SECTION 6: ANIMAL BIOTECHNOLOGY

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Review paper

REGIONAL DISTRIBUTION OF CATTLE, SHEEP AND GOAT FARMS IN THE REBUPLIC OF MACEDONIA, ACCORDING TO FARM SIZE AND BREED STRUCTURE

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Abstract
In the Republic of Macedonia the multitude of farms are cattle farms with an average capacity of up to 5 heads (74,6 %). The highest number of heads form all categories are bred in the Pelagonia region (19,6%), followed by the Skopje region (15,68%). The Vardar region has the least number of cattle (5,49%). Cattle crosses represent the majority of their kind in R. Macedonia (43,91%), followed by the Holstein - Frisian breed (39,65%). Out of the total number of sheep farms in the country, 41,69% are farms with a size of 50 heads, whereas farms with the size 51 - 100 and 101 - 500 heads are present with 20% and 30,4% respectively. Farms with a size of 301 – 500 heads consist 5,32% of the total number of sheep farms and farms with 501 -1000 and above 1001 heads are present with 2,30 % and 0,30% respectively. The highest number of sheep are bred in the Eastern region (22 %), after which is the Pelagonia region (16,9 %) and the Skopje region with the lowest number of 4,5%. Highly represented in the breed structure of the sheep population in R. Macedonia are the Ovcepolka (26,9%) and Sharplaninska pramenka (18,4%) strains.Out of the total number of goat farms in R. Macedonia, farms with a size of 1-5 heads consist the greatest percentage (65,09%), followed by farms with a size of 6-20 heads which participate in the total number with (26,05%). The eastern region has the highest number of goats (46,7%). In the breed structure of the goat population, highest participation was noted for indigenous goat breed (67,17 %). The presence of the Saneen breed is 11,75%, the Alpine 6,55 % and mixed breeds deriving from them are present with 4,19% and 10,34% respectively.
Key words: farm size, breed structure, regional distribution.

Introduction

Long-term development of livestock production in the future will primarily be determined by: environmental conditions, tradition of livestock production, farm size and economic factors. As a very important sector of agriculture livestock production occupies an important place in the structure of farmers' income, but also in the overall economy. The ability to converse plant food in high quality food products for human populations gives special significance to the livestock production. The agricultural sector is one of the most important sectors in the Macedonian economy. The share of this sector in the gross domestic product of the Republic of Macedonia is around 10% and increases to 15% if we take into account

the food industry. Livestock accounts for 27,5% of the value of agricultural production (Economic calculation in agriculture, SSO, 2002-2010). Cattle, sheep and goats breeding in the country are mainly performed by individual farmers. The production is orientated for its own consumption, but part of this production is marketed to dairy plants and slaughterhouses. It is noticeable, existence of a number commercially oriented family farms, large specialized livestock production enterprises, whose number is declining. According to the State Statistical Office of the Republic of Macedonia, in 2010 the highest percentage of cattle, sheep and goats (97,1% of cattle, 95,8% of sheep and 98,7% of goats) were located in individual farmers, while the rest goes to business sector (SSO, Yearly Report, 2011). Main aim of this paper is to determine regional distribution, farm size and breed structure of cattle, sheep and goats in the country as main indicators which will serve to evaluate production capacity of different regions in the country but also to recommend future activities in order to improve and increase production from ruminants. Operational data form Food and Veterinary Agency (FVA) in 2012 are used to determine regional distribution, farm size and breed structure of cattle, sheep and goats in the country.

EU trends in cattle, sheep and goat sector

Success in milk production, or total milk produced is primarily influenced by the technology of breeding and breed genetic capacity. Specialized dairy cattle farms in the EU, according to the system of EU farm typology and classification, is the cattle farm in which more than 2/3 of the income is realized from milk production. In these farms most of the agricultural lands are processed to produce food for their own needs. Production technology and farm management is directed towards the production of milk and specialized farms are poorly diversified. Most of the farms in the EU-15 are breeding at least 50 or more heads, which was not the case in the EU-10, where most of the farms were located in the herd size up to 10 or fewer heads. But, in the period 2000-2007 only large farms in the EU-15 with more than 100 heads of cattle were able to buy new breeding heads, while farms with more than 10 heads, farms in the EU-10, failed to raise livestock, while those farms with under 10 cattle heads have reduced the quantity and slowly disappeared. As a result of a number EU policies the number of dairy cattle in the last decade decline. Overall decline in the number of milking cows in the EU-15 in the period 1990-2010 is almost 33%, with the lowest average rate of decline registered in Denmark, France, Ireland and the Netherlands. During 2000-2010 decline in the number of milking cows continues, following parameters are noted: decrease of 12% (EU-15), 20% (EU-10) and 14% (EU-27) (Evaluation of CAP measures applied to the dairy sector, 2011).

This situation, the distribution of farms and their size affects milk production, even 87% of the milk in the EU-15 and 56% of the EU-10 comes from specialized farms. Despite this fragmentation in the production, large reduction of the quantity and the emergence of the tendency to increasing farm size and increase production indicating strong restructuring of the EU cattle milk production (e.g. Germany, the trend of reducing the cattle number from 5,2 million in 1996 to 4,2 million cows in 2006, but there was milk yield increase from 5,8 t per head to 7,3 t. In this period there was also a change in the number of farms from 186000 in 1996 to 92000 in 2006, and the increase in the average number of cattle on a farm from 28 heads in 1996 to 46 heads in 2006), (European Commission, 2010b).

Dairy sheep production is usually based on local breeds, well adapted to the production areas which are in many cases less favoured. Sheep use natural resources of low interest for other species, helping the maintenance of ecological equilibrium and natural landscape. In addition, they contribute to the maintenance of economical activity and population in rural areas (Ugrante and Gabina, 2004). At the European level, sheep and goat breeding represent minor agricultural activity representing 3,6% of the total value of livestock production in the EU-27. However, in certain Member States, sheep and goats farms occupy a significant part of the overall agricultural area: 31% in UK and 20% in Ireland, Spain, Romania and Italy. Sheep sector is characterized by a reduction of the quantity of sheep meat breeds (33% less reproductive sheep heads for meat production during the period 2000-2009), while the total number of sheep intended for dairy production has grown steadily (+43% number of milking sheep). Generally it can be seen a downward trend in the total number of sheep from 1990 (-1% per year) and slightly accelerated decline after 2005 (-3%). The number of goats was reduced by 11% in the period 1990-2009. This reduction was still compensated by productivity gains, which resulted in increased production of goat's milk. As main recent developments in dairy sheep breeding in EU can be noted: 9% increase of milk production from 1995, almost 2/3 of the milk produced is purchased and processed by commercial dairies with considerable variation among Member States, exports of EU-produced cheese is 3/4 of world trade with cheese; stable prices for sheep's milk in recent years, with differences between Member States. As the trend in most EU countries where sheep are milked there has been a rapid development of "productive breeds" in Spain is Assaf breed, where this breed according to the national census exceeds local dairy breed Churra, while sheep breed Lacaune is considered to be represented by more than 200000 reproductive sheep heads. Similar is the situation in Greece and Slovakia where the races Lacaune and East Friesian breed are the only sheep breeds on the rise (de Rancourt and Carrère, 2011). In dairy goat breeding following trends are noted: 10% increase in milk production from 1995, goat milk purchase compared sheep milk is lower intensity and noticeable number of producers process milk on their farms, marginal international trade of goat cheese, constant price growth of goat milk in the Member States that are major producers of goat milk (Evaluation of the CAP measures for the sheep and goat sector–Executive summary, 2011).

Livestock sector at primary level

Cattle breeding

A dairy cattle breeding is closely associated with technology of breeding and overall crop production. It is an expression of two biological and technological differently organized systems (milk and meat) which are interconnected, where meat production is in negative correlation with milk production. Considering the characteristics of the production, especially milk production, many factors will affect the volume of production. Data as: heads quantity, breed, farm size, breed and head regional distribution, as well as breeding technology provide the appropriate information for production capacity in terms of milk production. According to operational data FVA in 2012 about 40,797 cattle farms are registered in the Republic of Macedonia, with a total of 263283 cattle in all categories, with average farm size of 6,5 cattle per farm, taking into account all cattle categories. The data indicate that the average size of a farm in the Republic of Macedonia is different from the average size of cattle farms in some EU member states, Greece (14 cattle per farm) and

Austria (86 cattle per farm), but it is still twice the average size cattle farms in Montenegro (Marković et al., 2011).

Regional distribution

According to its structure and orientation cattle farms are small, traditional and low productive, with milk yield from 2,000 up to 3,000 liters (SSO, 2011). Largest number of cattle, according to the operational data of FVA in 2012 (Figure 1), in all categories are present in Pelagonija region (19,6%), followed by Skopje (15,68%), while the Vardar region is characterized with lowest distribution of bovines (5,49%). In other regions the distribution of cattle ranged from 9,29% (Southwest) to 14,73% (Polog).

Farm size

High percent (74,6%) of cattle farms in the Republic of Macedonia bred up to five heads. Also quite low percentages (21,1%) of farms are farms from 6 up to 20 heads. Approximately 4 % of cattle are farms with more than 20 highly productive heads and slightly higher annual milk production, and only 1% of the cattle farms, which usually are carriers of milk production and quality genetic material, are farm with over 50 breeding heads. Data structure of farms in 2012 is given in Figure 2 and Figure 3.

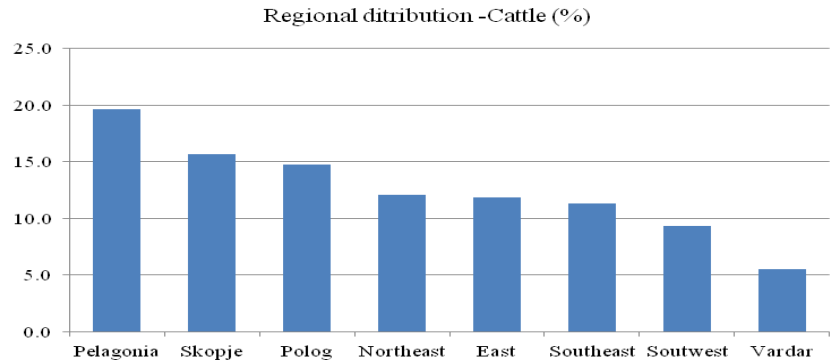

Figure 1. Cattle regional distribution

A similar trend in the farm size is observed in certain regions of the country (Figure 3). The unfavorable structure, regarding farm size, is present in: Southwestern (83,42%), Polog (79,13%) and Northeastern (70,73%) region where predominant farms are cattle farms with less than five heads. In these regions farms which should be carriers of cattle dairy production (from 51 up to 100 heads) are almost absent, although in these regions is very high percentage of registered holdings (Southwest 13,3%, Polog 21,8% and the Northeast 12,9%) regarding total number of registered farms in the country. In other regions also highly present are farms under five heads, but in these regions, there is a slightly better distribution of farms according to their size. Smallest number of farms in the category under five heads was noted in the Eastern region (63,89%). Category of cattle farms from 6 up to 20 heads ranges from 17,50% in the Southeast up to 27,37% in the Eastern region of country. While in Skopje and Northeastern region in this category (from 6 up to 20) are registered 26% of the farms. In other regions this category of farms is under 20% with the lowest (15,16%) value noted in the Southwestern region.

Farms that have a potential for milk production, according to farm size are farms with size of 21 up to 50 heads (small family farms). Generally speaking, compared to other categories of cattle farms their participation is quite small. Lowest percent of farms from this category is present in the Southwest (1,16%), and the highest percent in the Eastern (6,80%) and Skopje region (6,0%). This category of farms in Pelagonia and Vardar region is present with only 5,63% and 5,88%, respectively. In the other three regions in the county distribution of those farms is approximately 4% and in the Polog region is 1,63%. As regions where there is a little bit higher farm distribution of cattle farms with 51 up to 100 heads are: Skopje (3,67) and Vardar (3,07) region, while in other regions this category is less than 2%, with the lowest value in the Southwestern region (0,19%). The presence of large farms (farms with more than 101 breeding heads) is quite small and unevenly distributed in the country, 1,20% of the farms in the Vardar region belong to this category and 0,38% of the farms in Pelagonia region. In other regions the presence of this category of farms is low, with lowest value in Polog region where 0,02% of the farms are representative of this category.

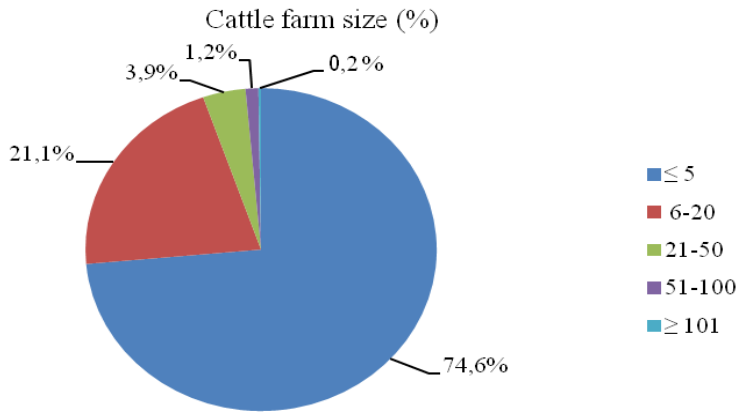

Figure 2. Cattle farm size

Breed structure

Breed structure, genetic capacity but also breeding technologies among other factors have a significant impact on total milk production. In order to determine breed distribution in the country cattle farms were classified according to cattle breeds present in the country. The total number of farms in the Republic of Macedonia, according to their ordinal number of the holding is 40,737, but since in one farm can be bred more cattle breeds, with separation of the breeds that are present in a farm and their registration, their number is higher. Bovine breeds distribution is shown in Figure 4. The results indicate that the largest share in the cattle population in the Republic of Macedonia belongs to crosses with 43,91%, Holstein-Friesian breed is present with high percentage (39,65%) in the country. Breed distribution according to regions is suggesting that crosses are present in a range from 67% in Skopje to 27% in Pelagonija and Polog region. It should be noted that the share of dairy breeds such as Holstein- Friesian, regarding total number of cattle is highest in Pelagonija region (64,2%) and Polog region (56, 3%), and lowest in the Skopje region (11,86 %). Based on the analysis and comparison of the data in the Republic of Macedonia in the period 2006-2012, there was

a reduction in the number of farms. The total number of dairy farms in the Republic of Macedonia in 2006 was 48741, to be reduced up to 40737 in 2012, according to their ordinal number of the holding. While in 2006 approximately 86% of farms were farms with 1-5 cows, and the proportion of large farms over 30 cows was 0,7%. In 2012, there was a slight improvement in the structure of farms, but again most of the farms (73,6%) belong to the category number up to 5 heads, cattle farm with 10 cattle were 87,6%, while about 3% belong to the category over 30 heads. Lowest percent (0,27%) belongs to farms breeding over 100 heads.

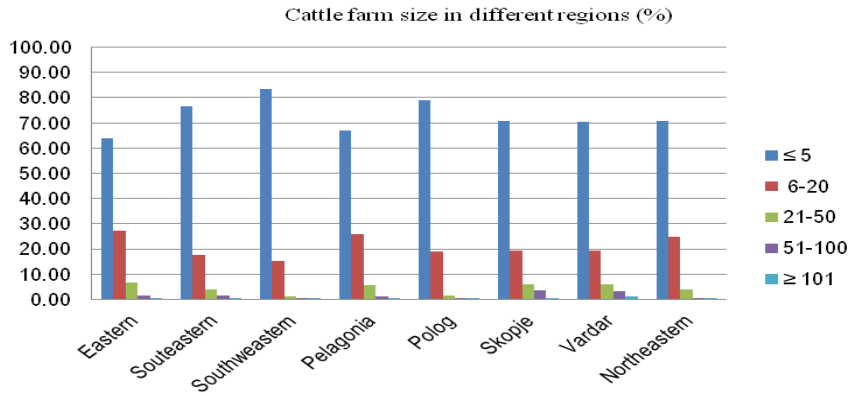

Figure 3. Cattle farm size in different regions (%)

Sheep breeding

Specific climatic conditions, the mountainous nature of the terrain, the advantage of the sheep to make full use of the capacities of rural (marginal) areas, then the ability to maintain economic capacities of the rural population, as well as the tradition of sheep breeding are ideal for development of sheep breeding in the Republic of Macedonia. The sheep milk, at least until now, mainly is intended for domestic market and it is the raw material for processing of different types of cheeses and other dairy products. Sheep milk production in the Republic of Macedonia in a longer time of period shows significant oscillations.

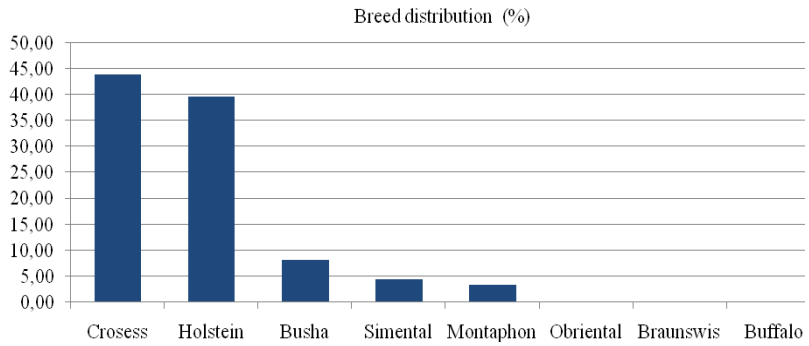

Figure 4. Breed distribution

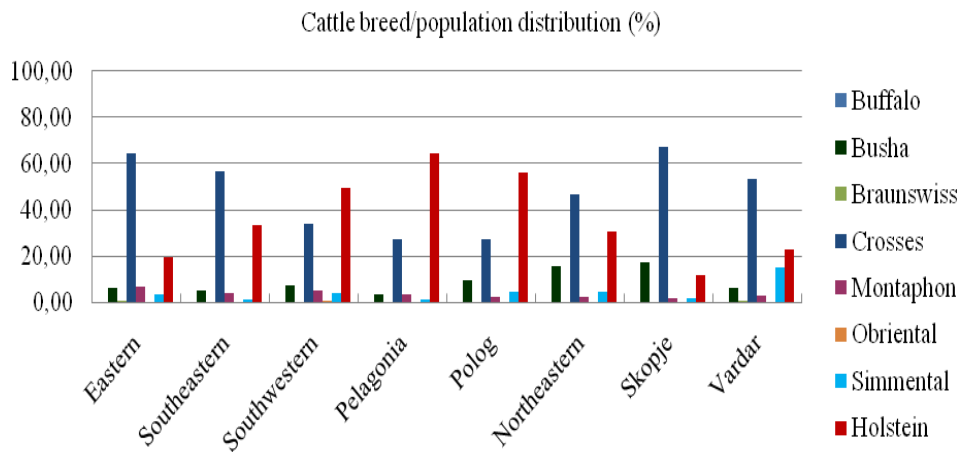

Figure 5. Cattle breed distribution in different regions

Totally produced sheep milk is affected by the number of breeding sheep in the country, genetic capacity and farm practice. Regarding ownership structure the highest percentage (97 to 98%) of the total milk production in the past period belongs to the individual farmers (SSO, 2011). While the rest amount of milk production belongs to the large agricultural enterprises and cooperatives. Considering the characteristics of sheep breeding technologies, several factors (climate, economic characteristics and farm size, as well as the technology of breeding) significantly affect the volume of sheep milk production. Mainly in sheep breeding are present two production systems (extensive and intensive) with multiple subsystems. In our conditions dominates extensive semi nomadic system. But the production of sheep milk generally distinguishes six systems that are based on the length of suckling period. Breeding technology in the Republic of Macedonia in most sheep farms is traditional, which means lambing once a season (January-February), lambs weaning (March-April) and milking period until the middle of July. System of sheep breeding in the Mediterranean pool often have a dual direction of production (excluding wool because of the low interest and low price), where two-thirds (or three-quarters) of income is due to milk and one-third (or one-quarter) of the income is a result of the production of meat. Although a high proportion of income in the sheep breeding is connected with milk production very few dairy sheep in the country are under the control of the productive and reproductive traits. Less than 0,3 to 0,5% of milking sheep, or in about 1500 dairy sheep in the country are under control of the productive and reproductive traits. Implementation of monitoring and determination the genetic quality and milk yield in sheep breeding is with low-intensity in the country. Undoubtedly this low coverage, lack of reproduction centers and low level of education of farmers adversely affect the development and utilization of the country's overall capacities in sheep breeding. According to operational data FVA in 2012 in the Republic of Macedonia 6671 sheep farms are registered with a total of 750,201 sheep heads in all categories, that the average size of sheep farms would be 113,4 heads per farm, taking into account all sheep breeding categories.

Regional distribution

According to the operational data of FVA, most of sheep population are bred (Figure 6) in the Eastern region (22,0%), followed by Pelagonija (16,9%), while the Skopje region is characterized with the least sheep heads (4,5%). In other regions the percentage of sheep heads ranges from 9,0% (Southeast) up to 14,27% (Southwest).

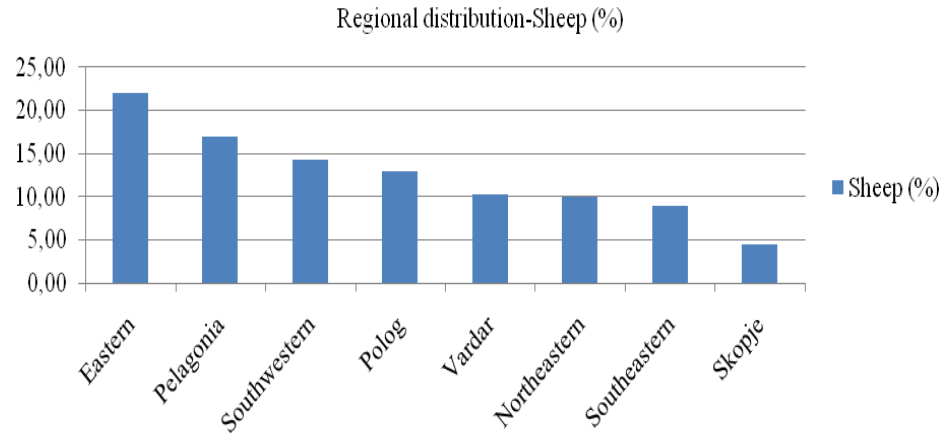

Figure 6. Sheep regional distribution

Farm size

Majority (41,69%) of sheep farms belong to group of farm which are breeding up to 50 heads, farms with a size of 51 up to 100 and from 101 up to 300 heads cover 20% and 30, 4%, respectively. Only 5,32% of sheep farms in the country belong to farms with 301 up to 500 heads. Very small part of the overall structure of the sheep farms belong to large farms from 501 up to 1000 and more than 1001 heads, 2,30 and 0,30%, respectively (Figure 7). Highest number of sheep farms (Figure 7) in the Republic of Macedonia is located in the categories up to 50 and from 101 up to 300 sheep. High density of farms (Figure 8) in the category up to 50 heads was noted in the Eastern (59,6%), Northeast (55,69%) and Skopje region (41,53%). In other regions this category of farms is between 20% and 33% with the lowest values in Pelagonija (12,13%) and Vardar (8,61%) region. Unlike other regions where most farms still belong farms under 50 heads, in four regions of the country (Pelagonija, Southwestern, Polog and Vardar) prevailing in farm size from 101 up to 300 sheep. In addition, 57,11% of the farms in Pelagonija, 47,19% of the farms in the Vardar region, 43,31% of the farms in the Polog and 35,38% of the farms in the Southwest region, belong to the category from 101 up to 300 heads. High distribution of farms in the category from 301 up to 500 heads were observed in Polog (14,29%) and Vardar (14,98%) region, while the same category had lowest distribution (2,27%) in the Northeast region. Farms with over 1001 heads are present in all regions with the exception of the Skopje region, and the highest presence (1,25%) of these farms was observed in Polog region.

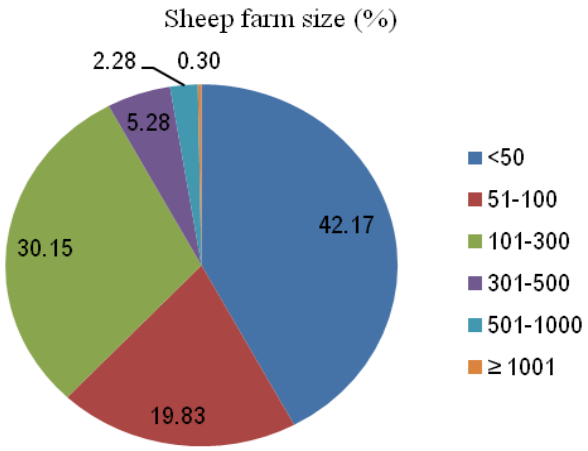

Figure 7. Sheep farm size

Breed distribution

Regarding breed structure of sheep population in the country, most frequent sheep population is Ovchepolian (201,673 heads, or 26,9%), and Sharplaninian (138 290 heads, or 18,4%) pramenka, with their usual concentration in the regions were those population are originated (Figure 9). Crosses of different breeds are present with 24% and Württemberg crosses are present with 16,3% of the total number of sheep. Highest concentration of crosses was observed in Skopje and Vardar (81,5%) region. Dairy breeds as Awassi, Blackhead Pleven, and East Friesian breed are insignificantly present. Crosses with Awassi breed have very low participation in the sheep population, accounting about 4% of the total number of sheep, with largest distribution in Pelagonija region (13 478 heads) (Figure 10).

Goat breeding

Considering the climatic conditions, as well as the experiences of other countries, the Republic of Macedonia has capacity for adequate development of goat breeding and through the formation of pure breed goat farms and application of intensive farming or semi intensive goat breeding systems. Goat breeding in the Republic of Macedonia posse's high development capacity including breed genetic improvement, higher milk production, which in turn will offer economic benefits to farmers. Main products from goat breeding in the country are different types of cheese that are commonly placed on the domestic market, but also production and sale of fresh milk. The highest part of income from goat breeding is accomplished by milk production, but a very small number of dairy goats are under control of productive and reproductive traits. Less than 0,2% of the population of dairy goats, or less than 1000 heads, are included in activities regarding monitoring and determining the genetic quality and milk yield. Undoubtedly this low coverage, lack of repro-centers and low level of education of farmers adversely affect the further development and utilization of the overall capacities of goat breeding.

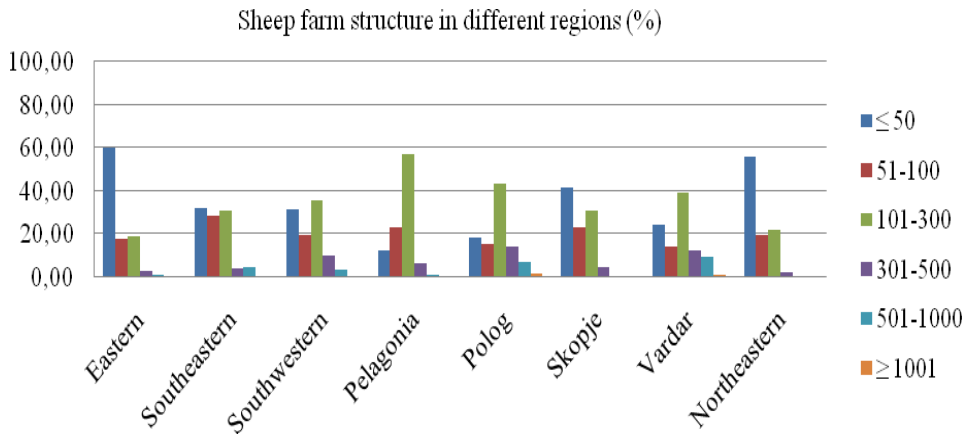

Figure 8. Sheep farm structure in different regions

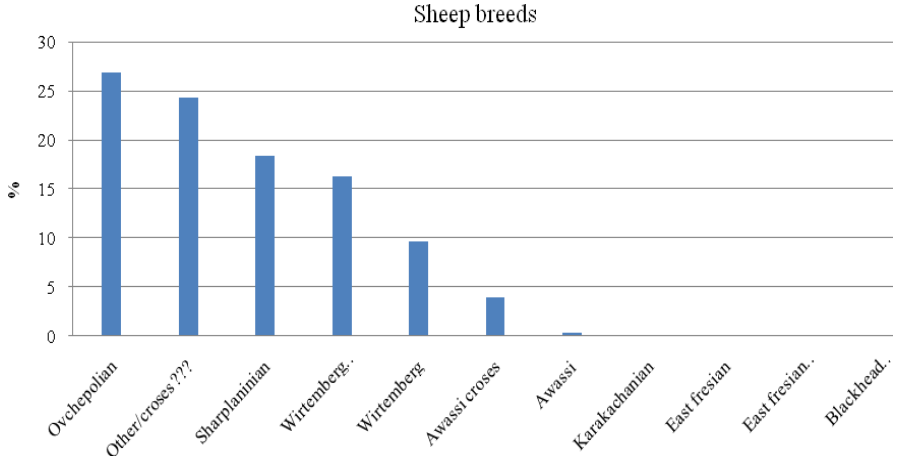

Figure 9. Sheep breeds in the country

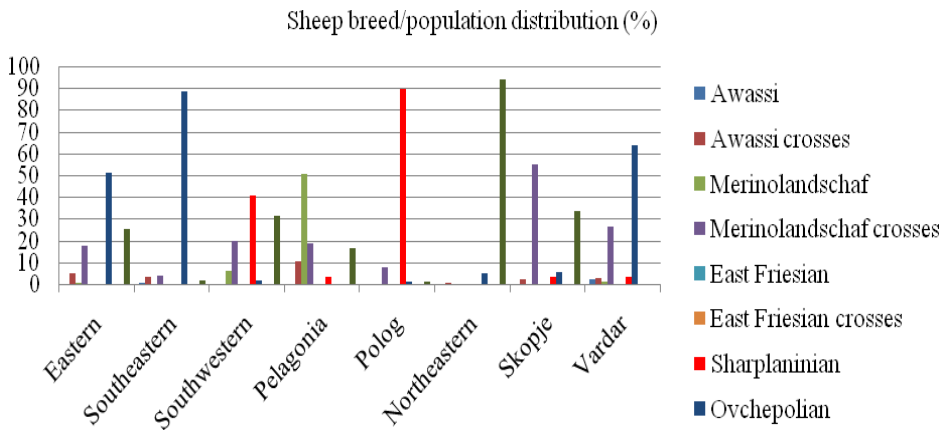

Figure 10. Sheep breed distribution

Regional distribution

According to the operational data FVA, Southeast and Eastern region are characterized by the highest number of goats which occupy 46,7% of the total number of goats in the Republic of Macedonia (Figure 11), low goats density was noted in Skopje region (4,1%), while in other regions they are present in a range from 6,0% (Polog region) to 12,8% (Vardar).

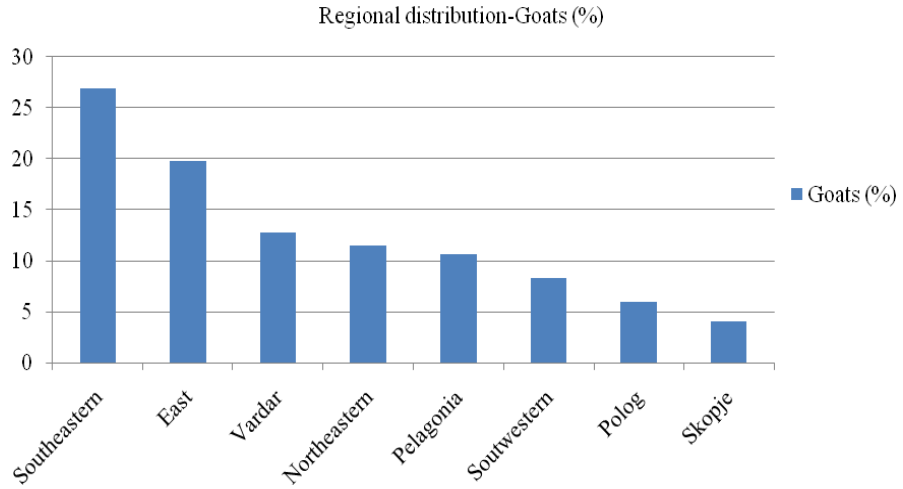

Figure 12. Goats regional distribution

Farm size

According to operational data FVA in 2012 (Figure 13) in the Republic of Macedonia 5746 goat farms are registered, with total of 48,492 goats, that will give average farm size of 8,4 heads per farm, taking into account all breeding categories. The majority (65,09%) of goat farms in the Republic of Macedonia are farms wit up to 5 heads, goat farm size from 6 up to

20 goats comprise 26,05% of the total number of farms. A small percentage (6,61%) of farms belong to goat farms from 21 up to 50 heads, while very low percentage belongs to farms which are breeding 51 up to 100 (1,76%), 101-200 (0, 40%) and more than 200 (0,09%) goats. Goat farms (Figure 14) up to 5 heads are mostly present in the: Eastern region (80,18%), Southeast (63,6%) and Skopje region (64,4%). In the Northeastern region 52,85% of farms belong to this category. In other regions this farm category is smaller and ranges from 48,18% in the Vardar, 46% in the Southwest and Polog up to 31,96% Pelagonija region. Although Pelagonija region is a region with the lowest distribution of farms up to 5 heads, this region compared to all other regions in the country has highest percent (43,35%) of goat farm in the category from 6 up to 20 heads. In the Southwest, Polog and Skopje region this category of farms is present with 39,34%, 37,89% and 30,37%, respectively. Lowest distribution (16,85%) of this farm category is present in the Eastern region. In the most important category of farms, farms with a size from 51 up to 100 heads, only the Vardar region have double-digit distribution (10,00%). Two-times smaller distribution, regarding Vardar region, has Pelagonija region (5,70%), while the remaining regions have even smaller distribution (under 5%). Distribution of farm category between 101-200 heads is very low in the country, but still 182 farms in the Vardar region belong to this category. In all other regions, the presence of this category of farms is below 1%. Farms larger than 201 heads are recorded in the Southeastern and Skopje region but with very with low distribution, 0,23% and 0,24% respectively, while in all other regions of these farms are not present.

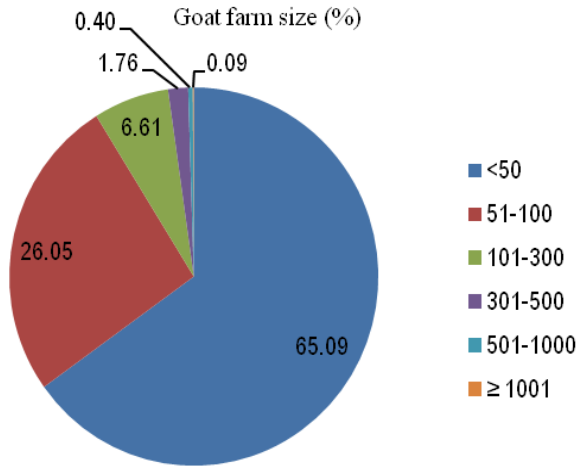

Figure 13. Goat farm size

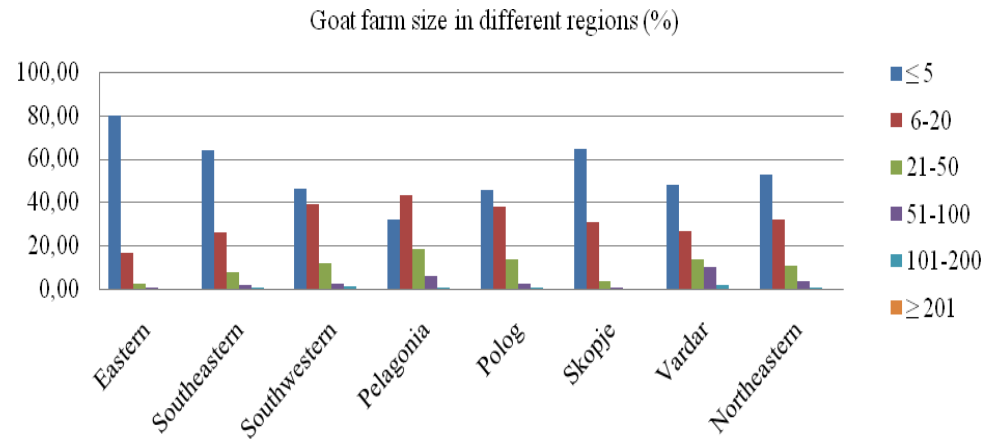

Figure 14. Goat farm size in different regions

Breed structure

Largest share in the goat population in the Republic of Macedonia has a domestic goat, representing 67,17% of the total goat number (Figure 15) and it dominates with over 85% in the Eastern, Southeastern and Southwestern region. Saanen goat participate with 11,75%, Alpine breed with 6,55%, while their crosses participate with 4,19% and 10,34% respectively (Figure 16).

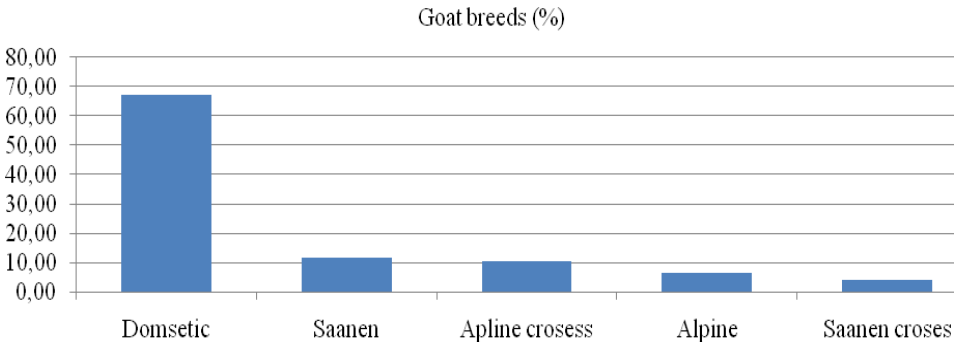

Figure 15. Goat breeds in the country

Breed distribution according to regions, suggesting that Saanen goat is the most present in Northeastern, Pelagonia and Skopje region. Dairy breeds and their crosses in the country occupy only one-third from total population but they are usually bred in small herds. Insignificant part of the population of dairy goats is under control of productive and reproductive traits, as well as the low level of primary and secondary education of farmers significantly contribute to reducing the development capacity of goat breeding. The absence

of the application of appropriate methods of breeding and nutrition of goats on the majority of the farms are limiting factors in terms of improving dairy capacity.

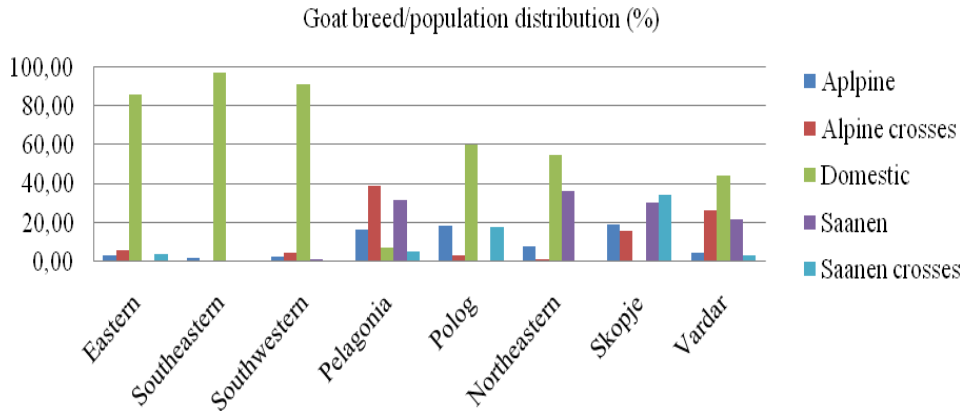

Figure 16. Goat breed/population distribution in different regions

Conclusions

Based on analyzed data, can be concluded that the greatest potential for cattle dairy production, given the largest presence of Holstein- Friesian breed, have Pelagonija (3183 l per cow) and Polog region (3138 l per cow). Significant presence of crosses in other regions in the country (from 33,8% up to 67,3%) is a solid base for the dairy industry. However, the small farm size is an unfavorable characteristic of cattle breeding in the Republic of Macedonia. This weakness becomes more pronounced if is noticed that during this analysis the total number of cattle in the country is included, rather than only dairy cows, affecting further reducing of farm size, and thus the total capacity to milk production. Also, the small population of dairy cows, which is under the control of the productive and reproductive traits, as well as the low level of primary and secondary education of farmers, has significant effect on dairy cattle breeding. Main characteristics of sheep breeding in the country is a preponderance of small (up to 50 heads) in the Eastern, Northeastern, and in the Skopje region) and medium-sized farms (101-300 heads in Pelagonija, Vardar and Polog region). Regarding the breed structure of sheep population in the Republic of Macedonia is characterized by a very small distribution of the dairy breeds and their crosses, regarding high presence of domestic breed and their crosses with meat-wool breeds (Württemberg). Insignificant part of dairy sheep population in the country is under the control of the productive and reproductive traits, as well as the low level of primary and secondary education of farmers have strong influence on sheep breeding. High distribution of goat farms with less than 5 heads except in Pelagonia region, dominance of domestic goat breed in the country regarding dairy goat breeding as well as low percent of goat population included under the control of the productive and reproductive traits are main weakness in this sector. Future activities in ruminant sector should be orientated in: increasing farm specialization and size and application of modern production technologies in dairy farming. Improvement of breed structure on farms, breeding dairy breeds, but also genetic

improvement of indigenous populations should be main direction in the country regarding ruminant breeding.

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РЕГИОНАЛНА ДИСТРИБУЦИЈА НА ГОВЕДАРСКИ, ОВЧАРСКИ И КОЗАРСКИ ФАРМИ ВО РЕПУБЛИКА МАКЕДОНИЈА, СПОРЕД ГОЛЕМИНАТА НА ФАРМАТА И РАСОВАТА СТРУКТУРА

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Апстракт

Во Република Македонија најголем број од говедарските фарми се фарми со просечна големина на фарма до 5 грла (74,6%). Најголем број од грлата во сите категории од видот говедо се одгледуваат во Пелагонискиот регион (19,6%), односно во Скопскиот регион (15,68 %). Најмал број на грла е забележан во Вардарскиот регион (5,49%). Мелезите се најзастапени (43,91%), додека Холштајн-фризиската раса е застапена со 39,65%. Од вкупниот број на овчарски фарми во земјата 41,69% отпаѓа на фарми со големина до 50 грла, додека фармите со големина од 51-100 како и фармите од 101-500 грла се присутни со 20% и 30,45%, респективно. Фармите со големина од 301-500 грла зафаќаат 5,32% од вкупниот број на овчарски фарми во земјата. Овчарските фарми со големина од 501-1000 грла и над 1001 грло се застапени со 2,30% и 0,30%, респективно. Најголем број на овци се одгледуваат во Источниот регион (22%), по кој следува Пелагонискиот регион (16,9%) и скопскиот регион со 4,5%. Највисоко застапена во расовата структура во овчарство е овчеполската овца (26,9%) и Шарпланинската праменка (18,4%). Од вкупниот број на козарски фарми во Р Македонија фармите со големина од 1-5 грла се најзастапени (65,09%), по кој следат фармите со големина од 6-20 грла кои пак учествуваат во вкупниот број со 26,05%. Источниот регион на земјата има најголем број на кози (46,7%). Во расовата структура на популацијата кози најголем процент е нотиран ка домашната автохтона коза (67,17%). Присуството на Санската коза е 11,75%, Алпина е застапена со 6,55% додека нивните мелезите се присутни со 4,19% и 10,34%, респективно.

Клучни зборови: големина на фарма, расова структура, регионална дистрибуција, говеда, овци и кози.

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EGG PRODUCTION AND EGG QUALITY PARAMETERS OF LAYERS FED DIETS WITH OR WITHOUT ADDED PHYTASE

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Abstract
Balancing the nutrients (especially Ca and P essential macro minerals responsible for high egg production and eggshell quality in laying hens), in the diets for layers selected for low body weight, but high egg sizes is a state of art. Whole cycle egg production results (egg number, laying percentage, feed consumption) and egg quality parameters (egg size, shell strength, yolk color and Hough Units) were compared in two groups of ISA Brown layers fed isoprotein and isocalloric diets without (NP) or with (AP) phytase enzyme at level of 0.01%. Trial group diet supplemented with phytase, contains lower level (on average 0.5%) of Non Phytate Phosphorus (NPP) source-Mono-Calcium-Phosphate (MCP). Non significant differences in daily laying percentage (81.02% vs 80.83%) and number of egg produced per hen housed (297.96 vs 297.99) were recorded. Analyzes revealed non significant differences in egg size and shell strength (60.96g vs 61.38g; 3851.05 vs 3928.67 g/cm^2) between the groups. Some significance, not attributable to the phytase supplementation, in yolk color (9.23 vs 10.53) and Hough Units (70.88 vs 67.64) were monitored for trial and control group, respectfully. Correlations between eggshell strength and egg weight were non significant. Modification of the feed formula through lowering the NPP and phytase supplementation is valuable approach that decrease the feed costs but does not influence number, size and quality of the eggs produced.
Key words: poultry, nutrition, phosphorus, phytase, egg production, egg quality.

Introduction

Balancing the nutrient in the feeding of the modern type egg layers is a state of art. High selection pressure toward better feed conversions have lead to decreased body weight, therefore lower feed intake capacities and higher egg sizes in these birds. These two characteristics are in high negative correlations therefore leads to need of fine tuning the nutrients essential for maintaining high performance (egg number) and high quality products (medium to big sized eggs with sufficiently strong shell). Beside Ca, Phosphorus (P) is accounted as essential mineral responsible for high egg production and egg shell quality in

laying hens. Organically-bound phosphorus salts of phytic acid (phytate phosphorus) is almost unavailable for the metabolism of birds (Klis et al, 1996; Kornegay 1996; Angel et al., 2002;) meaning that birds can hardly use phytate P due to evolutionary lack of enzymes that will degrade the phytate molecules. On the other side Non Phytate Phosphorus (NPP) sources as feed ingredients, are rather expensive but also limited, non-renewable resources, therefore inorganic part of P ingredients in the feed are often reduced with efforts of helping the birds to reach for phytate P in the grain sources (corn, barley, wheat, soya e.t.c) through supplementation with exogenic phytase enzymes. It is well known that low levels of available phosphorus have negative effect on production records, and that supplementations of exogenous phytase can help overcoming these negative effects. For example adverse effects of a low P (1.3 or 1.1 g/kg NPP) diets were overcome with phytase supplementation in the experiment of Francesch et all, (2005). There are numerous reports of many authors that supplementing diets with an exogenous phytase enzyme does not affect production level. In many of the studies improved performances in terms of better feed conversion ratios are reported, and significantly improved shell quality (Jalal and Sheideler 2001; Lim et al., 2003; Liebert et al., 2005; Liu et al, 2007; Rubio et al 2009; Skřivan et al, 2010; Meyer and Parsons 2011; Singh et al, 2011). Panda et al (2005b) compare different levels of non-phytate phosphorus (NPP) sources in the diets of White Leghorn at the beginning of the production period and conclude that low level of 0.15% NPP (180 mg/b/d) in the diets enables reasonable production performance but decrease the live weight, during 28 to 44 weeks of age and that higher amount are needed for improve bone mineralization. Same authors, (2005a) in different article reported the results obtained by adding phytase to the low NPP diets (1.2 and 1.8gNPP/kg). Addition of phytase to 1.2gNPP/kg diet significantly enhanced the body weight and equals groups fed diets containing 1.8-3.0gNPP/kg feed. Adding phytase to diets with NPP levels beyond 1.8 g/kg revealed no additional advantage in hen day egg production, food intake, food efficiency, shell weight, shell thickness and shell strength while egg weight, specific gravity and Haugh units were not influenced by phytase supplementation. In the trial of Koelkebeck and Boling, (2009), supplement feed with enzyme phytase resulted in substantially improved egg production. Comparative analyze of the production results in advance aged Hy-Line White-36 layers (60-72 week of age) fed diets with phytase in comparison with the group fed conventional diet was performed by Zaghari et al (2008). They found no significant difference in egg production, egg shell thickness and breaking strength. Their final conclusion was that phytase improves P utilization in older hens. Mohammed et all (2010), in their article report improvement in production (egg number) of Hy-Line White-36 hens fed diets containing rice bran and supplemented with phytase. They find better feed conversion and shell thickness but lower egg size in the groups fed phytase supplemented diets. In the experiment of Yildiz et al (2010), layers at age 22-42 weeks were fed basic diets (16% CP, 2800kcal/ME/kg and 13.86mg/kg^{-1} Mn) supplemented with three different levels of Mn (0, 35and 70mg kg^{-1}) and phytase (0, 100 and 500U). No significant differences were find in body weight, egg production, egg weight, feed conversion ratio and egg quality characteristics. This experiment was conducted to follow the production data of two flocks of layers, fed diets with or without added phytase, where diets lower in NPP were supplemented with phytase, through the whole production cycle. R. Macedonia is in the region where high summer

temperature causes problems in the shell thickness especially in the flock of advanced age, therefore phytase could be a possible solution for improvement of the shell quality.

Material and methods

Two farm houses (trail and control) were housed with ISA Brown pullets fed isoprotein and isocalloric diets with (Added Phytase - AP, trial or F+) or without (No Phytase- NP, control or F-) added exogenous phytase enzyme. Total and available phosphorus phase feeding level (Phase0 – F0, >28; Phase1- F1, 28-50 and phase2-F2, >50 weeks of age) was calculated in the diets formula according the recommended nutrient requirements (table 1). Lower level (on average 0.5%) of Non Phytate Phosphorus (NPP) sourced from Mono-Calcium-Phosphate (MCP), and exogenous phytase (Ronozyme P 5000) supplementation at level of 0.01%, was used in the trial group. In-house environmental and management conditions were similar for both groups of layers. Egg production results and egg quality parameters were compared in trial and control group. Egg quality analyzes were performed in the laboratory for egg marketing quality control at the Institute for animal biotechnology of the Faculty of agricultural science and food, using Egg Multi Tester EMT 5200 and Eggshell Gauge (Robotmation Co. Ltd., Tokyo, Japan). This equipment offers unbiased, computerized measure of the physical characteristics of eggs (weight, yolk color, Hough Units) and eggshell (egg breaking strength). Statistic analyses on results were performed by computer program STATISTICA module ANOVA and Fisher's LSD test to determine the level of statistical significant difference between the groups for the parameters of interest.

Table 1. Diet formulas used in the trial

	NP Diet F0 -	AP Diet F0 +	NP Diet F1 -	AP Diet F1 +	NP Diet F2 -	AP Diet F2 +
Ingredient	%	%	%	%	%	%
Corn	42.76	43.65	44.49	45.70	44.58	45.49
Barley	5.00	5.00	9.20	9.20	10.00	10.00
Soya been meal	30.42	30.25	24.43	23.97	23.75	23.58
Rape seed meal	4.00	4.00	4.80	4.80	5.00	5.00
Vegetable oil	5.81	5.51	5.20	4.84	4.28	3.97
Limestone	9.56	9.56	9.56	9.63	10.20	10.25
Salt	0.26	0.26	0.27	0.27	0.22	0.22
Sodium bicarbonate	0.21	0.21	0.17	0.17	0.29	0.29
MCP	1.50	1.03	1.20	0.73	1.04	0.56
DL-Methionine 98%	0.10	0.10	0.13	0.13	0.10	0.10
Enzyme – Ronensime phytase 5000 (+ or -)	0.00	0.01	0.00	0.01	0.00	0.01
Betaine – Methionine	0.05	0.05	0.02	0.02	0.03	0.02
Cholin – Chloride	0.08	0.08	0.03	0.03	0.01	0.01
Vit.-Min. premix	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
ME KCal / kg	2740.00	2740.00	2750.00	2740.00	2700.00	2690.00
Dry matter	89.00	89.00	88.80	88.80	88.70	88.70

Humidity	11.00	11.00	11.20	11.20	11.30	11.30
Crude ash	13.87	13.44	13.51	13.07	14.00	13.58
Crude fat	7.48	7.20	6.94	6.60	6.04	5.76
Crude proteins	17.95	17.96	16.27	16.17	16.14	16.15
Crude fiber	4.20	4.22	4.18	4.19	4.21	4.23
Lysine	0.96	0.96	0.85	0.85	0.83	0.83
Methionine	0.44	0.44	0.39	0.39	0.39	0.39
Met + Cystine	0.75	0.75	0.68	0.68	0.68	0.68
Ca	3.80	3.80	3.80	3.80	4.00	4.00
P (total)	0.67	0.57	0.60	0.50	0.57	0.47
P available	0.39	0.30	0.33	0.25	0.30	0.21
P Phytase liberated	0.00	0.09	0.00	0.08	0.00	0.09
P avail + P Phytase liber	0.39	0.39	0.33	0.33	0.30	0.30
P organic	0.03	0.02	0.03	0.02	0.02	0.02

Diet F0 +, F1 + and F2 + = diets supplemented with Phytase & F0 -, F1 - and F2 - = diets without Phytase

Results and discussion

Two flocks performed quite similar in terms of daily laying percentage (81.02% vs 80.83%) proving that different diets have no significant effect on production performances (Figure 1). These findings are in-line with the previously published results (Ciftci et al, 2005; Liebert et al, 2005, Yildiz et al, 2010). Number of egg produced per hen housed was almost similar (297.96 vs 297.99) as well as the feed consumption (table 2).

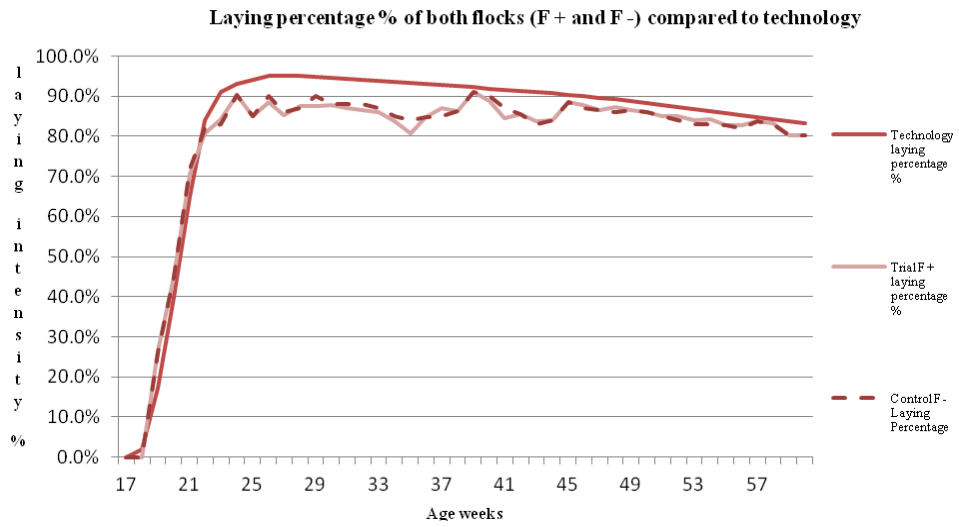

Figure 1. Egg production curves for both flocks

Table 2. Production parameters of two flocks

Production parameters	Control farmhouse F -	Phytase supplemented feed farmhouse F +
Feed consumption/hen housed	41.99	41.66
Number of egg produced/hen housed	297.96	297.99
Average production %	81.02	80.83

Egg quality parameters were not significantly influenced by different diets. Average egg size was 61.38g for NP (F-) group, while hens fed phytase supplemented F+ (AP) diet group, produced slightly smaller eggs (60.96g). This non significant difference in egg size was noticed through all feeding phases (F0, F1 and F2) and is comparable with the result published by Zaghari et al (2008). Shell strength of the two groups of layers at all three feeding phases were on average 3988.40 and 3871.13, 3861.68 and 3756.36, 3946.80 and 4041.60 for the first (>28 weeks of age), second (age 28-50 weeks), and third phase of production (>50 weeks of age) for both groups (without added phytase – NP and with added phytase – AP), respectively (table 3).

Table 3. Egg quality parameters

	Shell strength g/cm^2		Egg weight (g)		Yolk color (Roche 1-15)		Hough Units	
	Means	STD	Means	STD	Means	STD	Means	STD
Added Phytase First phase AP F0 +	3871.13	840.99	59.26	6.81	9.55	1.15	67.52	8.93
Added Phytase Second phase AP F1 +	3756.36	1028.88	62.44	8.53	8.94	0.70	74.46	7.68
Added Phytase Third phase AP F2 +	4041.60	692.78	61.16	7.91	9.25	0.47	69.68	7.32
No Phytase First phase NP F0 -	3988.40	892.23	59.82	8.70	10.79	1.21	64.03	7.90
No Phytase Second phase NP F1 -	3861.68	992.57	62.77	9.15	9.69	1.37	72.50	7.70
No Phytase Third phase NP F2 -	3946.80	832.66	61.78	3.00	11.99	0.65	64.50	6.17
Average for AP or F +	3851.05	897.19	60.96	7.78	9.23	0.91	70.88	8.63
Average for NP or F -	3928.67	913.13	61.38	8.25	10.53	1.45	67.64	8.52
Average for both groups	3890.52	902.30	61.17	7.99	9.89	1.38	69.23	8.69

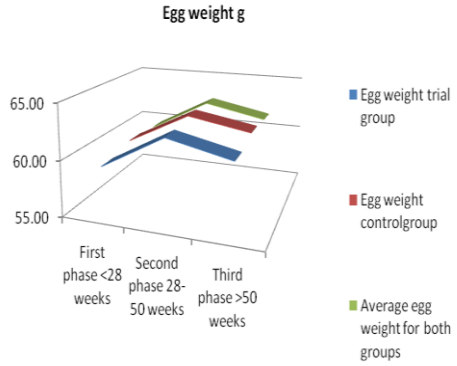

Figure 2. Egg weight data

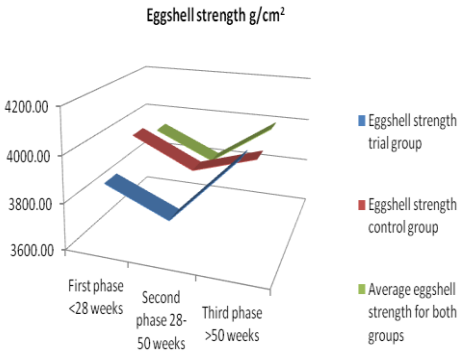

Figure 3. Eggshell strength data

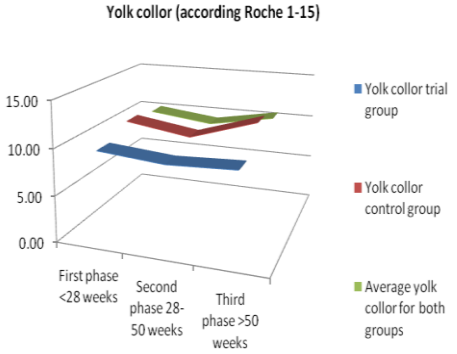

Figure 4. Eggs yolk color data

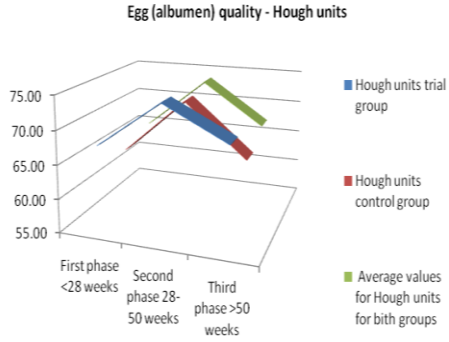

Figure 5. Egg albumen quality (Hough units) data

Our results for LSD test of the egg quality parameters, revealed no significant difference between F - and F + groups in egg weight (table 4) and shell strength (table 5) that is inline with the published results of Panda et al (2005a). Significant difference in the comparison between groups for the yolk color, presented in table 6, are hardly atributable to the effect of phytase but is probably effect of the added coloring agents in the vitamin mineral premix, at the stage of mixing the feed in the feed plant. LSD test of Hough units revealed significantly lower values at the feeding phase 1 (F1) in both diet groups compared to the other feeding phases (F0 and F2). This is most probably due to the seazonal effect (high summer temperatures) that coincidence with this feeding phase. Small, negative but non significant correlations were noticed between egg weight and egg shell strength (r=-0.090524).

Table 4. Egg weight LSD test between the groups

LSD Test; Variable: EGG_WEIG (fitaza.sta)						
Marked differences are significant at p < .05000						
	M=59.256	M=62.436	M=61.160	M=59.824	M=62.772	M=61.780
AP F0		0.174	0.533	0.807	0.133	0.409
AP F1			0.672	0.253	0.883	0.828
AP F2				0.658	0.593	0.863
NP F0					0.197	0.517
NP F1						0.742
NP F2						

Table 5. Eggshell strength LSD test between the groups

LSD Test; Variable: SHELLSTR (fitaza.sta)						
Marked differences are significant at p < .05000						
	M=3871.1	M=3756.4	M=4041.6	M=3988.4	M=3861.7	M=3946.8
AP F0		0.666	0.625	0.659	0.972	0.828
AP F1			0.408	0.373	0.686	0.580
AP F2				0.877	0.601	0.818
NP F0					0.626	0.904
NP F1						0.805
NP F2						

Table 6. Yolk color LSD test between the groups

LSD Test; Variable: YOLK_COL (fitaza.sta)						
*Marked differences are significant at p < .05000						
	M= 9.5522	M= 8.9360	M= 9.2500	M= 10.792	M= 9.6920	M= 11.990
AP F0		0.048*	0.455	0.000*	0.650	0.000*
AP F1			0.432	0.000*	0.013*	0.000*
AP F2				0.000*	0.270	0.000*
NP F0					0.000*	0.003*
NP F1						0.000*
NP F2						

Table 7. Hough Units LSD test between the groups

LSD Test; Variable: HOUGH_UN (fitaza.sta)						
*Marked differences are significant at p < .05000						
	M=67.522	M=74.456	M=69.680	M=64.026	M=72.500	M=64.500
AP F0		0.003*	0.470	0.126	0.030*	0.312
AP F1			0.107	0.000*	0.381	0.001*
AP F2				0.057	0.340	0.143
NP F0					0.000*	0.872
NP F1						0.008*
NP F2						

Conclusions

Feeding layers with lower level of NPP has no detrimental effects on egg production and egg quality parameters if the diet is supplemented with exogenous phytase enzyme. Supplementation of phytase enables the hens to use P from the grains therefore allows decreased NPP (inorganic sources of P, in this case MCP) levels for at least 0.5%. Additional effect of such feeding technology approach is reduced feed costs due to lower production costs but also due to lower feed consumption or better feed conversion.

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ПРОИЗВОДСТВО НА ЈАЈЦА И КВАЛИТЕТ НА ЈАЈЦА ОД НЕСИЛКИ ХРАНЕТИ ПРИ ДИЕТА СО И БЕЗ ДОДАДЕНА ФИТАЗА

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Апстракт

Балансирање на хранителните материи (посебно Са и Р како есенцијални макро минерали одговорни за високата продукција на јајца и квалитетот на лушпата кај несилките), во смеските за несилки селекционирани на мала жива маса, но голема маса на произведените јајца, е уметност. Производните резултати во текот на целиот циклус (број на јајца, интензитет на несивост, консумација на храна) и параметрите за квалитетот на јајцата (големина на јајцата, цврстина на лушпата, боја на жолтокот и Hough-ови единици) беа споредувани кај две групи на ISA Brown несилки без додавање (НП) или со додавање (АП) ензим фитаза на ниво д 0.01%. Смеската на опитната група со додадена фитаза, содржеше намалено количество (во просек 0.5%) од суровината Моно Калциум Фосфат (МКФ) како извор на НеФитински Фосфор (НФФ). Не беа забележани сигнификантни разлики во интензитетот на несивост (81.02% vs 80.83%) и бројот на произведени јајца по вселена несилка (297.96 vs 297.99). Анализите покажаа несигнификантни разлики помеѓу групите во големината на јајцата и цврстината на лушпата (60.96g vs 61.38g; 3851.05 vs 3928.67 g/cm^2). Слаба сигнификантна разлика, која не може да се припише на додавањето на фитаза, во бојата на жолтокот (9.23 vs 10.53) и Hough-овите единици (70.88 vs 67.64) е монитирана кај опитната и контролната група респективно. Кореалциите помеѓу големината на јајцата и цврстината на лушпата беа несигнификантни. Модификацијата на составот на смеската преку намалување на НФФ и додавање на фитаза е вреден пристап кој ги намалува трошоците за исхрана без да влијае на бројот, големината и квалитетот на произведените јајца.

Клучни зборови: живина, исхрана, фософор, фитаза, јајцепроизводство, квалитет на јајце.

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COMPARING PRODUCTION DATA OF LAYERS UNDER COVENTIONAL AND RESTRICTIVE FEEDING PROGRAM

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Abstract
Experiment was conducted to compare *ad libitum* feeding and quantitative feed restriction technique applied on egg producing ISA Brown layers. Pullets of same age were placed in two farm houses under similar environmental conditions and fed *ad libitum* until the age of 40 weeks. One of the flocks was put under gradual decline of daily feed allowance (controlled quantity of daily feed consumed), starting from 40 weeks of age onward, while the other flock remain on the usual *ad libitum* feeding technique. Both flocks were kept until the end of the exploitation period (80 weeks of age). Nutrient composition of the feed was defined according the recommended requirements and age of the layers. Records of the production (number and size of the eggs), mortality, feed consumption, feed conversion and egg quality parameters were collected during the experiment. Feed consumption of layers under restrictive feeding program was 41.85kg and of the full fed ones 42,87kg of feed (based on the hen housed number). This means 1kg of saving in terms of feed or roughly 0.3EUR/bird/cycle. Expressed in terms of standard 100 000 layer farm it is 30000EUR/cycle. Daily feed consumption was 113.12g of daily feed consumption for the trial flock and 117.39g in control group of layers. Laying percentage was not significantly different in control and trial flock (81.59 vs 80.55%). Feed conversion rate was 2.355 vs 2.299 kg feed/kg egg mass or expressed per egg 143.87g vs 140.44g feed/egg in the control (*ad libitum*) and restrictive feeding group, respectively. Egg size was same for both groups (61.08g vs 61.08g egg size). No differences were noticed in the egg quality parameters (Hough Units, eggshell strength, yolk color) between both groups. Restrictive feeding applied on layers at age of 40 weeks lead to lower feed consumption, lower production costs and has no negative effect on the egg production (egg number, size and quality).
Key words: poultry, restrictive feeding, egg production, egg quality.

Introduction

Modern layers are genetically sized toward smaller body weight but higher egg mass produced under optimal management and feeding conditions. Feed consumption is affected

by feed quality, temperature, age and level of egg production and many other variables. But it is interrelated and bird driven only if full-fed programs are applied. Even if there are thoughts that some restriction could be applied in order to control the level of daily feed consumption by the manager, additional efforts in this direction is lacking. It is due to need of additional investments in equipment, know how e.t.c. but there are also easily managed approaches that does not need many extra efforts and still offered some gain in terms of profitability. Poultry breeders know well that broiler breeders are subjected to restrictive feeding program that, if properly applied, does not influence the production and on the contrary influence the efficiency thus profitability. Overconsumption of feed under the full-feeding protocols always results in a excessive body fat buildup even in a layer genotypes negatively influencing feed conversion rates without benefit for the egg producers. Today's high feed price makes such restrictive feeding techniques actual to be applied in egg producing farms. Restrictions of 12 to13% in feed quantity were suggested by Swanson and Johnston (1975). They claim that this is possible at early age before peak of production, without negative effects on egg production records and with substantial economic savings. Kuney and Swanson (1979) reported possibilities for applying restriction at later stage (after 40 weeks of age) but they mentioned the importance that this restriction should be introduced gradually to reduce the stress of restriction.

The effect of restrictions on the levels of 11% and 8% during the first laying cycle and 12% and 10% during the second cycle were analyzed by Kuney and Enos (1980) in comparison with *ad libitum*-fed layers. In their reports restrictive feeding programs significantly improved feed efficiency escorted with non significant depressions laying rate and increased shell thickness. Quantitatively restriction (feeding 105 g/bird/day) initiated at different stages of production on performance and economic returns of White Leghorn layers were analyzed by Cunningham and Polte (1984). Conclusions were that that early feed restriction resulted in reduced egg production and size while restriction started at age of 38 and 45 weeks lead to egg production comparable to the *ad libitum* fed hens and lower feed costs.

In the conducted experiments Cunningham (1984) concluded that if start of restriction is at age of 36 weeks and is not severe, but rather offers 105g/bird/day, production results are comparable full feeding program offering less fed usage and improved profitability, demonstrating that maximum production levels do not always mean maximum returns.

Feed restriction programs applied in the experiments of Miles and Jacqueline (2000), resulted in slight decrease egg size, but support the theory that feed restrictions should be applied at later stages of production when majority of the eggs being produced fall into the large size category.

Material and methods

Basic idea of the trial lies in, maybe, the forgotten technology of restrictive feeding of layers where total input of feed (as un approach for reducing the costs of production) is reduced and as a consequence lower feed costs are made, but production results are maintained at the satisfactory level. Such approach logically leads to reduced feed inputs, lower levels of manure produced, but having in mind the genetically high egg producing predisposition of the modern laying genotypes, keeping the production records on restrictive amount of daily feed allowances is a challenge. Such restriction feeding

techniques are usual for broiler breeders and are seldom practiced on layer stocks. According the published records, there are different opinions about the age of the layers when this feed restriction should start and all of them have positive and negative sides.

The age of 40 weeks is the age when the birds gave most of their genetic potential of egg production in terms of reaching high peak (92-3-4% intensity of egg production at age of 28-30 weeks) and also sufficiently long period (10-12 weeks) of producing over 90%. At this age birds start to have a tendency of producing oversized eggs (and this happened especially at the latest stages of production when their body size reaches maximum – leading to maximal daily consumptions, lower intensity of egg production, but high daily egg mass output due to the size of the eggs) under *ad libitum* feeding technique. This period of egg production usually compensate for the smaller egg size at the beginning of the laying period therefore contributing to the average feed to egg mass conversion rate figures. Unfortunately these oversized eggs are very often escorted with poor shell quality leading to breakage, leakage and distribution problems, thus, failing compensate with extraordinary profit from premium price. Since egg number and size are partly genetically determined characteristic, it could be modified through feed management techniques. Usual approach of keeping the size of the eggs at these latter stages of production at desired level is through lowering the feed density but then it rises up the feed conversion ration and in terms of money it is a game of costs vs returns.

Based on all previously mentioned assumptions, comparative trial with two flocks of ISA Brown pullets that were housed in two farm houses at age of 16 weeks 16818 pullets in control farm house and 17587 in the trial farm house) is conducted.

Birds were fed formulas based on the recommended nutrient levels (table 1) and managed (lighting) appropriate to the age and weight, following the management manuals. The only difference was restrictive feeding program applied to the trial flock after the age of 40 weeks. Basically, daily feed allowances were recalculated, and defined amount of feed was delivered to the layers after the age of 40 weeks. This means that two flocks were kept under same condition (*ad libitum* feeding technique) at the beginning of laying period till age of 40 weeks. After this age the trial farm house flock was subjected to gradual feed restrictions through limited daily feed consumption. Control of the daily allowance of feed (Diet F1-up to 50 weeks of age and F2 – >50weeks) was performed calculating the final desired feed consumed (Table 2).

The technology of restrictive feeding applied in the trial suppose gradual introducing of 115g daily feed allowance per bird in the period of 14 days after the age of 40 weeks, than slowly reducing it to 11-112g/bird/day. These amounts are only calculative figures and the exact amounts were calculated on the spot before the beginning of the trail feeding program depending on the history of feed spent / bird housed up to the age of 40 weeks (this should be on the level of 17.5kg/bird housed). Beside this basic assumption for the calculation of the daily feed allowance for the period after week 40, lies in the feed consumption goal of reaching 48kg feed/ bird housed at age of 80 weeks.

Table 1. Nutrient composition of different diets (phase feeding) used in the experiment

	Diet F0	Diet F1	Diet F2
Ingredient	%	%	%
Corn	43.00	44.55	44.07
Barley	5.00	9.00	11.00
Soya been meal	34.15	29.61	28.65
Vegetable oil	5.70	5.00	4.00
Limestone	9.50	9.53	10.00
Salt	0.25	0.28	0.24
Sodium bicarbonate	0.22	0.17	0.29
MCP	1.46	1.21	1.11
DL-Methionine 98%	0.10	0.10	0.10
Betaine – Methionine	0.04	0.02	0.03
Cholin – Chloride	0.08	0.03	0.01
Vit.-Min. premix	0.50	0.50	0.50
Total	100.00	100.00	100.00
ME KCal / kg	2750.00	2750.00	2720.00
Dry matter	88.90	88.90	88.80
Humidity	11.10	11.10	11.20
Crude ash	13.55	13.50	13.89
Crude fat	7.48	7.01	6.01
Crude proteins	17.99	16.45	16.01
Crude fiber	4.10	4.10	4.20
Lysine	0.95	0.85	0.83
Methionine	0.45	0.40	0.39
Met + Cystine	0.75	0.67	0.67
Ca	3.77	3.81	3.98
P (total)	0.68	0.61	0.60
P available	0.40	0.35	0.33

Based on all previously mentioned assumption and figures, definite quantity of feed to be spent in the period after 40 weeks is calculated and daily feed allowance derivates out of this total figure, as it is presented in the table below (table 2). If the feed spent by the week 40 is higher than 17.4kg stated in table 2, then the amount that should be delivered to the birds after this age should be proportionally decreased.

Actual goal was to have same production % (laying intensity) in both farm houses (control and trial) keeping the size of the eggs at the same level that in overall will lead to better feed/egg mass conversion ration in favour of trial group. Egg production and egg quality parameters were analyzed to compare control feeding technique with *ad libitum* feeding technique.

Table 2. Example for calculation of the restrictive (control) feeding technique

Number of hen housed	18 000		
Age (period)	>40 weeks	40-80 weeks	18-80 weeks
Total feed consumption for the period (kg)	17.4	30.6	48
Daily consumption (g)	112.9	109.3	111
Total quantity of feed needed (kg)	313 200	550 800	864 000
Weekly quantity of feed needed (kg)	14 236	13 770	13 936
Daily quantity of feed needed (kg)	2 034	1 968	1 990
Mortality up to 40 weeks (3%)	540		
Number of remained hens at age 40 weeks		17 460	
Daily feed consumption (g)		(1968 / 17460) = 112.7	

Results and discussion

Both group of layers performed quite well and close to the technological levels presented in the ISA BROWN manuals (graph 1) during the period of investigation (409 days). In general no significant differences were noticed in comparison of the production parameters in the control (*ad libitum*) and restrictive feeding group. Average laying percentage was 81.59 for the control and 80.55% for the trial group of layers (table 3) and only high mortality rates (graph 2) during the extremely hot summer days were noticed as a problem, but affects both flocks. It could be noticed that rate of mortality was even slightly lower in the trial than in the control group probably due to lower body weight and less body fats.

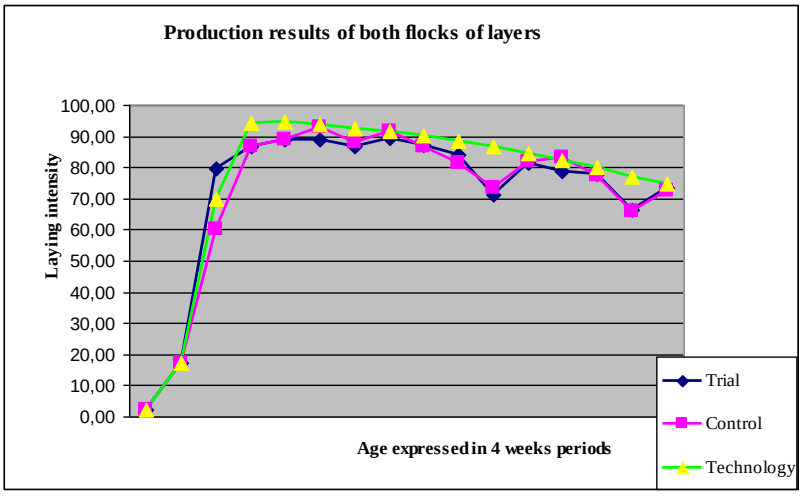

Figure 1. Egg production curves for both flocks

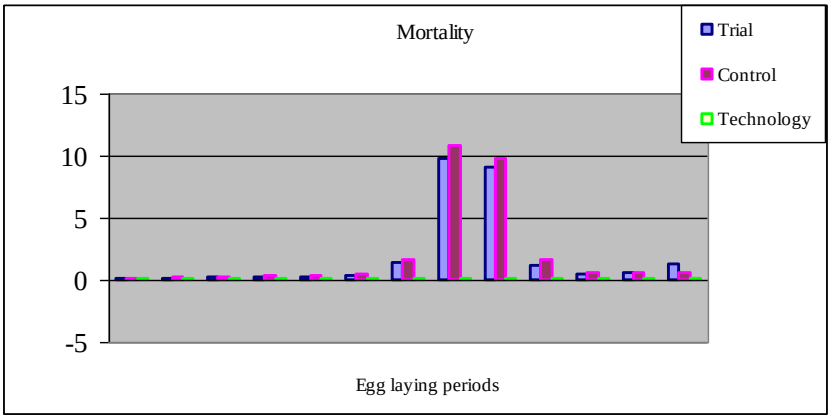

Figure 2. Mortality rates for both flocks

As could be noticed from the records of production for both flocks (table 3), full feeding has no advantage over restrictive (controlled amount of daily feed allowance) feeding technique in terms of production data. Number of eggs and kilograms of egg mass produced per hen housed are equal. Careful monitoring of production results revealed that control feeding techniques has some advantages over full feeding approach.

Feed spent per average hen and hen housed in the control (*ad libitum* fed) group is higher compared to the trial group of hens and this is directly attributable to the applied restrictive feeding technique. What is the most important this higher amount of feed consumed did not reflect the number of eggs produced because it was same in the control and trial flock.

Feed conversion is also one of the crucial production parameters. Expressed in terms if feed conversion rate trial group was superior to control one (2.299 vs 2.355) resulting in less feed per egg spent, therefore lower cost price of the eggs. Figures for amount of feed spent per produced egg are in favour of trial group. Namely, control flock spent 143.87g feed per produced egg, that is more than the feed spent per egg produced in the trial group (140.44g) meaning that the trial group spent 3.4g less feed compared to the hens of control group. These fact leads to better financial results of the layer farm activity if restrictive feeding technique is applied.

Size of the eggs produced influences all important productivity and profitability parameters. Number of egg and size of the produced eggs in the control and trial flock are presented in the table 4 below. These egg size records, followed in both groups, revealed that actually no difference in the average egg size were found between both groups (61.08g vs 61.08g). Only notification for the egg size is that 0.5% less L sized eggs, 0.7% more M sized eggs and 0.1% less S sized eggs were produced in the trial group similar like in the experiment of Miles and Jacqueline (2000). Additionally, less dirty and broken eggs were monitored in this group. No significant differences were monitored in the egg quality parameters presented in the table 5.

Lower feed consumption can be realized through proper and sound management of feed quantity on a daily basis. Feed consumption records presented in table 3 the showed that layers under restrictive feeding program consumed 41.85kg and the full fed ones 42,87kg of feed (based on the hen housed number). This means 1kg of saving in terms of feed or

roughly 0.3EUR/bird/cycle. Expressed in terms of standard 100 000 layer farm it is 30000EUR/cycle. This is a final result of lower daily feed consumption. These figures in our experiment were 113.12g of daily feed consumption for the trial flock and 117.39g in control group of layers.

Results from our study (comparable results between restricted and full feeding programs) resemble the results reported in the study of Cunningham and Polte, 1984 where restriction was started at age between 38 and 45 weeks. In their study just like in our case trial (restrictive fed hens) group consumed smaller quantity of feed and produced smaller (non significantly) egg size due to production of higher number of medium sized eggs.

Table 3. Production parameters of two flocks

Production parameters	Control flock	Trial flock
Avrage number of layers in the trial period	15016	15986
Total Quantity of feed consumed	720950kg	735990 kg
Quantity of feed spent / average number of layers	48kg	46.01 kg
Quantity of feed spent / housed number of layers	42.87	41.85
Average daily feed consumption (g)	117.39	113.12
Number of eggs / average hen	333.70	327.83
Number of eggs / hen housed	301.66	300.89
Kg eggmass / average hen	20.38	20.03
Kg eggmass / hen housed	18.20	18.20
Feed conversion - kg feed / kg egg mass	2.355	2.299
Average egg weight (g)	61.08	61.08
Average laying intensity %	81.59	80.55
Feed spent (g) / egg	143.87	140.44

Table 4. Number and size of the produced eggs

Egg size	Number of eggs		%	
	Control flock	Trial flock	Control flock	Trial flock
XL	232240	243180	4,63%	4,64%
L	2312770	2396630	46,15%	45,73%
M	1748290	1865770	34,89%	35,60%
S	144720	145800	2,89%	2,78%
DIRTY	497670	516600	9,93%	9,86%
BROKEN	75350	72720	1,50%	1,39%
TOTAL	5011040	5240700	100.00%	100.00%

Table 5. Egg quality parameters

Egg quality	Control flock	Trial flock
Average eggshell strength value	3897.00	3835.00
Average yolk color value (according LaRoche)	8.9	10.40
Average Hough Units value	71.80	71.20
Average weight of the analyzed eggs	60.20	60.15

Our data confirms that gradually applied restrictive feeding programs reduce the drop of production due to the stress at point of starting with changed (restrictive) feeding program and were in agreement with the findings of Kuney and Swanson 1979, even that in their experiment time limited approach in restriction was applied.

Recorded data in our trial confirms that restrictive feeding technique is appropriate management tool for situations when high feed price exist. Lower feed consumption, lower production costs, less environmental pollution without affecting the productivity parameters are the outcomes of applying this feeding technique. Equal number of eggs and equal average egg size (61.08) in both groups both groups support this fact.

Conclusions

Production parameters are not affected in the layer flock subjected to restrictive feeding program after the age of 40 weeks, if the restrictions are reasonable, sound and gradually applied. Restrictive feeding technique is efficient tool for improving the efficiency of egg production in terms of feed conversion ratio, thus leading to lower feed costs and improved profitability, especially in the upcoming periods of high world market grain, ingredients and feed prices. It should be considered every time when egg producers are facing high feed prices because it helps maintaining the profitability of the industry to desired level. Based on obtained results of this trial, such feeding technique is approved to be used after 40 weeks of age in layers.

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СПОРЕДБА НА ПРОИЗВОДНИТЕ РЕЗУЛТАТИ КАЈ НЕСИЛКИ ПРИ КОНВЕНЦИОНАЛНА И РЕСТРИКТИВНА ИСХРАНА

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Апстракт

Експеримент е организиран за да се спореди *ad libitum* техниката и техниката на квантитативна рестриктивна исхрана применета на јајценосните ISA Brown несилки. Јарки на иста возраст се сместени во два објекта во слични услови на одгледување и хранети *ad libitum* до возраст од 40 недели. Едното јато е ставено на постепено намалување на дневната дажба (контролирано количество на дневно консумираната храна) по навршување на 40 недели возраст, додека другото јато е оставено на вообичаената *ad libitum* технологија на исхрана. Обете јата се одгледувани на овој начин се до крајот на експлоатациониот период (80 неделна возраст). Нутитивниот состав на смеските е дефиниран во согласност со препорачаните потреби и возраста на несилките. Записи за производството (број и големина на јајца), морталитет, консумација на храна, конверзија на храна и параметрите за квалитетот на јајцата се собирани за време на експериментот. Консумацијата на храна на несилките каде е применета рестриктивна исхрана изнесуваше 41.85kg а кај оние кои беа хранети по волја 42,87kg храна (изразено врз база на бројот на вселени несилки). Ова значи 1kg заштеда во храна или околу 0.3EUR/птица/циклус. Изразено во термини на стандардна фарма со големина од 100000 несилки истото изнесува 30000EUR/циклус. Дневната консумација изнесуваше 113.12g за опитната и 117.39g за контролната група на несилки. Интензитетот на несивост не се разликуваше сигнификантно во контролното и опитното јато (81.59 vs 80.55%). Конверзијата на храната беше 2.355 vs 2.299 kg храна/kg јајчена маса или изразено по произведено јајце 143.87g vs 140.44g храна/произведено јајце кај контролната (*ad libitum*) група и групата на која е применета рестриктивна исхрана, соответно. Не се забележани разлики во параметрите за квалитет на јајцето (Хофови единици, цврстината на лушпата, бојата на жолтокот) помеѓу групите. Рестриктивната исхрана применета на несилки на возраст од 40 недели резултираше со намалена консумација на храна, намалени производни трошоци без негативно да влијае на производните параметри (број, големина и квалитет на јајцата).

Клучни зборови: живина, рестриктивна исхрана, јајцепроизводство, квалитет на јајца.

UDC: 636.32/.38:575.22(497)
Early communication

POLYMORPHISMS IN OVINE αS_1-CASEIN GENE AMONG AUTOCHTHON STRAINS OF PRAMENKA BREED SHEEP IN BALKAN

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Abstract
Alfa s_1 casein represents the main milk protein fraction which has an important role in the transport calcium phosphate in milk . There are four alleles (A, B, C and D) present in the α_{s1}-casein gene. Polymorphisms were detected within ovine α_{s1}-casein gene using PCR – RFLP. One hundred seventeen DNA samples were isolated from 6 different strains of Pramenka sheep from different parts of the Balkan Peninsula: Karakachanka (Macedonia), Svrljiska (Serbia), Bardoka (Kosovo), Istarska (Croatia), Dubska (Bosnia and Herzegovina) and Pivska (Montenegro). Three distinct patterns (designated nonA/nonA, nonA/A and A/A) were observed. Allelic frequencies were 0.08/0.92 A/nonA. No polymorphisms were observed in Bardoka and Istarska strains and all animals carried the nonA/nonA genotype. The highest diversity, related to α_{s1}-casein gene, were in the Karakachanka strain where 7 animals had nonA/A genotype, while 2 carried genotype A/A.
Key words: sheep, Pramenka, α_{s1}casein, polymorphisms.

Introduction

α_{s1} casein, composed of 215 amino acids is the main milk protein fraction. It has an important role in the transport of calcium phosphate. There are four alleles (A, B, C and D) present in the α_{s1} casein gene. In domesticated ruminants α_{s1} casein is polymorphic and the variants are related to the milk yield and quality (1). The aim of this study was to determine the polymorphisms in ovine α_{s1} casein gene present six autochthonic strains of Pramenika breed on the Balkan Peninsula which is most economically important sheep breed in this region. Polymorphisms were detected using Polymerase Chain Reaction – Restriction Fragment Length Polymorphisms (PCR – RFLP) by digestion with Mbo II restriction enzyme (2).

Material and methods

One hundred seventeen DNA samples were taken from 6 different strains of Pramenka sheep breed from different parts of the Balkan Peninsula as follow: Karakachanka from Macedonia (20 samples), Svrljiska from Serbia (20 samples), Bardoka from Kosovo (19 samples), Istarska from Croatia (19 samples), Dubska from Bosnia and Herzegovina (20 samples) and Pivska from Montenegro (19 samples) (3). Genomic DNA was extracted from blood samples using the method reported by somebody (yr). Briefly, which include Proteinase K which proteolysis DNase causes cell lyses, phenol-chloroform purification from proteins and alcohol precipitation. The region of interest from ovine α_{s1} casein -casein gene where the polymorphisms occur was amplified.

The PCR reaction mixture for amplification of the part from the ovine α_{s1} casein - gene. Briefly, 25 µl containing: 200 ng of genomic DNA, 1x PCR reaction buffer, 2.5 mM $MgCl_2$, primers: α_{S1}-F GGTGTCAAATTTAGCTGTTAAA and αS_1-R GCCCTCTTCTCTAAAAAGGTTT in final concentration of 1 pM and 2 U of Taq DNA polymerase. The conditions for amplification were: 3 minutes 95^0C (hot start), and 35 cycles of: denaturation at 95^oC for 30 sec, annealing at 55^oC for 30 sec and amplification at 72^oC for 45 sec.

The final elongation was performed at 72^oC for 10 minutes. The amplified products were checked on 1.5 % agarose gel electrophoresis (AGE). The digestion of amplified DNA was performed using Mbo II restriction enzyme in a mixture with total volume of 25 µl which contained: 1x Restriction buffer, 15 µl of amplified DNA and 5 U of RE. Restriction fragments were analyzed by 3% agarose TBE gel electrophoresis.

Results and discussion

The amplified fragment of interest from alfaS1 gene was 372 bp in length. (Fig. 1). Three distinct patterns (nonA/nonA, nonA/A and A/A) were observed in the PCR-RFLP analysis of ovine αS_1-casein gene (Fig. 2). The nonA allele exhibited two fragments at 306 bp and 66 bp as expected based on known sequences (2). In addition to 66 bp fragment, the A allele created bands of 160-bp and 146 bp which slightly interfere between them. NGenotype frequencies of 117 sheep samples from 6 different strains of Pramenka sheep were 0.02 for A/A, 0.12 for nonA/A and 0.86 for nonA/nonA. Allele frequencies among estimated DNA samples were in ratio 0.08/0.92 A/nonA.

In the Bardoka and Istarska strains, no polymorphisms were observed and all animals were heterozygous for the nonA genotype. Only one animal exhibited a DNA sample with the nonA/A genotype among the Svrljsika and Dubska strains. The Pivska strain exhibited five nonA/A genotypes. The highest diversity in the αS_1-casein gene was present in the Karakachanka strain where 7 animals had nonA/A genotypes, while 2 carried the A/A genotype. These observations are partially consistent with the published data related to the genetic diversity among the reported strains of Pramenka breed determined by microsatellite and mitochondrial DNA analysis (3,4). It those studies it was reported that the Karakacanka strain formed a genetically divergent population and has a largest deviation in the frame of Pramenka breed based on DNA microsatellites analyses (3,4).

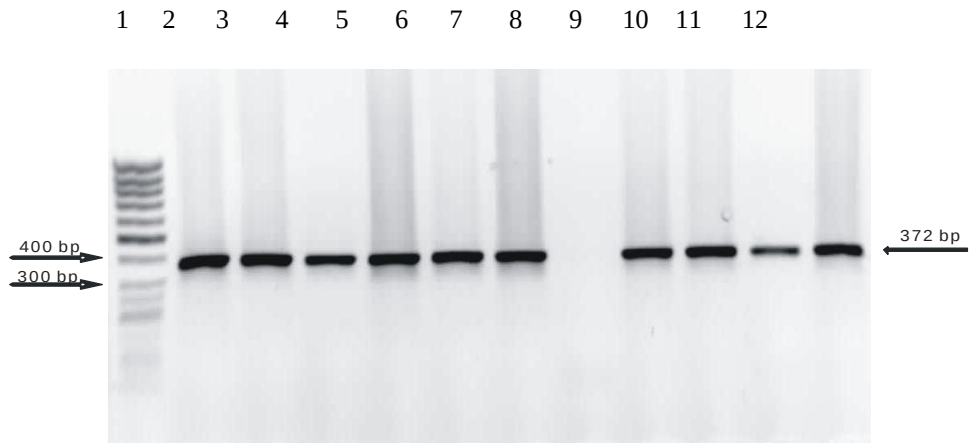

Figure 1. 1.5% AGE of PCR fragment (372 bp), line #1 – DNA ladder, lane #2-7 and 9-12 - samples; line #8 – negative control.

Three distinct patterns (nonA/nonA, nonA/A and A/A) were observed in the PCR-RFLP analysis of ovine αS_1-casein gene (Fig. 2).

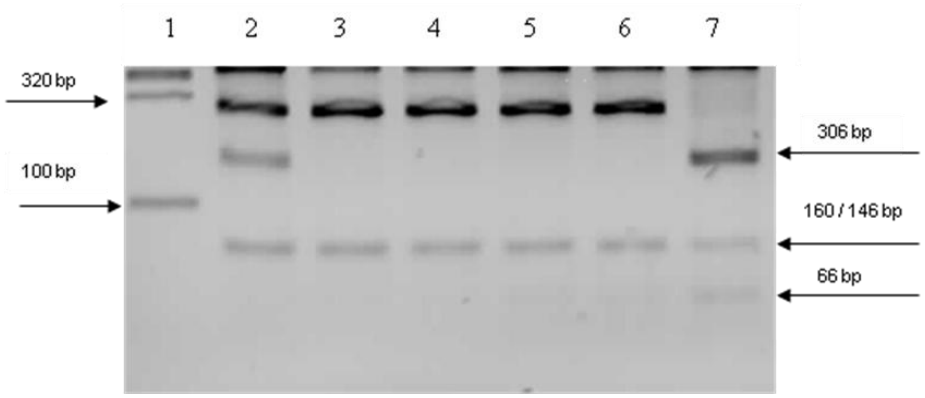

Figure 2. 3% AGE of *Mbo II* digested PCR products: line #1 – DNA ladder; line #2 - A/nonA, lines #3-6,– nonA/nonA; line #7- A/A.

Conclusions

The heterogeneity of Karakachanka strains in terms of DNA microsatellites, mitochondrial DNA and polymorphisms of αS1 casein gene compared to other strains of Pramenka sheep is likely due its geographical isolation and because it is a very small population it is subject to the effects of inbreeding.

Acknowledgement

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ПОЛИМОРФИЗАМ КАЈ ОВЧИОТ αS_1 КАЗЕИН ГЕН МЕЃУ АВТОХТОНИТЕ СОЕВИ НА ПРАМЕНКА ВО БАЛКАНОТ

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Апстракт

Алфа S 1 казеинот ја претставува најважната фракција од млечните протеини кој има улога во преносот на калциум фофатот во млекото. Досега се откриени 4 алели на генот за алфа S1 казеин. (A, B, C и D). Зполиморфизмите беа детектирани со PCR-RFLP. Сто и седумнаесет примероци на ДНК беа изолирани од 6 соеви на расата праменка од различни краишта на Балканскиот полуостров и тоаЧ Каракачанка (Македонија), Сврљишка (Србија), бардока (Косово), Истарска (Хрватска), Дубска (Босна и Херцеговина) и Пивска (Црна Гора). Три различни случаи се идентификувани и обележени како nonA/nonA, nonA/A и A/A. Алелната фреквенција изнесуваше 0.08/0.92 за A/nonA. Кај Истарскиот и бардока сојот не се откриени полиморфизми и сите животни се носители на генотипот nonA/nonA. Највисока диверзификација поврзана за генот за алфа S1 казеин е утврдена кај Каракачанскиот сој каде седум животни имаат nonA/A генотип, а 2 животни се носители на генотип A/A.

Клучни зборови: овци, праменка, алфа S1 казеин, полиморфизми.

UDC: 597.551.2-111.11:543-545.2
Early communication

ELECTROPHORETIC DETERMINATION OF FEMALE SPECIFIC PROTEIN IN KOI CARP AS A TOOL FOR GENDER IDENTIFICATION: TECHNIQUE COMPARISON

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Abstract
The development of plasma biomarkers has proven to be more challenging than initially anticipated. In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. Gender identification among fish species based on morphological features is very difficult and therefore the electrophoresis of plasma proteins can be used as a useful molecular tool. In this survey, native polyacrylamide gel electrophoresis (Native-PAGE) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were applied to analyze plasma proteins of koi carp (*Cyprinus carpio haematopterus*). The electrophoregrams showed that using SDS-PAGE as a technique is possible to discriminate male from female samples based on the presence of female specific protein (FSP) which can be used as a suitable marker. The Native-PAGE is not showing differences between male and female plasma samples. In the electrophoregrams obtained from SDS-PAGE, the molecular weight of FSH is approximately 143 kD and it is in according with pervious findings from the studies of different fish species.
Key words: koi carp, seum proteins, female specific protein, PAGE.

Introduction

Different methods have been successfully developed and used to collect diverse types of information associated with proteins. This is especially the case for the identification and quantification of proteins in complex samples, such as plasma. Numerous studies have demonstrated the impact of proteomics on answering key biological questions; especially those that help us understand vital functions of a living system. (Surinova *et al.,* 2011). Plasma proteins are kind of mirror on the physiological status in the organism.

Mulcahy, (1967) described changes in the plasma proteins of salmon, which were correlated with infection by Ulcerative Dermal Necrosis (UDN). The plasma electrophoretic patterns in health and UDN, differed quantitatively in the relative amounts of protein in the major protein fractions, and qualitatively in the occurrence of new fractions in some of the diseased fish. It provides the basis for a diagnostic method of UDN outbreaks.

In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. Currently, electrophoresis of sarcoplasmic proteins, plasma proteins, liver proteins and a number of enzymes often have been used by some researchers as an aid in the species or gender identification of fish (Pinerio *et al.*, 2001).
Despite the fish being of commercial significance and an interesting specimen for academic studies as well, limited studies exist on aspects of physiological changes associated with reproductive cycle and gametogenesis. Kumar Lau *et al.*(1999) presented eletrophoretetic profile of plasma proteins at different stages of maturation and attempts to trace the presence of female specific protein or vitellogenin. This study intends to show that the plasma proteins of same fish species can show electrophoreticaly different pattern related to gender.

Material and methods

Twenty-one koi carp *(Cyprinus carpio haematopterus)*, with a mass between 190 and 1200 g, were obtained from an experimental fishery at the Graduate school of environmental sciences at Ohio State University, Columbus USA. The blood was taken from dorsal aorta of live individuals. The blood samples were centrifugated at 1500 rpm for 10 minutes to separate the plasma. The obtained plasma was used for electrophoretical analysis of proteins.
SDS-PAGE was performed according to Laemmli (1970) method. Plasma proteins were separated on 16 x 12 cm and a 1 mm thick gel. The gel consisted of 3% stacking part on which proteins were stocked and a 12.5% running part on which proteins were separated. Each plasma sample was mixed with a buffer composed of 10% glycerol, 2% mercaptoethanol, 2% SDS and 0.01 bromphenol blue. Protein concentrations were adjusted to 2 μg/μl and 20 μl of samples were loaded on the stacking gel. The electric power of 300 Volts was used until the line of brome phenol blue came to the lowest part of the gel. The duration of electrophoresis was around 3 hours. After the electrophoresis, the proteins were stained with 0.04% Commassie Brilliant Blue R-250 in 40% ethanol and 5% acetic acid, and then distained in 10% acetic acid. Middle range protein ladder from 29 – 205 kD was used as a standard during the electrophoresis.
Native PAGE was done using the procedure established by Efremov *et al.* (1981). Plasma proteins were analyzed on gel with the dimensions of 16 cm x 12 cm x 0.1 cm using 12% acrylamide in 6 M urea. Each plasma sample was mixed with a buffer composed of 20% sucrose, 10% mercaptoethanol, 10% acetic acid and 0.01 brome- phenol blue. Protein concentrations were adjusted to 2 μg/μl and 20 μl of samples were loaded on the stacking gel. During the electrophoresis the electric power of 300 Volts was used for 5 hours.. After the electrophoresis, the proteins were stained with 0.04% Commassie Brilliant Blue R-250 in 40% ethanol and 5% acetic acid, and then distained in 10% acetic acid. As a comparative samples were used plasma samples from bassfish, yellow perch and bovine plasma sample.

Results and discussion

In the Figure 1 and 2 are shown the electrophoregrams from SDS-PAGE and Native PAGE of fish plasma proteins.

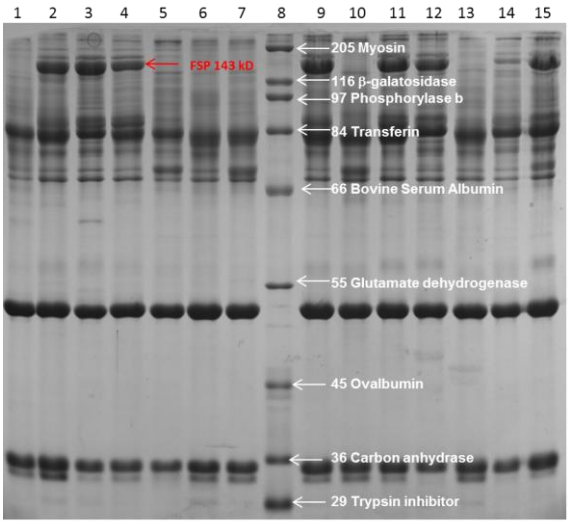

Figure 1. 12.5% SDS-PAGE on plasma proteins from 14 samples of koi carp. #1 male/827 g, #2 female /586 g, #3 female/697 g, #4 female/734 g, #5 male/502 g, , #6 male/294 g, #7 male/635 g, #8 Broad Range Standard (myosin 205 kD, galatosidase 116 kD, phosphorilase b 97 kD, transferrin 84 kD, bovine plasma albumin 66 kD, glutamate dehydrogenase 55 kD, ovalbumin 45 kD, carbon anhydrase 36 kD and trypsin inhibitor 29 kD) #9 female/663 g, #10 male/1200 g, #11 female/747 g, #12 female/750 g, #13 male/194 g, #14 male/325 g, and #15 female/287 g.

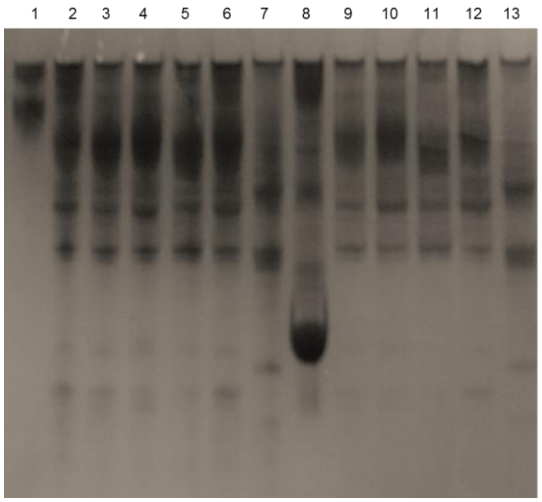

Figure 2. 12% Native-PAGE on plasma proteins from 14 samples. #1 bass fish, #2 female /586 g, #3 female/697 g, #4 female/734 g, #5 male/502 g, #6 male/294 g, #7 male/635 g, #8 bovine plasma proteins, #9 male/1200 g, #10 female/747 g, #11 female/750 g, #12 male/194 g, #13 yellow perch.

Electrophoretic plasma protein profile of female *Cyprinus carpio haematopterus* – koi carp obtained by SDS-PAGE reveals appearance of female specific protein. Previously it was characterized as 143 kDa female-specific glycolipoprotein (FSP) which is a calcium-binding protein (Komagata *et al*,. 1991). The protein is evidently absent in the plasma of males. There is an only one male sample (#14), where the FSP is slightly appearing. Native PAGE didn't show an appearance of FSP but it shows the differences between plasma protein samples from different fish species (Yilmaz *et al*, 2007). The electrophoregrams also showed the individual differences among the plasma samples, but they were not subject of interest of this study. Electrophoresis of plasma proteins have been widely used in the classification of fish. These kinds of studies brought about a new look to taxonomical evaluation. Discrimination of related taxa can be easily made according to their electrophoretic results of plasma proteins (Theophilus and Rao, 1998).

Conlusions

SDS-PAGE can be used as a techinique for gender determination of koi carp based on identification of the presence of female specific protein in plasma sample.

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ЕЛЕТРОФОРЕТСКА ДЕТЕРМИНАЦИЈА НА ЖЕНСКИ СПЕЦИФИЧЕН ПРОТЕИН КАЈ *KOI CARP* КАКО АЛАТКА ЗА ИДЕНТИФИКАЦИЈА НА ПОЛОТ: СПОРЕДБА НА ТЕХНИКА

З. Т. Поповски, Б. Р. Димитриевска, Е. Мискоска-Милевска, К. Порчу, К. Банџо

Апстракт

Со развојот на биомаркерите во плазма се докажува дека тие стануваат многу поинтересни отколку што се претпоставувало. Порано, идентификацијата на различните видови риби било воглавно базирано на анализирање на надворешните морфолошки карактеристики. Одредувањето на полот кај различни видови риби засновано на морфолошки особини е многу тешко и затоа електрофорезата на плазма протеините може да се користи како соодветна молекуларна алатка. Во оваа истражување, нативната полиакриламид гел електрофореза (Нативна ПАГЕ) и содиум-додецил-сулфат полиакриламид гел електрофорезата (СДС-ПАГЕ) беа користени за анализа на плазма протеините кај кои крапот (*Cyprinus carpio haematopterus*). Електрофореграмите покажуваат дека СДС-ПАГЕ како техника може да прави разлика помеѓу машки и женски единки базирано на присуство на женски специфичен протеин (ЖСП), кој може да биде искористен како соодветен маркер. Нативната ПАГЕ не покажува разлики помеѓу машките и женските плазма примероци. На електрофореграмот од СДС-ПАГЕ, молекулската маса на ЖСП е околу 143 кД и е во согласност со претходните истражувања кај други видови риби.

Клучни зборови: koi carp, серум протеини, полово специфичен протеин, PAGE.

UDC:636.4(497.7)
Original scientific paper

QUALITATIVE AND QUANTITATIVE EVALUATION OF OXYTETRACYCLINE RESIDUES IN COW'S MILK IN TETOVO, MACEDONIA

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Abstract
This study was carried out from 2011 to 2012 to evaluate qualitative and quantitative oxyteteracycline residues in cow's milk produced in different rural areas of Tetovo in Macedonia. Our research has evaluated presence of oxytetracycline residues in raw milk produced from 20 milking cows' farms in Tetovo region in Macedonia. About 156 milk samples were controlled by application of ELISA test for qualitative evaluation of oxitetracycline residues. All positive samples were frozen and analyzed with high performance liquid chromatography (HPLC) method for qualitative evaluation of oxytetracycline in Institute of Food Safety and Veterinary in Tirana. By this control performed using ELISA was evaluated that 4.5% (7/156) of raw milk samples were positive for oxytetracycline residues. All positive milk samples were tested with HPLC to evaluate the quantity of oxitetraxycline. The detected levels of positive samples were as following: 60ug/l, 80ug/l, 140ug/l, 220ug/l, 3000 ug/l, 1200 ug/l and 1400ug/l (ppb). Positive results are confirming treatment of milking cows with high doses of oxytetracycline which may contain the drug in the milk for lengths of time.
Key words: residues, oxyteteracycline, milk, cows, Tetovo.

Introduction

Oxytetracycline is a broad-spectrum veterinary antibiotic and is used to kill many pathogenic bacteria. For a long time it is used in lactating and non-lactating dairy cattle for the treatment of bacterial infections such as enteritis, pneumonia, diphtheria, infections infection caused by chlamydia, genital infections, urethritis and other infections in animals. Regulatory limits protect consumers from over-exposure to oxytetracycline above maximum residue limit (MRL) of 100ug/l milk (Lewis, 1997; Alica et al., 2003). As antibiotic with broad-spectrum of tetracycline, oxytetracycline works by interfering with the ability of bacteria to produce proteins that are essential to them. Effect of this antibiotic is related to stops the spread of the infection and the remaining bacteria are killed by the immune system or eventually die (Bishop et al., 1994). However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections (Grave et al., 1993). Using oxyteteracycline for treatment of different animal infections remains a very effective way to control many infections in milking cows. Even

thought the advantages of treatment the oxyteteracycline residues are having adverse effects on people allergic to antibiotics. Use of this antibiotic build up antibiotic-resistant organism in humans and cause inhibition of starter cultures used to produce cultured milk products such as yogurt and cheeses (Alica et al., 2003). For these reasons it is important effectively control of oxitetracycline residues in milk and therefore, regulatory authorities have enacted maximum residue limits (MRLs) for oxytetracycline in milk (EMEA; 1995). Many countries have introduced in national residues monitoring programs the control for residues of oxytetracycline in cow s' milk used for human consumption. On this context, Republic of Macedonia is realizing each year national residues monitoring plan according to EU specific legislation for residues control. Detectable concentrations of antibiotic residues in milk supplies higher than the MRLs are not allowed to be used to by consumers. One of main cause for having residues in cow s' milk is treatment of metritis and other genital infections having the incidence ranged from 10 to 15 % (Lewis, 1997). The most commonly observed diseases recorded in the dairy farms are mastitis in average incidence until 30% (Alica et al., 2003). According to the results of other studies disease conditions such as dystocea, retained fetal membrane, metabolic problem and foot problem recorded in dairy farms are having the incidence above 15%. Other authors are reporting that more than 50% of the farmers interviewed utilized oxytetracycline, and only 5% of the farmers were aware of dry cow therapy for controlling mastitis (Forst et al., 1991). Administration of antibiotics was accomplished using the routes of intramuscular, intramammary, intrauterine and peros in 52.9%, 30.9%, 8.8% and 14.7% of the farms respectively (Grave et al., 1999). For above reason oxitetraxycline in milking cows is commonly used and risk of residues of this group of antibiotics is higher.

Material and methods

Study was focused in 20 cattle farms located in Tetovo district in Macedonia. 156 raw milk samples were collected every month from different dairy farms in Tetovo. Attention was paid to collection of milk samples analyzed were of different farms from different locations. Milk samples were kept in the refrigerator (4°C) until analysis and were analyzed within two days at most.

Use of ELISA for qualitative detection

For detection of oxytetracycline residues in raw milk samples collected from milking cows in Tetova are used MaxSignal® Oxytetracycline ELISA Test Kit. It is a competitive enzyme immunoassay for the qualitative and quantitative analysis of milk samples. The method is based on a competitive colorimetric ELISA assay. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample. Use of MaxSignal® Oxytetracycline ELISA Test is performed according to instruction closed to kit box.

HPLC analysis for qualitative detection of positive samples

All positive samples confirmed by MaxSignal® Oxytetracycline ELISA Test are tested with HPLC to quantify the residue of oxytetracycline. To perform HPLC procedure are used chemicals and material: Acetonitrile and methanol were of HPLC grade; oxalic acid dihydrate Suprapur and Na2HPO4 heptahydrate; ethylene diamine tetraacetic acid (EDTA) disodium salt, citric acid monohydrate (Thermo Fischer Scientific) were of purity grade. Solid phase extraction (SPE) column Oasis HLB, 3 cc, 60 mg was purchased from Waters (Milford, USA). The vacuum unit for SPE was purchased from Supelco. The other hardware included an analytical balance (Kern, Balingen, Germany), a cooling centrifuge (Mechanika Precyzyjna, Poland), and a rotary vacuum evaporator (Bűchi, Flawil, Switzerland), (Petkovska et al., 2006).For the qualitative and quantitative evaluation, the external standard method was used. Each sample was analyzed in duplicates way at the least, every series containing a blank sample. Simultaneously, aliquots of the milk samples with the addition of standard solutions of known concentrations were measured. The detection and quantization limits were established based on the standard deviation of the blind test and the slopes of the calibration curves, repeatability was based on 20 parallel determinations and the recovery was based determinations of the milk sample with the addition of the solution of standards of known concentrations (50µg/l and 100µg/l). Basic statistical processing was done using the Unistat software, Version 5.1 (Unistat Ltd. 1998).

Results and discussion

Raw cow s' milk samples before analytical process was controlled with ELISA to find out if the oxytetracycline residue is present or not. Even thought the MaxSignal® Oxytetracycline ELISA Test is used also for quantitative evaluation of oxytetracycline residues, many of studies recommended use of it only for qualitative detection. In our study we use this kit to find out the presence of oxitetracycline in milk. Then all positive cases were performed with HPLC to quantify the level of oxytetracycline residues. The antibiotic residues of positive samples which showed residues oxytetracicline above MRLs were 5/156 or 3.2%. Oxytetracycline was found being present in all positive samples in a concentration from 60-1400µg/l. Levels of oxiteteracycline in five positive milk samples above MRL were respectively 140ug/l, 220ug/l, 3000 ug/l, 1200 ug/l and 1400ug/l. The positive milk samples were analyzed by HPLC for oxitetraxycline quantification. A given sample was regarded as positive for oxytetracycline if its retention time and peak corresponded to that of the standard. Retention time was considered a reasonably unique identifying characteristic of a given samples (Ding and Mou; 2000; Cinquina et al., 2003).

Table 1. Milk samples collected in dairy farms and positive case of oxytetraxycline residues from 2011-2012 in Tetovo, Macedonia

No. farms	No. milk samples	Detection of oxitetraxycline residues with HPLC (%)	Above MRL (100 µg/L) in %
20	156	4.5% (7/156)	3.2% (5/156)

Table 2. Qualitative evaluation of raw cow s' milk samples confirmed positive by HPLC

Milk samples	Qualitative evaluation by HPLC
1.North part of Tetovo	60 ug/l
2.West part of Tetovo	80 ug/l
3.West part of Tetovo	140 ug/l
4.West part of Tetovo	220 ug/l
5.South part of Tetovo	300 ug/l
6.West part of Tetovo	1400 ug/l

The area inscribed by the peak isproportional to the amount of substance separated in the chromatographic system. To get the concentration of oxytetracycline, a reference standard of a known concentration had been injected in to the HPLC and concentration of the sample was extrapolated from the curve peak area. Studies in Europe carried out for detection of oxytetracycline residues in raw milk produced by milking cows reported values of incidence from 0, 5% to 2, 7 % (Grave et al., 1999; Allara et al., 2001). There are many studies confirming the low incidence from 0, 01%- 1.5%. In some cases in Germany and USA the incidence of level of oxytetracycline was higher than 5% and the cause was attributed the genital infections in milking cows (Heeschen et al., 1996). Comparing of chromatograms of reference standards, oxytetracycline HCl and some samples those were positive for oxytetracycline from the dairy farms were performed in these study to detect level of this antibiotic in milk. The range for oxytetracycline residue level was 0µg/l to 1600µg/l (Grave et al., 1999). The antibiotic residue positive samples which showed residues of oxytetracycline were 7 (4.5%).

Conclusions

The oxytetracycline residues of milk samples collected in dairy farms in Tetovo in Macedonia showed positive cases of oxytetracycline in 4.5% of total samples confirmed by MaxSignal® Oxytetracycline ELISA Test. Performing analytical control with HPLC oxytetracycline residues above MRLs were confirmed in 5 samples or 3.2%. The most of milk samples originated from west part of Tetova confirming values of incidence of oxytetracycline above the MRL and the risk of oxytetracycline residues in cow s' milk produced in this area.

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КВАЛИТАТИВНА И КВАНТИТАТИВНА ЕВАЛУАЦИЈА НА РЕЗИДУИ ОД ОКСИТЕТРАЦИКЛИН ВО КРАВЈО МЛЕКО ВО ТЕТОВО, МАКЕДОНИЈА

Менсур Камбери, Капелан Сулај

Апстракт

Оваа студија беше спроведена од 2011 до 2012 да се оценат квалитативните и квантитативните остатоци на oxyteteracycline во кравјо млеко произведени во различни рурални подрачја на Тетово во Македонија. Нашето истражување го оценува присуство на остатоци од антибиотик во суровото млеко произведено од фармите со 20 молзни крави во тетовскиот регион во Македонија. Вкупно 156 примероци на млеко беа контролирани со примена на ЕЛИСА тест за квалитативна евалуација на остатоци од oxitetracycline. Сите позитивни примероци беа замрзнати и анализирани со високо перформансна течна хроматографија (HPLC), метод за квалитативна евалуација на антибиотик, анализата беше реализирана во Институтот за безбедност на храната и ветерина во Тирана. Со оваа контрола користејќи ELISA се оцени дека 4,5% (7/156) од суровото млеко одредени примероци беа позитивни за остатоци на oxitetracycline. Сите позитивни млеко проби беа тестирани со HPLC да се оцени количеството на oxitetraxycline. Откриени нивоа на позитивните примероци беа: 60ug / л, 80ug / л, 140ug / л, 220ug / л, 3000 ug / л, 1200 ug / l и 1400ug / л (ppb). Позитивните резултати го потврдуваат третманот на молзење крави со високи дози на oxytetracycline кој може да содржи лекови во млекот за одреден временски период.

Клучни зборови: остатоци, oxyteteracycline, млеко, крави, Тетово.

UDC: 636.4 (497.7)
Review paper

REVIEW OF THE ACHIEVED RESULTS IN THE PIG PRODUCTION OF THE REPUBLIC OF MACEDONIA

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Abstract
In the last ten years the pig production of the Republic of Macedonia has a positive trend. This study was realized during 2010 and 2011 under the state herd book project. The registrations and the major part of analyzes are conducted via WinPig software. The used methodology was based on the daily individual registration of the "feeding days". Six pig farms "from farrowing to finish" were the subject of the investigation. The information's were adjusted and the results by farm were calculated. The most of farm results show positive trend of the analyzed traits. In 2010 and 2011 from the analyzed 11344 and 12294 inseminations the conception rate was 85,4 and 84,0%, respectively. The number of total born piglets was 11,7 and 11,92, the number of born alive was 10,98 and 11,2, the number of still born was 0,74 and 0,72, respectively from 9272 and 9918 realized farrowing's in 2010 and 2011. The number of weaned piglets was 9,4 and 9,9, the lactation mortality rate was 14,8 and 12,0% with individual weight of 6,2 and 6,7kg, respectively from 9270 and 9729 realized weaning's in 2010 and 2011. The number of empty days per litter was 16,8 and 13,8, the farrowing index was 2,30 and 2,33, under lactation length of 26,8 and 27,2 days in 2010 and 2012, respectively. Finally the number of weaned piglets per sow per year increased for 1,4 piglets in 2011 compared with 2010 (21,7 p/s/y in 2010 vs. 23,0 p/s/y in 2011).
Key words: pigs, livestock production, farm results.

Introduction

Several analyzes were conducted since 1990's related with the pig performances on-farm in the Republic of Macedonia (Belicovski *et al*., 1993, Belicovski *et al*., 1994, Belicovski *et al*., 1995, Belicovski *et al*., 1996, Vukovic *et al*., 2004). They shows that pig husbandry in the country is driving through the time with various results depend of explored farm and year. Generally, in the last ten to twenty years the pig production of the Republic of Macedonia has a positive trend in many relevant performances. The quality of the carcasses on the slaughter line (Vukovic *et al*., 2006 and Vukovic *et al*., 2010) shows that the market pressure had positive effect on the farms who improve the production in all segments and reach the European level.

Farm results in the developed counties mostly are compared under the several performances like: farrowing rate(%), number of born alive per litter, number of weaned piglets per sow per

year, non-productive days per litter, farrowing index and others which becomes internationally accepted like an indicators of overall swine farming success. The most commonly used indicator is the number of weaned pigs per sow per year (w/s/y). This figure could express the overall farming results having in mind that factors affecting weaned pigs per year are many and they are incorporated into regular management of the pig farms (www.daff.qld.gov.au, 2012). The reached average level in some EU breeding organizations on the large number of farms was 28,8 w/s/y in 2011 (www.pigprogress.net, 2012) and some others in 2010 predict new targets of 35 w/s/y since at that time the 5% of the best farms realized production of 32 to 34 weaned piglets per sow per year (www.wattagnet.com, 2010).

Material and methods

This study was realized during 2010 and 2011 under the state herd book project. The registrations and the major part of analyzes are conducted via WinPig software. The used methodology was based on the daily individual registration of the "feeding days". Six pig farms "from farrowing to finish" from Republic of Macedonia were the subject of the investigation in the equal periods in 2010 and 2011, respectively. The information's were adjusted and the annual results by farm were calculated for the following traits: the number of services, the conception rate (%), the total number of born piglets/litter, the number of born alive piglets/litter, the number of still born piglets/litter, the number of weaned piglets/litter, the pre-weaning mortality(%), the farrowing index, the non-productive days (days) and the number of weaned piglets per sow per year. The source of analyzed data were recorded as a result of the total number of services per farm and year in 2010 (n=11344) and in 2011(n= 12294). The distribution of services per farm, year and total is presented in the Graph 1. The litter size traits and other analyzed traits were observed from 9272 and 9918 realized farrowing's in 2010 and 2011 and from 9270 and 9729 realized weaning's in 2010 and 2011.

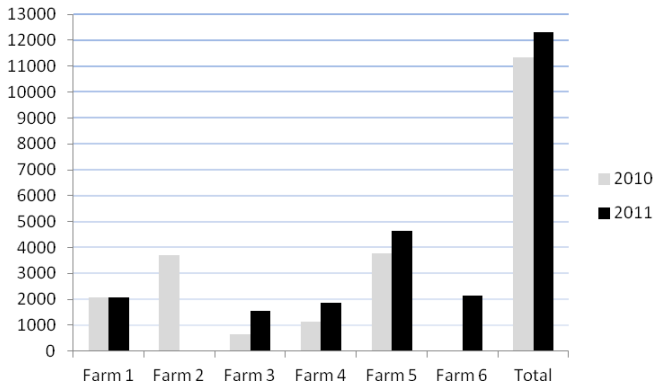

Figure 1. Number of services per farm and year

Results and discussion

In 2010 and 2011 from the analyzed 11344 and 12294 inseminations the average conception rate for all analyzed farms was 85,4 and 84,0%, respectively (Graph 2). The greater and the

smaller conception rate are determined in 2010 (Farm 3 vs. Farm 1). The annual trend per separate farms and overall is negative or variable since generally farms shows decreasing of the insemination success in 2011. The observed differences per year are not large, however they could express the variations per farms in management and could indicate possible negative influences of the genetic and non-genetic factors. The overall results for the conception rate in observed farms generally shows acceptable results above 80%. Compared to the observed conception rate (%) in sows per Macedonian farm published in the past (Belicovski *et al*., 1993) the analyzed results were much higher.

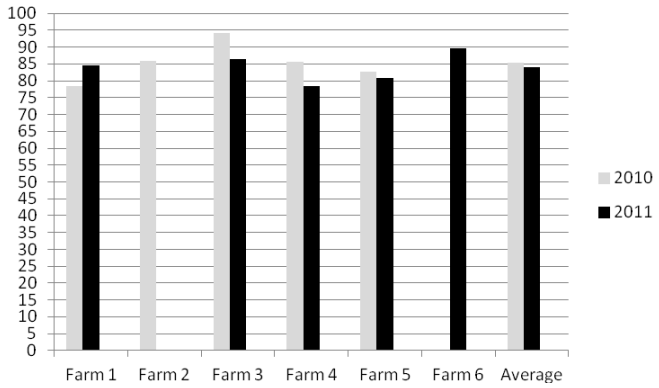

Figure 2. Conception rate (%) in sows per farm and year

The average number of total born piglets per litter was 11,70 and 11,92, the average number of born alive per litter was 10,98 and 11,20 and the average number of still born per litter was 0,74 and 0,72 (Graph 3, Graph 4 and Graph 5), respectively in 2010 and 2011. Trend per year shows that observed gain on the total born and born alive is 0,22 piglets per litter and much lower changes of the average number of still born piglets (-0,02 per year). The observed trend is close to the predictions for genetic improvement of those traits on the phenotypic level (Vukovic et al., 2002).

Observed average number of born alive per litter realized in the Macedonian pig farms in 2010 and 2011, compared with those published before (Belicovski *et al*., 1993) were much higher (10,98 and 11,20 vs. just 9,30 piglets).

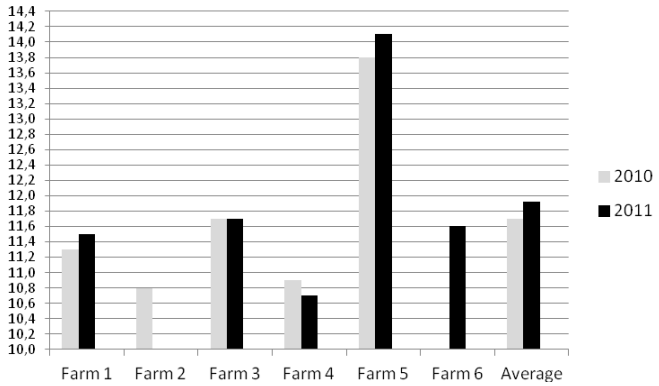

Figure 3. Total number of born piglets/litter per farm and year

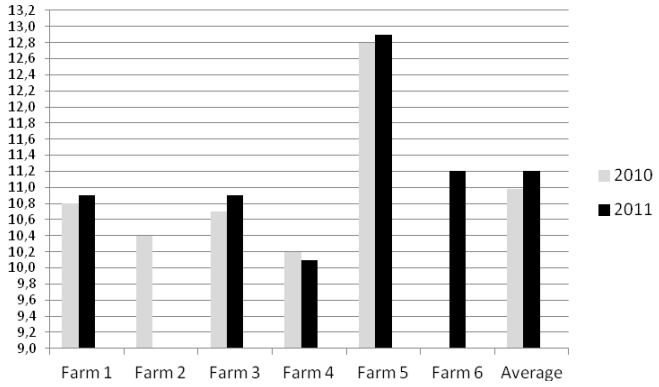

Figure 4. Number of born alive piglets/litter per farm and year

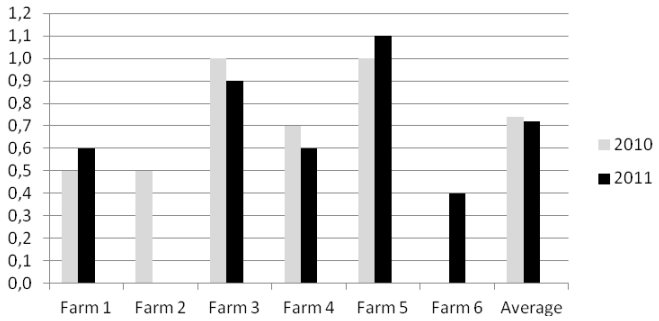

Figure 5**.** Number of still born piglets/litter per farm and year

The number of weaned piglets was 9,4 and 9,9, the pre-weaning mortality rate was 14,8 and 12,0% in 2010 and 2011, respectively (Graph 6 and Graph 7).

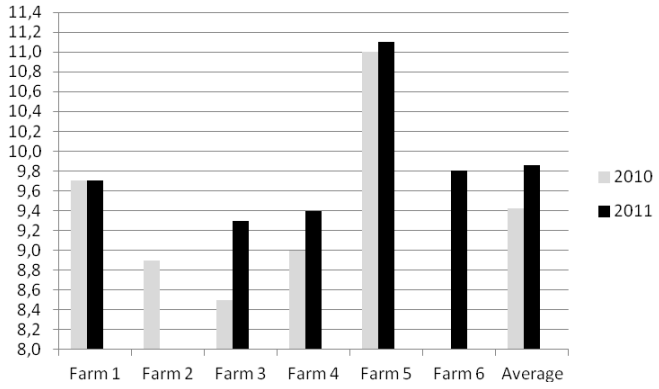

Figure 5. Number of weaned piglets/litter per farm and year

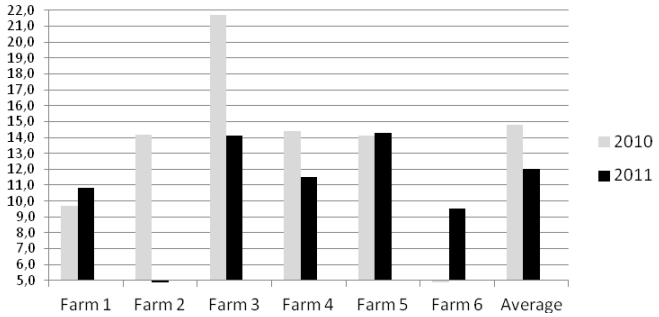

Figure 6. Pre-weaning mortality per farm and year (%)

Decreasing of the pre-weaning mortality rate of -2,78% generally support the greater number of weaned piglets per litter in the observed years (0,44 piglets per litter). The expressed result means significant improvement in the farrowing management and utilizing of the higher number of live born piglets per litter on the phenotypic level. In average, the realized level is much higher with the level observed in the period 1990-1992 (Belicovski *et al*., 1993) for the number of weaned piglets per litter (7,77 vs. 9,42 and 9,86 piglets) and much lower for the pre-weaning mortality rate (15,29% vs. 14.8 and 12,00%).

The results of the number of empty days per litter (16,8 and 13,8 days) and the farrowing index (2,30 and 2,33) in 2010 and 2011, respectively are presented in the Graph 8 and 9.

The observed level farrowing rate in average (2,22 vs. 2,30 and 2,33) and individually per separate farms in two periods (1990-1992 according Belicovski *et al*., 1993 and the actual investigation for 2010-2011) shows positive trend. Annually on the farm level, the combination of the observed non-productive days per farm (Graph 9) and litter size traits at farrowing (Graph 3, 4 and 5) and at weaning (Graph 6 and Graph 7) the overall results per farm and year could be compared under the complex indicator - number of weaned piglets per sow per year (Graph 10.).

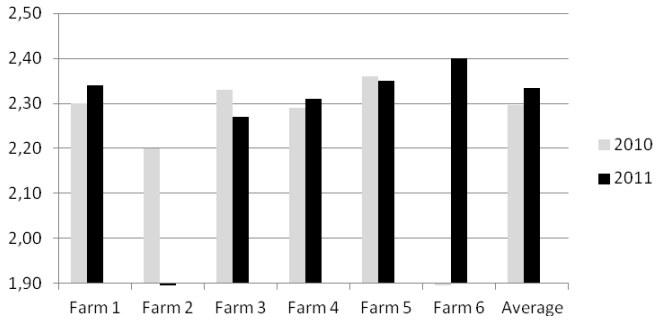

Figure 7. Farrowing index per farm and year

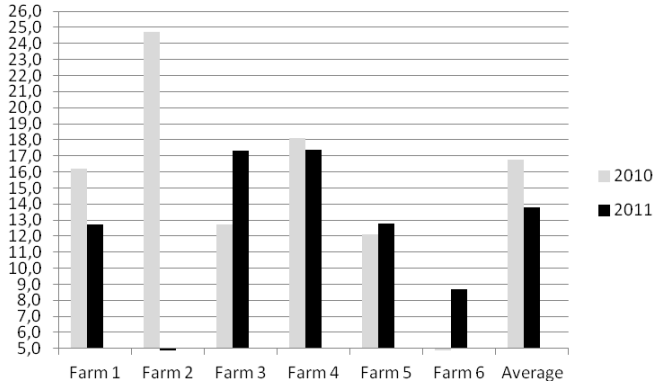

Figure 8. Non-productive days per farm and year (days)

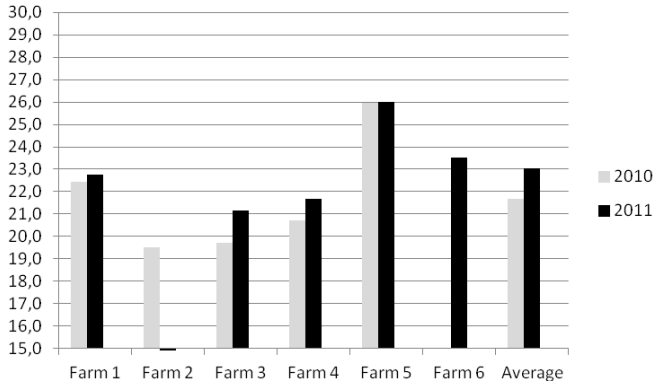

Figure 9**.** Number of weaned piglets/sow per farm and year

According conducted investigation the number of weaned piglets per sow per year increased for 1,4 piglets in 2011 compared with 2010 (21,7 p/s/y in 2010 vs. 23,0 p/s/y in 2011). In addition if we indirectly calculate the number of w/s/y under the input parameters used from the investigation finalized in early nineties (Belicovski *et al*., 1993) we could come to the average number of only 17,25 piglets. Is very clear that in 20-21 years absolute progress on the phenotypic level is 4,45 and 5,75 w/s/y, respectively. The realized progress is going much faster in the last years, however if we linearly divide the absolute gain for two observed years the trend is 0.22 to 0.28 w/s/y which is consistent with previously predicted trends (Vukovic et al., 2002).

The overview of the achieved results in the investigated years are showed in the Table 1. According those results and expressed trends compared with previous investigations in the Republic of Macedonia (Belicovski *et al*., 1993, Belicovski *et al*., 1994, Belicovski *et al*., 1995, Belicovski *et al*., 1996, Vukovic *et al*., 2004) we could summarized that Macedonian pig husbandry significantly improve the farm performances. The observed results are expressing progress in the better control of the factors responsible for proper swine farm

management (usage of superior genetic, artificial insemination, better feeding and housing and much higher level of applied knowledge in daily production).

Table 1. Overview of annual results of the Macedonian pig farms (2010-2011)

Parameteres/traits	2010	2011	Difference
Conception Rate %	85,4	84,0	-1,40
Preg. days/Lit	115,4	115,6	0,14
Total born/Lit	11,70	11,92	0,22
Live born/Lit	10,98	11,20	0,22
Born dead/Lit	0,74	0,72	-0,02
Pigs weaned/Lit	9,4	9,9	0,44
Wean.weight/pig	6,2	6,7	0,55
%Pre-w mortality	14,8	12,0	-2,78
Suckl. days/Lit	26,8	27,2	0,36
Non-prod. days/Lit	16,8	13,8	-2,98
Lit/Sow/Year	2,30	2,33	0,04
Pigs weaned/sow/yr	21,7	23,0	1,35
Number of serves	11344	12294	950
Farrowing	9272	9918	646
Total Weanings	9270	9729	459

Conclusions

In the last twenty years the pig production of the Republic of Macedonia has a positive trend of annual improvements observed under common farm performances. Compared with the European's Macedonian pig farms figures are easily coming closer by achieved results.

The average level of production results observed in 2010 and 2011 per Macedonian farms were: the conception rate of 85,4 and 84,0%, the number of total born piglets per litter 11,7 and 11,92, the number of born alive per litter 10,98 and 11,2, the number of still born per litter 0,74 and 0,72, the number of weaned piglets per litter 9,4 and 9,9, the pre-weaning mortality rate of 14,8 and 12,0%, the number of empty days per litter 16,8 and 13,8, the farrowing index 2,30 and 2,33, respectively.

The number of weaned piglets per sow per year increased for 1,4 piglets in 2011 compared with 2010 (21,7 p/s/y in 2010 vs. 23,0 p/s/y in 2011). The realized trend in this complex trait was 0,22 to 0,28 w/s/y in the last twenty years on the phenotypic level.

Acknowledgement

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ПРЕГЛЕД НА ПОСТИГНАТИ РЕЗУЛТАТИ ВО СВИЊАРСКОТО ПРОИЗВОДСТВО ВО РЕПУБЛИКА МАКЕДОНИЈА

Вукович В., Анднов С .

Апстракт

Во последните десет години на свињарското производство во Република Македонија има позитивен тренд. Оваа студија се реализира во текот на 2010 и 2011 година преку проектот за воведување на матично книговодство. Регистрацијата и поголемиот дел од анализите се извршени преку WinPig софтвер. Користената беше методологија која се базира на дневата поединечна регистрација на "хранидбени денови". Шест свињарски фарми "од прасење до тов" беа предмет на анализа. Информациите беа обработени а резултатите беа пресметани по фарма. Поголемиот дел од резултатите по фарма покажуваат позитивен тренд од анализираните својства. Во 2010 и 2011 година од анализираните 11.344 и 12.294 инсеменации степенот на концепција беше 85,4 и 84,0%, респективно. Вкупниот број на родени прасиња беше 11,7 и 11,92, бројот на живо родени беше 10,98 и 11,2 , бројот на родени беше 0,74 и 0,72, односно од 9272 и 9918 реализираат прасења во 2010 и 2011 година. Бројот на одбиени прасиња беше 9,4 и 9,9, лактационата смртност беше 14,8 и 12,0%, со поединечна тежина од 6,2 и 6,7 кг, респективно од 9270 и 9729 реализираат обивања во 2010 и 2011 . Бројот на празни денови по легло беше 16,8 и 13,8 , индексот на прасење беше 2,30 и 2,33, должината на лактацијата изнесуваше 26,8 и 27,2 дена во 2010 и 2012, респективно. Конечно бројот на одбиени прасиња по маторица беше годишно зголемен за 1,4 прасиња во 2011 година во споредба со 2010 година (21,7 P / S / у во 2010 наспроти 23,0 P / S / у во 2011 година).

Клучни зборови: свињи, добиток, фармски резултати.

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Original Scientific Paper

DEFINING OF BREEDING GOALS OF HOSTEIN CATTLE IN THE R. OF MACEDONIA

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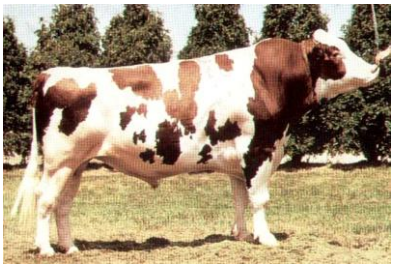
Abstract
According to the Low of Animal Production of R. of Macedonia from 2008, for every breed of cattle in our state is nessesery to create a separate breeding program. Black and white (Holstein) population is a dominant highproductive breed of cattle, which is aimed for high dairy production. Facing the obtained results from controlling of several bigger cattle farms in our state, the following breeding traits and goals for our hostein population are recommended: milk production – more than 8.000 kg per cow for standard lactation, milk fat content – more than 4%, milk protein content – more than 3,4%, body weight of cows – 600-700 kg, height of withers of cows – 135-145 cm, udder – spacious, simetric, with good support of ligaments and lifted from the surface, well adapted for machine milking, with the average milk speed 2,3-2,5 kg/min. Also, the other important breeding goals are: producing healthy cows with good adaptation, less somatic cells content in milk, healthy and flexible animals with good longlasting, with healthy udder, good fertility and big capacity for growing. Because of the fact that breeding goal is an economic category, in future it is essential to increase dairy production by more forage crops, by better consumption ability with the pararel increasing of body capacity. The previous is important from the aspect of the production economy, as well as from the ecological agricultural and dairy production and products with better quality in the R. of Macedonia.
Key words: cattle, selection, Holstein, breeding goals, breeding program.

Introduction

Holstein or Black and White breed is the most productive dairy breed in the world, which has relatively large body capacity and ability to consume large forage crops, as well as with good adaptation and accommodation ability. Therefore, Holstein cows are spreaded al aover the world, from the East to the West, from the meadows to the mountain regions. This breed is rearing mainly for intensive dairy production, which is connected with intensive plant production.



Picture 1 Picture 2

Picture 1. Black and white (BW) type of Hosltein cattle . Picture 2 Red and white (RW) type of Holstein cattle

Black and white (Holstein) population is a dominant breed in the world, with almost 20% from the total cattle population in the world (Trajkovski and Bunevski, 2006). In the R. of Macedonia, from the total number of cattle, 42% belong to Holstein breed. It is a high productive breed aimed for high milk production.

Breeding strategies

In different parts of the world, there are several different breeding strategies of cattle production, which vary from the different points of view:

- Balance between profitable, market oriented and sustainable production,
- Results of Holsteinization (± effects),
- How to continue? Breeding for functional traits, or using the heterozis effects or ?

Anyway, the influence of dual-purpose breeds of cattle in intensive and extensive production system is increasing in the Europe and in the world (Simmental and its crossing with RHF, or Simmental with beef breeds.

Common strategies for dairy production in the world are: a) “high input” strategy (in USA, Canada, Israel, Japan, etc.);b) “low input” strategy (N. Zealand, Australia, etc.);c) “medium input” strategy, with “high performance” cattle and “permanent pasture using” strategy (Switzerland, etc.);d) using dual-purpose cattle breeds – “dual-purpose cow strategy” (mainly in middle Europe: Baravia, Austria, etc.).

Defining the breeding goals

Theoretically, the breeding goal represents the directed genetic improvement of the certain charactedistics realized in successive generations of animals which accomplish the desired production through the future, but expectes economic, social and ecological production conditions (Groen, 2000). The clear definition of breeding goals means what we have to expect in the future. So, definition of breeding goals is the first step of every breeding program for each breed of cattle. But for each productive type and breed of cattle, there are different separated breeding objectives, depending by defferent zootechnical, economical and market conditions. From the point of view from the farmers, every breeding goal ought to satisfy 2 main conditions: 1) maximal profit in current productive conditions, and 2) with realization risk in the working limits (Groen, 2000). According to the breeding goals, the following breeding and selection program ought to be adapted for each breed of cattle, with the emphasis on defining traits, agregate genotype, variances, covariances, fixed and random

effects, heritabilities and other parameters. Finaly, all breeding goals are directly or indirectly connected with the profit function on the farm, so they have an ecomonical value.

Size of Holstein population and cattle breeding in the R. of Macedonia

From the total population of cattle in our state (269.443 heads – Annual Statistical Review of RM, 2011), 41,57% or totaly 112012 heads belong to Holstein population.

Table1. Population of black and white cattle in the R. of Macedonia from 2008 to 2010 (Veterinary Chamber of the Ministry of Agriculture, 2010)

Breed of cattle/Year	2008		2009		2010	
	No. of heads	In %	No. of heads	In %	No. of heads	In %
Black and white cattle	101843	41,80	109525	42,49	112012	41,57
Total No. of cattle	243667	100,0	257724	100,0	269443	100,0

Our cattle rearing is based mainly on small individual farms which number takes 95% from total cattle breeding, and on small number of large cattle farms (with more than 100 heads) which are specialized for intensive cattle production. Individual farmers have a small number of cattle per farm – in 2011 the national average is 6,5 heads of cattle per farm. In our state there are totally 45.127 registered dairy producers, which posses 91% from total cattle heads and have 1 to 9 cows per farm (graph 1), which is in correlation with their small ownership of agricultural land per farm. From the other side, larger farms are the nucleus of high-productive cattle genetics, mainly of black and white cattle.

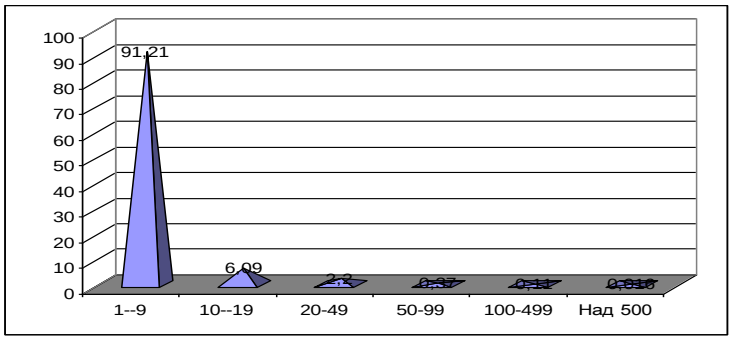


Figure 1. Structure of cattle farms according to their average number of cows per farm in RM (MAFW, 2010)

Material, method of work, obtained results and discussion

In the following tables are presented the results from the controlled dairy farms of Holstein cattle in 2011 in the R. of Macedonia.

From table 3 can be considered that the average milk production in Holstein population in 2011 for all controlled lactations on 5 biggest farms in the RM is 6171 kg, but calculated on III lactation is 6640 kg milk. Compared with the other Holstein population in the other countries, it is similar with the neighbouring countries, but lower than western and north European countries (from 8000-10000 kg milk) (Trajkovski and Bunevski, 2006).

Table 2. Results of the controlled 5 biggest farms for milk production traits in Hosltein farms in 2011

Farms	Average milk yield per cow for all lactation, kg	Milk yield calculated on III lactation, kg	Milk fats, %	Milk proteins, %	Dry non-fat matters in milk, %	Relative weigth of milk
Farm 1	6947,5	7085.3	4,21	3,62	9,76	1,0338
Farm 2	7915,0	8587,8	3,68	3,59	9,59	1,0325
Farm 3	5504,0	6329,9	3.72	3.65	9.69	1.0321
Farm 4	5842,0	6171,8	4,64	3,79	10,06	1,0336
Farm 5	4645,6	5021,4	4,00	3,48	9,19	1,0307
Average	6170,82	6639,3	4,05	3,626	9,658	1,0325

Also, comparing with the other reproductive traits for Holstein population in the other countries, it is similar with the neighbouring countries, but lower than western and north European countries, due to the selection work and realization of the breeding programs for that breed of cattle.

Table 3. Results of analyzes of several reproductive traits of controlled cows on 5 biggest farms in 2011 in RM

Parameter	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Average
Length of pregnancy, days	282,5	283	283	282	283	282,7
Age at first insemination, months	15,9	16,5	15,2	16,0	16,8	16,08
Age at first delivering, months	25,8	25,6	24,6	29,6	40,5	29,22
Twinning, %	1,1	1,5	2,5	1,0	1,3	1,48
Average number of semen doses per insemination	2,3	2,5	1,5	2,5	2,4	2,24
Annual mortality of calves, %	3,5	1,1	4,0	7,8	3,9	4,06

Breeding program for Holstein cattle in the R. of Macedonia

Every breeding program has the following steps of its realization: 1) defining the breeding goals, 2) defining the breeding program, defining the selection criteria, 4) creating of breeding scheme, 5) creating a breeding structure, 6) creating the mating plan, 7) progeny controlling, and 8) economical analyses of the breeding program.

According to the Low of Animal Production (2008), in 2010 was prepared the Common Breeding Program for Livestock Selection (CBPLS) by the Ministry of Agriculture, and a Breeding program for Holstein cattle in Macedonia was recommended in 2011. In the Breeding program, totally more than 30 traits are taken in account for the Hosltein selection, devided in several groups of traits:

a) Productive traits: milk production (in kg), milk fats (in % and kg), milk proteins (in % and kg), dry matters in milk (in %), and specific weight of milk (in %),

b) Reproductive traits: calving period (in days) and service period (in days);
c) Exterier traits: body mass (in kg), wither height (in cm), body length (in cm) and chest circumference (in cm),
d) Linear traits – 18 traits according to the World Holstein Association Program,
e) Other traits: milking spead, delivering difficulties, desease resistance, number of Somatic cells (SCC), fertility, growing, longlasting etc.

Breeding goals for Holstein cattle in the R. of Macedonia

The main breeding objective for Holstein cows in economical dairy production. That high milk yield could be produced by rearing of big cows (with 140 cm of wither heigth), with large and long bodies, which can have a good ability of consumption of big amount of forage crops. The udder ought to be large, spacious, well balanced between fore and rear quarters and balanced udder index, with good support ligaments and proper size of teats which are simetric settled on the udder quarters, as well as well adapted for machine milking. Legs ought to be thin, with derisable pasture, and with strong and healthy hoofs. Holstein cows ought to have a high milk production on different climate and environmental conditions. Specialy desireable animals are those with a good resistance on deseases, with good longlasting, good fertility, early maturing, easy delivering, good conductivity at milking and with well growing (daily gain). Holstein heifers ought to have a fast development and to have 390-420 kg of body weight at 15-16 months of age at the first insemination, i.e. 24 to 26 months of age on their first delivering.

The main breeding goals for Holstein population in the R. of Macedonia are presented in table 4.So, the main breeding goals in selection of Holstein cows are directed to the genetic improvement of:

- Production traits (kg of milk, kg and % of milk fats, kg and % of milk proteins),
- Reproductive traits (with the special emphasis on calving interval in days),
- Linear (exterior) traits of their conformation, and
- Other traits connected with their functionality, resistency, milk quality, longlasting and efficiency.

Table 4. Main breeding objectives for Holstein cows in the R. of Macedonia

Milk production:	– more than 8.000 kg per cow for standard lactation
Milk fat content:	– more than 4%,
Milk protein content:	– more than 3,4%
Body weight of cows:	– 600-700 kg
Height of withers of cows:	– 135-145 cm
Udder:	– spacious, simetric, with good support of ligaments and lifted from the surface, well adapted for machine milking
Average milking speed:	2,3-2,5 kg milk/min
Other important breeding goals:	producing healthy cows with good adaptation, less somatic cells content in milk, healthy and flexible animals with good longlasting, with healthy udder, good fertility and big capacity for growing

According Boicherd (2010), cattle selection has been long oriented towards production, in a non sustainable way. Now cattle have to face new challenges for sustainable production with its three pillars: economic, societal and environmental. So, breeding objectives should be adapted to efficiently account for all traints involved in sustainability. Boicherd (2010) showed that after the long period of selection on production, most functional traits have been deteriorated, sometimes up to a critical point, and need to restore. This is particularly the case for fertility, mastitis resistance, longevity, metabolic diseases (e.g.: - 1% conception rate (CR) per year in Holstein). From the other side, most of the functional traits have a low heritability (0.02-0.10) and are difficult to select.

Future of Holstein breeding goals and selection

Cattle selection has been long oriented towards production, in a non sustainable way. Cattle have to face new challenges for sustainable production with its three pillars: economic, societal and environmental. Breeding objectives should be adapted to efficiently account for all traits involved in sustainability. Dairy production today has: a) very high level of performance traits, b) increasing herd sizes, c) increasing occurrence of diseases, d) time for observing cows limited. So, we have to select a so called "functional and healthy cow'. Genetic improvement requires: 1) definition of traits, 2) Recording schemes, 3) Knowledge of genetic parameters, 4) Economic weights, 5) Inclusion in breeding goal. From the International comparison of dairy bulls, MACE EBVs from Interbull April 2012 will forse five major traits in cows selection: protein in kg (as an indicator of production); overall Udder (indicator of conformation); longevity; SCS (indicator of udder health); calving to First Service (indicator of fertility). Even there is no global effect from using of Genomic Selection (GS) yet, in future, animals are evaluated from their DNA information, with markers covering the genome. GS is already applied on dairy cattle and will be extended of other situations in the near future. It is a unique opportunity to:

1) Have a more balanced genetic trend - genetic trend up to doubled! Opportunities for a more diversified objective,

2) Select for new traits considered as unachievable until recently – right now where is a disconnection between performance recording (in a reference population) and Selection (of candidates without performance) = Flexibility!,

3) Accuracy is the same for males and females,

4) For some traits recorded on a high scale, the accuracy is little dependent on heritability.

In near future we have to expect the rapid use of GS and its beneficions.

Conclusions

According to the controlled traits and obtained results of milk production and reproduction traits in Holstein cows in Macedonia, the following breeding traits and goals for our Holstein population are recommended: milk production – more than 8.000 kg per cow for standard lactation, milk fat content – more than 4%, milk protein content – more than 3,4%, body weight of cows – 600-700 kg, height of withers of cows – 135-145 cm, udder – spacious, simetric, with good support of ligaments and lifted from the surface, well adapted for machine milking, with the average milk speed 2,3-2,5 kg/min. Also, the other important breeding goals are: producing healthy cows with good adaptation, less somatic cells content in milk, healthy and flexible animals with good longlasting, with healthy udder, good

fertility and big capacity for growing. So, in the future selection of dairy cattle more emphasis ought to be given to the functual traits, not only on milk production.

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ДЕФИНИРАЊЕ НА ОДГЛЕДУВАЧКИТЕ ЦЕЛИ НА ХОЛШТАЈН ГОВЕДАТА ВО РЕПУБЛИКА МАКЕДОНИЈА

Буневски Ѓоко, Коцевски Драгослав, Џабирски Владимир, Тројачанец Сњежана, Порчу Кочо

Апстракт

Согласно Законот за сточарство на РМ од 2008 година, потребно е за секоја поважна раса говеда во нашата земја да се креира посебна одгледувачка програма. Црно-белата (холштајн) популација на говеда претставува доминантна благородна раса говеда која интензивно се одгледува за производство на млеко. Согласно производните параметри кои се добиени во контролираните поголеми фарми во Р. Македонија, предложени се следниве одгледувачки цели за холштајн говедата: млекопроизводство – над 8000 кг/крава за стандардна лактација, содржина на млечна маст – над 4%, содржина на протеини во млекото – над 3,4%, телесна маса кај кравите – 600-700 кг, висина на гребенот кај кравите од 135 до 145см, виме - пространо, уедначено, со добра потпора од лигаменти и подигнато од подот, прилагодено за машинско молзење, просечната молзност треба да биде 2,3 - 2,5 кг млеко/мин, а други битни карактеристики се: добиваање здрави грла со добра адаптациона способност, помал број на соматски клетки, здрави и флексибилни животни со добра долговечност, со здраво виме, добра плодност и голем капацитет за раст. Поради фактот што одгледувачката цел е економска категорија, во иднина е многу важно производството на млеко да се зголемува со поголемо учество на волуминозната добиточна храна. Тоа би се постигнало со зголемување на способноста за консумација на животните, со паралелно зголемување на форматот на грлата. Тоа е важно како од аспект на економичноста на производството, така и од аспект на еколошкото земјоделско производство на млеко во Р. Македонија со подобар квалитет на производите.

Клучни зборови: говеда, селекција, холштајн, одгледувачки цели, одгледувачка програма.

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USAGE OF BIOPROTECTIVE CULTURES IN VACUUM-PACKAGED CHICKEN BREASTS IN CASING

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Abstract
The advantage of bioprotective cultures using arises from the fact that it is not necessary to declare E- number which is obligatory for additives and causes skepticism among the consumers. The purpose of this research is to determine the influence of bioprotective cultures B-2 SafePro *(Lactobacillus sakei)* and B-LC-48 *(Lact. curvatus)* in vacuum packaged chicken breast on: pH values, sensory characteristics, total bacteria count and the amount and appearance of purge in vacuum packaging. Smoked chicken breasts in casing are used in this research, which are sliced and packaged in vacuum. Depending on the bioprotective culture used and the manner of its application, before vacuum packaging, the following five product groups are made: 1-reference products without bioprotective culture, 2-A-products with spray application of bioprotective culture B-2 SafePro, 2 -B products with droplet application of the bioprotective culture B-2 SafePro, 3-A-products with spray application of bioprotective culture B-LC-48 and 3-B products with droplet application of bioprotective culture B-LC-48. Smoked chicken breasts treated with droplet application, from the bioprotective culture B-2 SafePro, compared to the other product groups, gave the most satisfactory results in all tested parameters.
Key words: bioprotective cultures, pH, sensory characteristics, microorganisms.

Introduction

The awareness of consumers, through education, constantly increases. If the food is hygienically unsafe (pathogenic microorganisms, contaminants, radiation) it might cause diseases and organic illness. On the other hand, additives are used to inhibit multiplying and developing of undesirable microorganisms. Constant consuming of food that contains additives of chemical origin could result in undesirable effect on human body. This conclusion requires new criteria in food sector especially in meat industry. Those criteria allow long shelf life and improving the sensorial characteristics of meat products without using additives of chemical origin. Something like this is possible only if bioprotective microorganisms are used. This implies inoculating with lactic-acid bacteria which inhibit the spoilage and pathogenic microorganisms. The purpose of this paper is based on the previous researches and literature informations for issues described in it. The attention in this research is directed towards determination the influence of bioprotective cultures *(Lact. sakei)* and B-LC 48 *(Lac.bac. curvatus)* in vacuum-packaged chicken breast

on: pH values, sensorial characteristics, total number of bacteria and the amount and appearance of purge in vacuum packaging.

The advantage of bioprotective cultures using results from the fact that declaring E-numbers is not necessary which is obligatory when using additives which causes skepticism among the consumers.

Canned meats (definition and division)

Canned meats are defined as a products manufactured from: meat, animal fats, viscera, residues of fat and connective tissues and additives which are exposed to the influence of heat in hermetically sealed containers (cans, glass, tubes, casings from allowed plastic and aluminum material) (Џинлески, 1990).

Depending on the regime of heat treatment, two types of canned meats are distinguished: semi-shelf stable and shelf stable canned meats (Korce-Pavlic D, 2003). Smoked chicken breasts in casing belong to the group of chunks of canned meats. These types are produced from cured or cured and smoked pork, beef and mutton, tongues, solid fats and additional ingredients.

Microorganisms in food production and preservation

Employing of microorganisms in food production and preservation is as old as the production of food is. Desirable and undesirable microorganisms are part of meat products micro flora. The undesirable micro flora contain the following microorganisms: *Esherichia coli, Listeria monocytogenes, Clostridium perfringens,* which are involved in meat spoilage processes, whereas slime producing bacteria are: *Achromobacter, Flavobacterium, Alcaligenes, Pseudomonas* and *Serattia.*

Those microorganisms could be transfered to meat through: soil, dust, equipment etc. That is the reason why this type of spoilage appears in the producing area, not only due to inappropriate process for cooling the meat but due to irregular hygienic conditions, as well. When the slime appears in the producing areas, with the aim to keep the meat safe, the following actions should be taken: meat is put into the cooling chambers where the temperature is around 0-2^{0}C, the air moisture is lowered while the air circulation is increased. Under these conditions slime producing bacteria can not be reproduced, meat dries and future development of the spoilage processes is stopped. (Џинлески Б, 1990).

Desirable microflora are: lactic-acid bacteria and *Micrococcus*. To stimulate the bacterial growth, increasing the medium acidity i.e. decreasing the pH value is necessary (Беличовска Даниела, 2007). Lactic-acid bacteria convert the carbohydrates in meat using them as an energy source. As a result of this, lactic acid is created which decreases the pH value resulting in products shelf life extending.

Employing the bioprotective culture b-2 safepro (lactobacillus sakei)

B-2 SafePro is a single strain culture containing *Lac. sakei* (microaerophilic bacteria) in freeze-dried form and can be applied in cooked and cured meat products. The theoretical and experimental knowledge date from the 1987 year, when, for the first time, the bacterium was isolated from fresh beef packed in modified atmosphere (MA).

So far the application of B-2 SafePro always resulted in inhibiting the following bacteria: Listeria *monocytogenes, Brochothrix thermosphacta, Leuconostoc sp* etc., which are a part of the spoilage micro flora, which appears as a result of temperature regime changes during keeping the products. During growth of B-2 in a product a part of the fermentable sugars are

converted into lactic acid. In the 1991 a research was conducted for the profile of acidification of *Lactobacilus sakei*. In a liquid medium (MRS-IM) at 10^0C, 1% sugars were added, significant in meat production. Acidification was observed in medium where sucrose (disaccharide consisting of fructose and glucose) is added, as well as in medium where glucose is added. Neither addition of lactose nor maltodextrin caused acidification (Jelle, 1991). *Lactobacilus sakei* has limited lipolytic activity against fat of animal origin and low proteolytic activity (Jelle, 1991). The strain does not produce biogenic amines, H_2O_2 or bacteriocin. B-2 is capable of growing at 2^0C, and its optimum growth temperature in MRS-agar is 35^0C. However exceeding the temperature limits of below 20 and above 40^0 C, results in longer lag phases and lower acidification rates. In the same temperature range, addition of salt, generally, results in slower acidification. Even salt concentrations as low as 2% slightly inhibits the activity of B-2. B-2 is not active in the presence of 10% salt. At optimum temperature conditions, the absolute maximum level of salt that allows growth of B-2 is 6% with inoculation level higher than 10^6 CFU/ml.

Concerning the other commonly used meat additives, B-2 has been shown to survive the addition of 450 ppm Na-meta bisulfate in vacuum packed fresh British sausage. However the culture did not grow to the same level (from 10^7 to 10^9 CFU/g) as in the sausage without Na-meta bisulfate. Addition of $NaNO_2$ (100 ppm prolonged the lag phase of B-2 for 1 day at 10^0C (Andersen, 1997). Beside the anti-microbic effects typically for B-2, its applying in dry and semi-dry meat products results in improving their sensorial characteristics.

The mode of action for the inhibitory effect of B-2 on *Listeria* and other microorganisms is not specifically known. However, based on the mechanisms generally described in the literature, it is assumed that so called „competitive exclusion“ is the major reason for success of B-2, though unknown mechanisms could be responsible, as well. „Competitive exclusion“ is due to a critical balance between the growth of the competitive culture versus the contaminant at the specific conditions. That balance could be upset if growth parameters are changed for both microorganisms. In the case of B-2 the major competitive factors are: faster growth at storage conditions compared to the contaminant, faster utilization of easily fermentable nutrients, fast distribution at meat surface, quick removal of oxygen i.e. lowering the redox potential, production of inhibitory organic acids and lowering the pH.

Employing the bioprotective culture b-lc-48 (lactobacillus curvatus)

B-LC-48 is a single strain culture containing (*Lactobacillus curvatus*) (facultative anaerobic bacteria) in freeze-dried form. This bioprotective culture is recommended for bioprotection of different types of Ready-To-Eat food products which are packed under vacuum or modified atmosphere and cold-stored. Due to competitive exclusion and bacteriocin production *L.curvatus* contributes to suppressing growth of indigenous lactic-acid bacteria and *Listeria monocytogenes*. B-LC-48 grows at 4-40^0C and survives freezing. The culture does not ferment saccharose and lactose and, consequently, if lactose or saccharose are added, the acid formation will be limited. B-LC-48 is packed in water proof and air proof aluminium foil pouch. Culture should be stored at temperatures below -17^0C to have a shelf life for at least 18 months. At +5^0C the shelf life is at least 6 weeks. The culture is applied by spraying its suspension onto the product surface after cooking. It is necessary during the treatment salt to be limited below 10%.

Material and methods

Smoked, sliced and vacuum-packed chicken breasts in casing are used in this research. They are produced according to the usual technological procedure which is applied in meat processing facility "Lecker" in countryside of Trebosh - Tetovo.

After heat treatment and cooling, the products are sliced. Then slices are vacuum packed in polyethylene bags. Depending on the bioprotective culture used and the manner of its application, before vacuum packaging, the following five product groups were made:

1-reference products without bioprotective culture,

2-A-products with spray application of bioprotective culture B-2 SafePro,

2-B products with droplet application of bioprotective culture B-2 SafePro,

3-A-products with spray application of bioprotective culture B-LC-48 and

3-B products with droplet application of bioprotective culture B-LC-48.

The following bioprotective cultures are used: B-2 SafePro, single strain culture of *Lactobacillus sakei* in freeze-dried form, and B-LC48, also, single strain culture of *Lact. curvatus* in a convenient freeze-dried form. These bioprotective cultures should be stored at temperatures below -17^0C in order the shelf life to be at least 18 months. They are packed in aluminium foil pouches (25 g). These bioprotective cultures are product of Chr.Hansen, Danemark.

Following methods are used:

Culture preparation and application

The culture must be dissolved in non-chlorinated water. One pouch (25 g) is dissolved in ½ liter of water. One half of the solution is used for spray application and the other half for droplet application. Automatic dispenser standardized to a quantity of 0,25 ml is used for droplet application. One droplet of 0,25 ml is enough for 100 g of meat product. After the preparations, droplet and spray application with solution of bioprotective culture B-2 SafePro is done, and then in the same way B-LC-48 application is completed. It should be noted that spray application with plastic atomizer does not offer complete accuracy in terms of the solution amount that should be applied to the product surface. The solution should be applied on both sides of the sliced product. Because these products are vacuum-packed distribution of culture onto the surface are less critical.

pH measuring: before heat treatment, after heat treatment and 30 days after production. Digital pH-meter Metler Toledo MP 120 is used. pH values, before and after heat treatment are obtained by direct measuring with probe, and results are displayed on the pH meter.

Sensory characteristics examination (appearance, color, odour, taste, texture and consistence), 30 and 60 days after production.

Total number of bacteria examination (microbiological examination), 30 and 60 days after production.

Purge description at 40 days after production (clear, colorless or milky, slimy, thick).

Purge amount into packages (%)

That information is obtained by digital scale measuring (American Weight Scales, Inc.), with accuracy ± 0,1 g in vacuum-packed products before and after opening the bag, after previously removed purge. The purge is removed from the surface and from the inside of the bag by paper. After that the product is put again in previously opened bag, and then measured again. The difference between the weight of the product, before and after opening

the bag, expressed in percentage in relation to the weight of the product before its opening, expresses the purge extracted from the product during its keeping.

Results and discussion

Bioprotective cultures solubility, pH value and sensorial characteristics of smoked chicken breasts, produced with and without bioprotective cultures, is evaluated. The amount of purge is described and calculated and microbiological analyses are conducted.

It is concluded that bioprotective culture B-LC-48 is more difficult to dissolve compared to B-2 SafePro.

The results of pH value measurement, 30 days after production are presented in Table 1.

Table 1. pH values of the products

Days after production	Product groups				
30	1	2-A	2-B	3-A	3-B
	6,18	5,46	5,45	5,68	5,8

The lowest pH value is found in smoked chicken breasts where bioprotective culture B-2 SafePro is applied. pH value of 5.45 has been observed in products with droplet application of bioprotective culture, while the products with spray application have shown a pH value of 5.46. The highest pH (6.18) has been found in referent group of products that had not been treated with bioprotective cultures. The lower pH value is a factor that has a significant influence on extending the shelf life of the product.

The results from sensorial evaluation made in the periods of 30 and 60 days after production are presented in Tables 2 and 3.

Table 2. Sensorial evaluation of products, 30 days after production

Groups	Appearance	Colour	Odour	Texture	Consistence	Taste	Total
Points	1-5	1-5	1-5	1-10	1-10	1-15	Max.50
1	4,83	3,3	3,3	8,17	8,5	10,50	39,1
2-A	4,8	4,17	3,8	8,33	8,33	12,40	41,33
2-B	4,17	4,20	4,2	8,5	8,5	12,50	41,70
3-A	4,33	4	3,83	8,17	8,17	12,50	41
3-B	4,33	4,17	3,5	8,17	8,17	12,17	40,5

The products treated with bioprotective culture B-2 SafePro with droplet application, in total, are sensorially the most acceptable (41.70 points) 30 days after production. They, also, exhibit the best results regarding colour, odour, texture, consistence and taste.The referent group of products, without bioprotective cultures, has the worst sensorial grade in total (39.1 point).

According to the percentage of purge, 40 days after production (Table 4), it could be concluded that it is the lowest (2.73%) in the group of products treated with B-2 SafePro with droplet application (2-B). The same group of products has, also, the best results regarding the characteristics of purge (clear and slime-free with an excellent odour),

compared to the rest of the product groups. The highest amount of purge (3.74%) has been found in referent group that has not been treated with bioprotective cultures. This group has the worst purge characteristics: slimy, milky and thick.

Table 3. Sensorial evaluation of products, 60 days after production

Groups	Appearance	Colour	Odour	Texture	Consistence	Taste	Total
Points	1-5	1-5	1-5	1-10	1-10	1-15	Max.50
1	3,50	3,33	2,78	8,11	8	6,55	31,98
2-А	4	4,44	4,33	8,22	8	11	39,42
2-Б	4,33	4,44	4,67	8,22	8,55	11,2	40,8
3-А	4,33	4,22	3,44	7,44	8,11	8,77	36,55
3-Б	4	3,89	3,22	8,11	8,44	8,66	36,78

The products treated with bioprotective cultureB-2 SafePro with droplet application, also, in total, are sensorially the most acceptable (40.8 points), 60 days after production. The referent group of products, without bioprotective cultures, has the worst sensorial grade in total (31.98 points). The results for purge amount found in the bags, along with the description of the purge, 40 days after production are presented in Table 4.

Table 4. Purge amount and its description, 30 days after production

Product groups	Purge amount (%)	Description of the purge
1	3,74	Milky, thick and slimy
2-А	2,89	Milky, slime free, excellent odour
2-Б	2,73	Clear, slime free, excellent odour
3-А	2,99	Milky and slime free
3-Б	2,92	Milky and slimy

Microbiological examinations of referent group (without bioprotective cultures) and control groups (treated with bioprotective culture), have been done at the Veterinary institute, Skopje, 30 and 60 days after production. No pathogenic bacteria have been found, both, 30 and 60 days after production. However, the total bacteria count has been proven to be higher in referent group of products (without bioprotective culture) than the control groups (treated with bioprotective culture).

Conclusions

Based on the research results, the following conclusions can be presented:

Smoked chicken breasts treated with bioprotective cultures have lower pH values than products which are not treated with bioprotective cultures. The lowest pH value is found in smoked chicken breasts treated with bioprotective culture B-2 SafePro, droplet application.

Products treated with biprotective cultures are sensorially more acceptable then untreated products. The products treated with bioprotective culture B-2 SafePro, droplet application, are sensorially the most acceptable.Percentage of purge in vacuum packages is lower in products treated with bioprotective cultures than untreated products. Purge amount (%) is

the lowest in products treated with B-2 SafePro, droplet application. The same products have the most favorable purge characteristics. Total bacteria count is evidently higher in referent group of products which are not treated with bioprotective cultures compared to the control groups, treated with bioprotective cultures.

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УПОТРЕБА НА БИОПРОТЕКТИВНИ КУЛТУРИ ВО ВАКУУМ ПАКУВАНИ ПИЛЕШКИ ГРАДИ ВО ЦРЕВО

Пејковски Златко, Ужевска Дана

Апстракт

Предноста на користењето на биозаштитните култури произлегува од фактот дека не е потребно декларирање на Е-бројот кој е задолжителен при употребата на адитиви и предизвикува скептицизам кај широката потрошувачка маса. Целта на ова истражување е да се утврди влијанието на биозаштитните култури B-2 SafePro (Lactobacillus sakei) и B-LC-48 (Lactobacillus curvatus) при нивната апликација во производот вакуумирани димени пилешки гради во црево врз: pH вредноста, сензорните карактеристики, вкупниот број на бактерии и количеството и изгледот на издвоената течност во вакуум пакувањата. Во испитувањата се користени димени пилешки гради во црево, нарежани и пакувани во вакуум. Во зависност од употребената биозаштитна култура и начинот на нејзина апликација, пред вакуум пакувањето, изработени се следниве пет групи производи: 1-референтни производи без аплицирана биозаштитна култура, 2-А-производи со спреј апликација на биозаштитната култура B-2 SafePro, 2-B-производи со апликација со накапување на биозаштитната култура B-2 SafePro, 3-А- производи со спреј апликација на биозаштитната култура B-LC-48 и 3-B- производи со апликација со накапување на биозаштитната култура B-LC-48. При тоа димените пилешки гради со апликација со накапување на биозаштитната култура B-2 SafePro, во споредба со преостанатите групи производи, даваат евидентно подобри резултати за сите испитувани параметри.

Клучни зборови: биопротективни култури, pH, сензорни карактеристики, микроорганизми.