDENDUM / CORRECTIONS AND ADDITIONS

Journal Biotechnology in Animal Husbandry

In the issue 1, vol. 30 (2014), page 125 to 136, mistakenly, paper entitled VARIABILITY IN SIZE AND SHAPE IN MUSCOVU DUCK WITH AGE: PRINCIPAL COMPONENT ANALYSIS, by DM Ogah, M. Kabir was re-released, instead of work titled PATH COEFFICIENT MODEL FOR ASSESSMENT OF WEIGHT USING LINEAR TRAITS AT BIRTH AND AT WEANING IN NIGERIAN INDIGENOUS PIG, by D. M Ogah, N.D. Yusuf, M.M. Ari, which is published in this issue.