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BEEF CATTLE WELFARE - RISKS AND ASSURANCE

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Review paper

Abstract: Beef production is widespread all over the world but the legislation regarding welfare area of beef cattle is not specifically addressed and fully implemented. Beef cattle welfare assurance affects not only animals but is also a question of ethics and products quality. Today, it is possible to determine welfare quality state in feedlots through relevant methodology such is Welfare Quality®Assessment Protocol applied to fattening cattle. It enables implementation of improvement strategy regarding identified risks and causes of poor welfare. Different literature sources, based on welfare risk assessment, indicate major welfare problems in cattle kept for beef production. According to them, respiratory diseases are usually linked to overstocking, inadequate ventilation, mixing of animals and failure of early diagnosis and treatment. In addition, digestive disorders are associated with intensive concentrate feeding, lack of physically effective fibre in the diet whilst behavioral disorders comes as a consequence of inadequate floor space, and commingling in the feedlot. Particular welfare problems are related to the implementation of animal husbandry methods-mutilation, which expose animals to pain and suffering. This paper gives a review of most important beef cattle welfare topics including recommendations for its assurance and improvement.

Key words: beef cattle, welfare, risks, assurance, legislation, assessment, food quality

Introduction

Beef meet is the fourth produced (by value) animal protein in the world after milk and pork. Production of beef meet in world has increasing trend over the past 55 years, from 23 millions of tonnes in 1960 to 57 millions in 2014 reaching its maximum level. Among countries, the United States is the largest producer of beef in the world followed by Brazil and the European Union. At the same time, those countries are the largest consumers of beef in the world. Recent years beef production in Serbia is not satisfactory although there are substantial potentials for it *(Aleksić et al., 2012)*. Our country has been traditional exporter of beef, meat products, and fattening young cattle into many countries. Nowadays, production of

meat is in constant decrease, which is consequence of reduction in total number of cattle as well as insufficient number of slaughterhouses with EU certificate *(Ostojić Andrić et al., 2012a).*

Generally, beef breeding is widespread all over the world and there are six main categories of those production systems: dairy farming, beef breeding herds, semi-intensive grazing systems, bobby calf production, veal farming and intensive fattening units. Each of these systems have advantages and disadvantages regarding the management and production efficiency as well as quality of products obtained *(Petrović et al., 2011).* In recent years however, great attention is paid to the aspect of health and welfare of reared cattle. The initiative of people to care about the welfare of farm animals is based on their moral attitude and concern for the right and wrong treatment of animals, with presumed opposition to over-exploitation and/or cruelty towards animals *(Ostojić Andrić et al., 2012b).* There is also growing concern for many consumers in Europe about farm animal welfare since it becoming increasingly recognized as an important attribute of food quality *(Blokhuis et al., 2008; Blandford et al., 2002; Ostojić Andrić et al., 2006).* Specifically considering beef products, *Veissiere et al. (2007)* report that consumers have relevant concern levels for animal welfare. Guided by the above mentioned, some markets developed farm assurance schemes which guarantying animal welfare friendly products, such as UK's so called "Freedom Food" *(Burgess et al., 2003).* The link between farm animal welfare and food quality becomes even more important with growing evidence that animal welfare has direct and indirect impacts on food safety and quality *(Blokhuis et al., 2008; Wyss et al., 2004).* When it comes to beef meat, poor welfare conditions in beef cattle rearing usually resulted in low meat quality due to stress (dark-cutting beef) and inappropriate handling and transport (bruises, leg fractures, injuries, diseases etc.) *(Aleksić et al., 2013).* It reflects negatively not only the appearance of flesh, but also its sensory characteristics and the ability for technological processing *(Delić et al., 2013).* It is also important to note that chronic exposure to stress has an immunosuppressive effect, decreasing disease resistance and increase using of antibiotics which potentially leads to drug residues in meat that can be harmful for human's health. This paper gives a review of most important welfare issues in beef cattle, including major risks and recommendations for its assurance.

Beef farming systems

Cattle in the EU are primarily reared on a grass and forage-based diet. In Member States, such as the UK, Ireland and France, grazing and grass finishing of cattle is prevalent, whereas Scandinavia primarily feeds cattle on harvested forages. In Central and Southern Europe, where grain yields are higher, cattle tend to feed on less grass and forage and more grain, but not nearly to the

extent of the United States. From an animal welfare perspective, beef cattle reared and finished on pasture benefit in terms of health and well-being and have the opportunity to express natural behavior. Cattle are adapted to a life spent grazing on pasture, which provides them with an appropriate diet for their ruminant digestive system. Beef cattle on pasture also have more opportunities for natural behavior such as grazing, walking, choosing different areas for lying and social interactions.

Definition of welfare

Welfare is commonly define as a list of needs (freedoms) which should be provided to the animal and which are contained in "The principle of Five Freedoms and Provisions" (FFP) given in Table 1. It is defined by the Farm Animal Welfare Council *(FAWC, 2014)* for whom the welfare of an animal includes its physical and mental state. These freedoms identify the elements that determine the animals' own perception of their welfare state and define the provisions necessary to promote that state (Webster, 2001). According to these freedoms the assurance of animal welfare can only be accomplished by proper production practices, specific not only to the animal species, but also to production systems and husbandry, climatic and farming conditions, housing and management methods, feeding, etc.

Table 1. The Principle of Five Freedoms and Provisions (FFP), *FAWC (2014)*

1. Freedom from hunger and thirst	access to fresh water and diet to maintain full health and vigour
2. Freedom from discomfort	provision of an appropriate environment including shelter and a comfortable resting area
3. Freedom from pain, injury or disease	prevention or rapid diagnosis and treatment
4. Freedom to express normal behaviour	provision of sufficient space, proper facilities and company of the animal's own kind
5. Freedom from fear and distress	ensuring conditions and management which prevents mental suffering

Assessment of welfare quality in beef cattle

Regardless of conditions that are present in animals' rearing, welfare assessment should be a scientific procedure and should include health, physiology, performance and behaviour measures *(European Commission, 2000).* One of the

novel method for welfare assessment in beef cattle is those developed under the *Welfare Quality Project® (2009)* which utilizes physiological, health and behavioural aspects to assess the welfare of fattening cattle on farm and at the slaughterhouse. Description of the measures that will be used to calculate the overall assessment of welfare are given in *Welfare Quality® Assessment Protocol for Cattle (2009)*, (Table 2). Starting from mainly animal-based measures, collected on farm or slaughterhouse, this assessment enables us to convert them to

Table 2. Collection of data for fattening cattle on farm *(Welfare Quality® Protocol, 2009)*

Principle	Welfare Criteria		Measures
Good feeding	1	Absence of prolonged hunger	Body condition score
	2	Absence of prolonged thirst	Water provision, cleanliness of water points, number of animals using the water points
Good housing	3	Comfort around resting	Time needed to lie down, cleanliness of the animals
	4	Thermal comfort	*As yet, no measure is developed*
	5	Ease of movement	Pen features according to live weight, access to outdoor loafing area or pasture
Good health	6	Absence of injuries	Lameness, integument alterations
	7	Absence of disease	Coughing, nasal discharge, ocular discharge, hampered respiration, diarrhoea, bloated rumen, mortality
	8	Absence of pain induced by management procedures	Disbudding/dehorning, tail docking, castration
Appropriate behaviour	9	Expression of social behaviours	Agonistic behaviours, cohesive behaviours
	10	Expression of other behaviours	Access to pasture
	11	Good human-animal	Avoidance distance
	12	Positive emotional state	Qualitative behaviour assessment

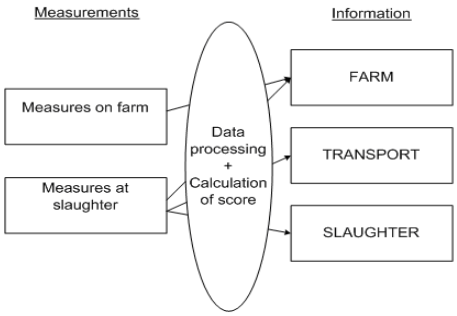

Figure 1. From measures to information ***(Welfare Quality® Protocol, 2009)***

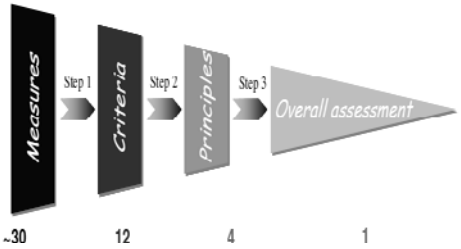

Figure 2. Integration of measures to an overall welfare assessment ***(Welfare Quality® Protocol, 2009)***

summary information about overall welfare state on given farms as it is shown in Figures 1 and 2. Potential use of the output generated includes not only information provided to improve welfare quality but is also available to consumers, advisors and retailers in beef industry *(Ostojic Andrić et al., 2013)*.

Legislation

There is no specific EU legislation considering the welfare of cattle kept for beef production *(Blandford et al., 2002; European Commission, 2001).* However, some general EU legislations relating to the protection of the welfare of calves as well as animals at the time of slaughter, killing and during transportation, are applicable. Veal production has been a controversial welfare topic within Europe and led to the implementation first in 1991, and later in 2008, of legislation laying down minimum standards for calves' protection (Council Directive 2008/119/EC, laying down minimum standards for the protection of calves). European Convention for the protection of animals for slaughter (1979) and Council Directive 93/119 EC on the protection of animals at the time of slaughter and killing were adopted in order to improve handling, restraint, stunning and slaughter conditions. Also, animal transportation is a very relevant issue for animal welfare and therefore being subject to specific legal requirements such as European Convention for the protection of animals during international transport (1968) and Council regulation 1/2005 on the protection of animals during transport and related operations.).

At the national level, welfare legislation may address minimum requirements for beef cattle, for example, in Austria (Tierschutzgesetz, 2004). In Serbia, first law on animal welfare was adopted in 2009 *("Official Journal RS", No.41/2009),* including set of regulations which refer to rearing conditions, traffic and record in terms of farm animal welfare *("Official Journal RS", No. 6/10)* and the procedure for deprivation of animal life in slaughterhouse *("Official Journal RS", No. 14/2010).* The limited extent of legal standards regarding beef production

contrasts with numerous welfare concerns, as highlighted, for example, by *SCAHAW (2001).*

Welfare risks in beef cattle production

For centuries, cattle were grown in a traditional manner, within small farms, mainly grazing. Since the second half of the nineteenth century, the development of industry and continuously raising of population pointed to the need of rapidly increasing production of protein products which led to the industrialization of cattle breeding and implementation of new solutions in animal husbandry. This new era in cattle breeding included a significant reduction in the housing area, inadequate or completely deprived movements and thus the impossibility of expressing natural behaviours and social interactions *(Ostojić Andrić et al., 2011; Hristov et al., 2011).* Today, there are serious indications that the increased frequency, particularly the so-called production diseases, is directly related to disturbed animal welfare. According to *Gregory (1998)* the most important welfare risks which occur in beef production are summarized in table 3.

Table 3. Most important stress and welfare issues in beef cattle *(Gregory, 1998)*

	Dairy cow	Beef breeding herd	Semi-intensive beef grazing systems	Feedlots	Veal units	Bobby calf production
Dystocia	✓	✓				
Cow–calf separation	✓				✓	✓
Mastitis	✓					
Lameness	✓					
Metabolic and digestive disorders	✓			✓*	✓*	
Poor body condition/ underfeeding	✓*	✓*				
Social stressors	✓		✓*			✓
Dehorning/disbudding/ docking	✓	✓				
Castration		✓*		✓		
Hot-iron branding		✓*	✓*	✓*		
Handling		✓	✓	✓	✓	✓
Transport	✓	✓	✓	✓	✓	✓

* Only applies to particular systems, countries, or regions.

In a broader context, as reported by *EFSA Scientific Opinion (2012)* major welfare problems in cattle kept for beef production were respiratory diseases linked to overstocking, inadequate ventilation, mixing of animals and failure of early diagnosis and treatment, digestive disorders linked to intensive concentrate feeding, lack of physically effective fibre in the diet, and behavioural disorders linked to inadequate floor space, and co-mingling (mixing of animals from different sources in the feedlot). In further text, only some of most important welfare risks will be discussed more detailed.

The impact of heat and cold stress

Beef cattle can tolerate and adapt to a wide range of air temperatures, and metabolic heat production increases with increasing feed intake. Thus, animals on the highest rations are least sensitive to cold and most sensitive to heat. Cold stress can be reduced by provision of appropriate shelter and a dry lying area. Therefore, it is recommended that beef cattle confined in houses or open feedlots should be provided with structures and facilities to reduce the effects of factors contributing to thermal stress such as excess air movement, precipitation, relative humidity and solar load. Provided that these are effective there is no need to make provision for the control of air temperature *(EFSA, 2012).*

Housing condition - floor quality

Beef cattle kept on slatted floors have a higher incidence of abnormal standing and lying movements and also a higher incidence of injuries than animals kept on straw or sloped, partially straw-bedded areas *(Absmanner et al., 2009).* Partial rubberisation or rubber mats on concrete floors, especially for lying areas, reduces the prevalence of lesions to claws and joints. However, wherever possible, cattle housed on slatted concrete floors should have access to a bedded area. *Lowe et al. (2001)* showed that Continental-cross steers of 450 kg kept on straw were significantly cleaner than steers kept on perforated rubber mats or conventional slats.

Mutilations - castration, disbudding/dehorning

Castration is carried out in cattle in order to: reduce aggressive and sexual behaviour, reduce the incidence of meat quality problems, particularly dark-cutting meat, encourage fattening, or avoid unwanted pregnancies *(Stafford and Mellor, 2005).* It is common practice in Ireland, UK, north western France and USA, where the males are fattened as steers. All castration methods cause intense acute pain and chronic pain that may last for some days and even up to 2 weeks (*Marti et al., 2010*). A study of *Bretschneider (2005)* showed that loss of weight also increased greatly with the age of castration, independently of the method used.

Approximately 35 % of beef cattle in European Union (EU) are disbudded and about 15 % are dehorned. Disbudding of young calves seems to be more

acceptable than dehorning from a welfare point of view and does not cause as much pain as dehorning older animals *(EFSA, 2012).*

It has been shown that very young animals feel pain, but they may actually feel more pain than adults due to the immaturity of the nociceptive system (*Fitzgerald, 1994*). On the other hand, in older animals, mutilation will result in a more extensive area of tissue damage and so may cause more pain and a more prolonged recovery period (*Bretschneider, 2005*). Restraining animals during mutilation procedure usually cause some distress in addition to the existing pain. This stress may be lower in animals under 6 months of age compared to older animals simply due to their size. Overall, this could mean that when calves are mutilated at a young age they may suffer less overall pain and distress than old larger animals *(King et al., 1991).* In most EU Member States, there was a reinforcement of using anesthesia for most mutilations but the use of analgesia in post-operative period is less common and should be more practiced *(Hewson et al., 2007).*

In conclusion, all mutilation measures should be followed by use of appropriate anesthetics and analgesic in order to avoid stress and pain as important welfare risks. Some non-invasive procedures such as immunocastration and genetic selection of polled animals should be widely implemented.

Social stress and abnormal behavior

Inappropriate human-cattle interactions are often seen as a source of social stress, especially, rough handling of animals in everyday managing, transport or during veterinary procedures. Nowadays, with increasing herd sizes and mechanisation, loose housing become more convenient in beef production, which resulted in less frequent contacts of animals with humans and increase their perception of humans as a potential danger. In these situations, fear reactions and antipredatory strategies, such as flight or fight, are typically observed during handling *(Waiblinger et al., 2006).* Several studies *(SCAHAW, 2001; Krohn et al., 2001)* have shown that early human contact with calves (during the first few days following weaning) is of great importance for establishing good human-animal relationship and most effective in terms of reducing fear of humans.

Interaction between animals in feedlot can also be a source of social stress *(EFSA, 2012).* Mixing and regrouping of cattle increase the incidence of agonistic behaviors and also have disadvantages from a health perspective. Older and more aggressive animals may cause trauma and severe stress to lower ranking calves. There is also a risk that young, immature, heifers may be harassed and become pregnant when kept with sexually mature bulls. In terms of behavioural disorders, beef cattle are often prone to tongue rolling and urine drinking, that usually occur as a consequence of inappropriate nutrition and feeding (high starch, fibre or proteins ratio in diet).

Growth-promoting hormones

In the United States (US) beef production growth promoters (hormones and beta-agonists) are widely administered in approximately two-thirds of all beef cattle *(WAP, 2014).* Producers administer these non-therapeutic drugs in view of reducing production costs as they allow animals to grow larger and more quickly on less feed. Growth promoters are problematic for animal welfare because they stress the animals' metabolism, diverting resources into growth rather than maintenance, increasing hunger and vulnerability to suboptimal management. Furthermore, some of these drugs are used as an easy alternative to good husbandry, suppressing disease but allowing other poor practices such as overcrowding.

Diseases and injuries

Many health problems of beef cattle can be attributed to errors in management *(Radostits, 2001).* Observation of the animals is particularly important as problems are likely to be expressed through animal behaviour, although many stockpersons do not recognise early signs of respiratory disease *(Gorden and Plummer, 2010).*

It has been demonstrated that colostrum-deprived and stressed calves, nervous animals and some breeds are more susceptible to bovine respiratory disease-BRD *(Pereira and Stilwell, 2011).* "Bullers" (hierarchal lower animals that are constantly harassed by pen mates) are 2.5 times more likely to have respiratory disease and 3.2 times more likely to die *(Taylor et al., 2010).* Animal weight when entering the feedlot is also a significant factor *(Thomson and White, 2006)* and co-mingling animals of different ages and size will predispose to BRD those that are smaller.

Most beef cattle diseases have a multi-factorial etiology. In addition to pathogens and animal-related conditions, other contributing factors include stocking density and environmental stressors that disturb homeostasis in the animal. If infection is not detected and treated early in the course of disease, what is frequently happen in large herds, than severe, chronic infection usually arises. Chronic pneumonias, for example, cause very poor welfare with pain, asphyxiation and ill thrift *(EFSA, 2012).*

Some diseases occur due to inappropriate feeding regime. Rumen bloat can occur when the percentage of legumes in the diet is high, but also growing cattle fed intensively on high grain rations (<15% physically effective fibre) are at a high risk of sub-acute ruminal acidosis (SARA).

Considering, beef breeds have been selected for a high meat production, there are often associated with a hypermuscularity which can cause leg disorders, increase calving difficulties and decrease cow longevity *(EFSA, 2012).*

Current state of beef cattle welfare

One of novel studies conducted in Austria, Germany and Italy on a total of 63 beef bull farms (deep litter or cubicle housing systems) and assessed by *Welfare Quality®Assessment Protocol for Cattle (2009)*, shown there are significant areas for improvement of beef cattle welfare *(Kirchner et al., 2013)*. The highest average welfare scores were obtained from 'Absence of prolonged hunger' (94/100 points) followed by 'Absence of pain induced by management procedures' (88/100) and 'Comfort around resting' (77/100). Most welfare concerns related to the criteria 'Absence of disease' (40/100), 'Expression of social behaviour' (44/100) and 'Positive emotional state' (48/100), thus indicating room for improvements. Two-thirds of the farms achieved the 'Enhanced' level, about one-third was estimated 'Acceptable' and only one farm 'Excellent'.

Conclusion

Beef production is a highly subsidized activity in the EU, with payments provided to livestock producers providing incentives to follow EU environmental and animal welfare principles. Traceability systems that include mandatory animal identification and product labelling have been progressively developed in the EU. Animal welfare legislation has been introduced, banning electric cattle prods, phasing out certain routine management practices including castration without pain relief, dehorning and branding as well as the introduction of housing requirements during the winter season. Although it seems to be a major shift, recent studies showed there are still many risks in beef production that need to be eliminated in order to provide welfare assurance of beef cattle. Further objectives in improving the beef cattle welfare should be directed towards satisfying the social and emotional needs of cattle, as well as the prevention and control of the most common diseases.

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Dobrobiti tovne junadi - obezbeđenje i rizici

D. Ostojić Andrić, S.Aleksić, M.M.Petrović, V.Pantelić, N.Stanišić, V. Caro Petrović, D. Nikšić, M. Petričević

Rezime

Proizvodnja junećeg mesa je široko rasprostranjena u svetu ali je zakonodavstvo koje se odnosi na oblast zaštite dobrobiti tovnih goveda još uvek nedovoljno specifično i ne primenjuje se u potpunosti. Obezbeđenje dobrobiti tovnih goveda od značaja je samim životinjama, a istovremeno je i pitanje etike i kvaliteta proizvoda. Danas je moguće utvrditi stanje kvaliteta dobrobiti u tovilištima putem pouzdane metodologije kao što je Protokol za ocenu kvaliteta dobrobiti tovne junadi. On omogućava primenu strategija unapređenja dobrobiti na farmama u odnosu na utvrđene rizike i uzroke loše dobrobiti. Različiti literaturni izvori, zasnovani na metodi ocene rizika, ukazuju na ključne probleme dobrobiti tovne junadi. Respiratorne bolesti obično su u vezi sa prenaseljenim objektima, neodgovarajućom ventilacijom, mešanjem životinja i neblagovremenom dijagnostikom i lečenjem obolelih životinja. Oboljenja digestivnog sistema nastaju kao posledica intenzivne ishrane koncentrovanim hranivima, u nedostatku vlaknastih hraniva. Neodgovarajuci podovi u objektima, mešanje životinja iz različitih grupa/uzrasta i loš postupak odgajivača dovode do poremećaja ponašanja i socijalnog stresa. Posebni problemi dobrobiti odnose se na primenu zootehničkih metoda-mutilacija, kojima se životinje izlažu bolu i patnji.

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IN SILICO ANALYSIS OF BETA-LACTOGLOBULIN GENE IN SOME SELECTED MAMMALIAN SPECIES

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Abstract: This study investigated in silico, the genetic diversity of Beta-Lactoglobulin (β-Lg) and their evolutionary and differentiation within and among selected mammalian species; and also examined the attendant effects of polymorphism on the functionality of the gene. A total of 21 β-Lg gene sequences with corresponding amino acids belonging to 6 species [cattle (4), buffalo (4), sheep (3), goat (3), pig (3) and horse (4)] were retrieved from GenBank (www.ncbi.nlm.nih.gov). All sequences were trimmed to equal length (500bp) corresponding to the same region. Sequences' alignment, translation and comparison were done with ClustalW using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66. The alignment revealed high polymorphism of sequences among extant species. The Dxy inferred using p-distance revealed that sheep and goat had the lowest distance of 0.05 with a maximum distance of 0.65 between goat and horse. The hypothesis of strict neutrality ($d_N = d_S$) was rejected for all extant species as allelic sequence evolution was driven by both purifying and positive selection. Only those of pig and buffalo were driven by positive selection. In-silico functional analysis of non-synonymous mutations using PANTHER revealed that, all the 12 amino acid substitutions (10 in cattle and 2 in sheep) did not impair protein function. The Neighbour-Joining phylogeny revealed trans-species evolution, but a species-wise phylogeny was obtained for UPGMA with consensus sequences. Thus, all probed SNPs from this study have no deleterious effect and can be tolerated by breeders when selecting stocks for milk improvement.

Key words: single nucleotide polymorphism, beta-lactoglobulin, in silico, phylogeny.

Introduction

To select for greater milk production, breeders have traditionally relied upon phenotypic evaluation and selection. In developed countries, phenotypic selection has yielded appreciable degree of successes in predicting genetically superior animal (*Staiger, 2007*). In United State, average milk production per lactation has doubled in the last forty (*Dekkers and Hospital, 2002*) while in United Kingdom, production has tripled in the last seventy years (*Simm, 1998*). More than half of this increased in milk production has been due to improvement in genetics. Despite these increases, phenotypic selection could be improved to give better accuracy by utilizing molecular genetics and bioinformatics selection techniques. Because milk production is a quantitative trait, heritability value is not a perfect predictor of genetic merit of an individual (*Staiger, 2007*). In addition, milk production can only be measured in females that have reached sexual maturity or calved; this makes it very difficult to analyze males and prepubescent animals. However, with recent development and advances in DNA technology to identify genes, QTL and SNPs, many of these pitfalls can be overcomed because DNA extraction and analysis is not limited by age, sex or time.

Milk proteins have been grouped into casein and whey. Casein accounts for about 80% while whey accounts for 20% of milk protein (*Hoffman and Falvo, 2004*). Beta-lactoglobulin (β-Lg) is a lipocalin, a widely diverse family, most of which bind small hydrophobic ligands and thus may act as specific transporters, as does serum retinol binding protein. In bovine, β-Lg gene is located on chromosome 11 (*Berry et al., 2010*).

Milk proteins exhibit genetic polymorphism at both protein and DNA levels and this polymorphism could be due to amino acid substitution or deletion of small peptides along the polypeptide chain (*Chin, 1998*). Today, a number of studies have indicated that milk production, composition and quality are affected by genetic variants of milk proteins (*Chin, 1998*). For instance, it has been reported that β-casein A2 and A3 and K-casein are associated with higher milk yield when compared with other variants (*Ng-Kwai-Hang et al., 1986*). Therefore, identification and probing of milk protein variants provides an important tool for complementing traditional breeding methods in improving the yield and quality of milk and dairy production (*Chin, 1998*). This study aimed at examining the genetic diversity of β-Lg gene *in-silico* especially on its evolution and differentiation within and among species and also examining the attendant effects of polymorphism on its functionality in the selected mammalian species.

Materials and Methods

Sequences Retrieval

A total of twenty-one (21) β-Lg sequences from six (6) species [cattle (4), buffalo (4), sheep (3), goat (3), pig (3) and horse (4)] were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The GenBank accession numbers of the sequences are: U60978, U60356, NM_001082494, NM_001082493 (Horse); AY962046, X54976, NM_213754 (Pig); Z19569, FR823504, X58471(Goat); FR821305, FR821309, AY515301 (Sheep); M19088, AJ296332, Z11996, X63139 (Cattle) and JF274007, FN377869, HQ398854, AJ005429 (Buffalo).

Multiple Sequence Alignment (MSA) and Translation

Sequences' alignment, translation and comparison were done with Clustal W as described by *Larkin et al*. (*2007*) using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66.

Test of Selection

The relative proportion of non-synonymous substitution per non-synonymous site (d_N) and the number of synonymous substitutions per synonymous site (d_S) were estimated using the method of *Nei and Gojobori* (*1986*). The ratio of non-synonymous to synonymous divergence "ω" or (d_N/d_S) was also tested for departure from the neutral expectation of unity using the codon based Z-distribution model as modified by Nei-Gojobori, applying proportion correction.

Functional Analysis

In-silico functional analysis of non-synonymous mutation was estimated using PANTHER (*Thomas et al., 2003*). PANTHER tools estimates the likelihood of a given non-synonymous (mutation) Coding SNP to initiate a functional change in protein. The subPSEC (substitution position-specific evolutionary conservation) score was also estimated based on alignment of evolutionarily related proteins. The probability that a given variant will cause a deleterious effect on protein function is estimated by $P_{deleterious}$, such that a subPSEC score of -3 corresponds to a $P_{deleterious}$ of 0.5 (*Brunham et al., 2005*). The subPSEC score is the negative logarithm of the probability ratio of the wild-type and mutant amino acids at a particular position. PANTHER subPSEC scores, derived from the probabilities of observing the variant amino acids in a PANTHER Hidden Markov Model (HMM), are continuous values from 0 to -10. When subPSEC = 0, the substitution is interpreted as functionally neutral, whereas more negative values of subPSEC predict more deleterious substitutions *(Brunham et al., 2005*). The published SNPs used for this study include: 10 (cattle): Gly64Asp, Ala118Val, Gln59His, Glu45Gln, Glu158Gly, Pro50Ser, Asp130Tyr, Ile78Met, Glu108Gly, Pro126Leu and 2 (sheep): Tyr20His, Arg148Glu.

Phylogenetic Analysis

Neighbor-Joining NJ tree was constructed each using P-distance model and pairwise deletion gap/missing data treatment. The construction was on the basis of genetic distances, depicting phylogenetic relationships among β-Lg nucleotide sequences of the investigated species. The reliability of the tree was calculated by bootstrap confidence values (*Felsenstein, 1985*), with 1000 bootstrap iterations using MEGA 5.1 software *(Tamura et al., 2011*). Similarly, UPGMA tree for each gene was also constructed with consensus sequences; using same model as that of the NJ tree. All sequences were trimmed to (500bp) equal length corresponding to same region before generating the tree.

Results and Discussion

In Table 1 is shown the estimated distance matrix for β-Lg between consensus sequences of 6 mammalian species. Maximum Dxy was between goat and horse and minimum Dxy was between sheep and goat. The average genetic distance Dxy is an index of divergence within and across species; where Dxy=distance between sequence x and sequence y. The higher the value of Dxy the far apart the two species are. Amongst ruminants, the maximum Dxy value of 0.62 obtained between cattle and buffalo is also evident on the dendrogram which shows evidence of trans-species evolution. The high value between these two species might have arisen from the different regions of DNA used for this study or probably, the included sequences are the divergent regions of the two species. The minimum value between sheep and goat is consistent with recent molecular grouping of sheep and goat. Also the maximum value between goat and horse is consistent with classical grouping as goat is expected to be closer to sheep, cattle, buffalo and pig than horse. This is similar to the findings of *Vincent et al*. (*2014*) on β-CN, who reported maximum Dxy between goat and horse.

Table 2 shows the means of d_S and d_N, omega (ω), z-statistics and probability. The 'ω' value reveals that evolution is driven by positive selection for only pig and buffalo whereas all other species are under purifying selection. Insight into the mechanism by which natural selection drives gene functional diversification across different species and lineages is a key issue in biology (*Toll-Riera et al. 2011; Yakubu et al. 2013a*).

Table 1: Evolutionary Divergence of β-Lg between Species

	Cattle	Buffalo	Goat	Sheep	Pig	Horse
Cattle		0.02	0.03	0.03	0.03	0.03
Buffalo	0.62		0.03	0.03	0.03	0.03
Goat	0.53	0.57		0.01	0.03	0.03
Sheep	0.55	0.57	0.05		0.03	0.03
Pig	0.55	0.57	0.57	0.59		0.03
Horse	0.63	0.59	0.65	0.64	0.61	

Values above the diagonal represent standard error estimate(s) while those below the diagonal are the average genetic distances between species.

Table 2: Mean Numbers of Nucleotide Substitutions per Synonymous Site (d_S) and per Non-Synonymous Site (d_N) With Their Ratio In β-Lg Among Selected Extant Species

Species	Codons	d_S (±SE)	d_N (±SE)	ω	Z-Statistics	P-value
Buffalo	33	1.05±0.08	1.14±0.05	1.09	5.34	0
Cattle	27	1.96±0.12	1.29±0.06	0.66	-0.14	0.89
Sheep	45	0.80±0.07	0.56±0.03	0.7	-0.39	0.7
Goat	44	0.77±0.07	0.53±0.03	0.69	-0.01	0.98
Pig	39	0.47±0.06	0.50±0.03	1.06	3.26	0
Horse	48	0.44±0.08	0.24±0.03	0.55	-0.18	0.85

ω = omega or (d_N/d_S), d_N = relative proportion of non-synonymous substitution per non-synonymous site, d_S= the number of synonymous substitutions per synonymous sites

The varying substitutions of amino acids within and across species might be as a result of separate divergence from their common ancestor. According to *Marini et al*. (*2010*), as orthologs diverge from their most recent common ancestor, their different evolutionary trajectories lead to divergence in the selective constraints on homologous sites. The comparison of the number of non-synonymous substitution per non-synonymous sites (d_N; amino acid altering) to the number of synonymous mutations per synonymous sites (d_S; silent mutation) also known as omega ($\omega = d_N/d_S$); is a useful estimate of gene selective pressure (*Yakubu et al. 2013a*). *Zhang et al*. (*2005*) noted that omega (ω) >1 implies positive selection, that is, selection has caused some amino acid substitutions that are non-deleterious and that the operative effect of purifying selection is not strong enough to overcome the effect of positive selection. In this study, the null hypothesis of strict neutrality (d_N-d_S) was rejected. The estimated (ω) values which range from 0.55-1.09 symbolize the operation of both positive and purifying selection. Only pig and buffalo had ω >1 signifying positive selection; while other

species with ω<1 are under purifying selection. *Yakubu et al*. (*2013b*), in their study on MHC reported that, positive selection favours new variants thereby increasing allelic polymorphism *(Bergstronm and Gyllensten, 1995*) which in turn favours the ability of antigen-presenting cells to bind a wide array of self and non-self-peptides, thus conferring higher resistance to infectious diseases (*Hedrick and Kim, 2000*). Mutation in pig and buffalo increases fitness (*Kosakovsky Pond, 2011*) and this is expected to reflect on performance. The d_N-d_S shows the surplus of beneficial or deficit of deleterious non-synonymous substitution. The high 'ω' values might enhance retinol binding, immune response and metabolism of phosphate in the mammary gland (*Kontopidis et al., 2003*)

Table 3 depicts predicted effect of non-synonymous amino acid variants in β-Lg gene. Several SNPs have been reported to serve as biomarkers for exploring the genetic bases of disease conditions (*Tariq et al., 2013*) and production traits. The prediction of SNPs status is promising in modern genetics analysis and breeding programmes as they have been used to identify those animals with higher breeding value. But it is still a great challenge to identify functional SNPs in production or disease related gene. However, computational approach has helped in overcoming this challenge and this has increased the success rate of genetic association studies. Major interest in both human and animal genetics is to distinguish mutations that increase fitness from those that reduce fitness (*Tariq et al., 2013*).

Table 3: Prediction of the Effect of Non-Synonymous Amino Acid Variants in β-Lg Gene

Mutated amino acid	SubPSEC score	P deleterious	P Substituted	Wild-type
Cattle				
Gly64Asp	-	-	-	Lys
Ala118Val	-	-	-	Lys
Gln59His	-	-	-	His
Glu45Gln	-	-	-	Asp
Glu158Gly	-	-	-	Lys
Pro50Ser	-	-	-	Asp
Asp130Tyr	-	-	-	Pro
Ile78Met	-	-	-	Trp
Glu108Gly	-	-	-	Lys
Pro126Leu	-	-	-	Asn
Sheep				
Tyr20His	-	-	-	Ile
Arg148Glu	-	-	-	Asp

In this study, a total of 12 SNPs were sourced and probed, however, all the probed SNPs were beneficial i.e. protein function is not altered in anyway. Therefore, for milk improvement programmes, all probed SNPs should be noted.

Figure 1 depicts the deduced amino acid by ClustalW excluding sites with missing/ambiguous data and gaps. A greater level of polymorphism was shown within and across species. The topology of distance-based β-Lg NJ-tree is a clear reflection of trans-species evolution (figure 2) which could be attributed to the different regions used for the study. Random clustering of sequences was exhibited by all the studied species except for horse whose entire sequences aggregated. This distinct embranchment shown by horse is premised on the identical promoter region. Generally, the random clustering may largely be due to regional variability such that those of similar region clustered together. On the contrary, the UPGMA tree revealed species-wise evolutionary history (figure 3); a congruence with classical taxonomy.

#BUFFALO_JF274007	EAVPAWHSCV	TCASPPPPQG	PWRSGGGDSF	SGGALSSHGW	GRCPRPISPG	SRRLPGRH	[184]
#BUFFALO_FN377869	GLT..Q.AGP	E.PEGAAHRR	SC.M.NEARS	LKKPSLVQAL	TKSLCLALH.	AKPV.D.R	[184]
#BUFFALO_HQ398854	YFGYFQYKVF	F.FVQGAGEK	HLLLINSI.S	LVWGPWGGLP	MWVCSLMLGD	NELAHQKQ	[184]
#BUFFALO_AJ005429	GLT..Q.AGP	E.PEGAAHRR	SC.M.NEARS	LKKPSLVQAL	TKSLCLALH.	AKPV.D.R	[184]
#CATTLE_M19088	PLT.VQ.AGP	Q.PEGAAHRR	SC.M.NEARS	LKKPSLVQAL	TKSLCLALH.	AKPV.D.R	[184]
#CATTLE_AJ296332	GLS..Q.AGP	E.PEAAAFRR	SC.MVSKAHS	PIWTPGVQAP	NGAWSWGCSR	FNFMLNKT	[184]
#CATTLE_Z11996	PLA.SRLVSS	V.ILCVVFKH	.RP.SNETPL	.DTP.LPYHP	DEWFTLTPAS	QGYELETP	[184]
#CATTLE_X63139	.GSL.RELGL	K.WEVLAFHW	SSALSCRGFP	..LL.LCCLP	SG.LQ.GARE	P.LVTRY.	[184]
#GOAT_Z19569	GLT..Q.AGP	E.PEGAAHRR	SC.M.NEARL	LKKPSLVQAL	TKSLCLALH.	AKPV.D.G	[184]
#GOAT_FR823504	MV.HELLPEW	WHFLERACLP	.CIPSRSA.L	.LTPSKPC.L	..A.H.PLDQ	HGQGS.AR	[184]
#GOAT_X58471	GLT..Q.AGP	E.PEGAAHRR	SC.M.NEARL	LKKPSLVQAL	TKSLCLALH.	AKPV.D.G	[184]
#SHEEP_FR821305	VV.HELLPEW	WHFLEQACLP	.CIPSKSA.L	.LTPSKPC.L	..ASH.PLDQ	RG.GS.AC	[184]
#SHEEP_FR821309	VV.HELLPEW	WHFLEQACLP	.CIPSKSA.L	.LTPSKPC.L	..ASH.PLDQ	RG.GS.AC	[184]
#SHEEP_AY515301	.FGILLSREF	KNFNNWVHYV	Q.EVHERFP.	YTCMAHDQLP	AGA.GRTDAQ	LDS.QDCA	[184]
#PIG_AY962046	RLAG.RA.ER	GEDGAVGWDW	A.SWTSRVG.	..HSAPGRSS	ILAANL.AE.	PDLSSSIL	[184]
#PIG_X54976	GHT..QRAGQ	EQPEGAAHRR	SC.A.NKACS	LKKPSLVQAL	TSSSCLAPH.	PQPE.D.P	[184]
#PIG_NM_213754	GHT..QRAGQ	EQPEGAAHRR	SC.A.NKACS	LKKPSLVQAL	TSSSCLAPH.	PQPE.D.P	[184]
#HORSE_U60978	GGT.VQ.PGR	E.SEGAAHRR	SCAR.NALTL	WRRLTQV.YQ	E.F.CLVLC.	R.HV.D.G	[184]
#HORSE_U60356	GST..Q.PGR	E.SEEAVHRR	LCVR.N.ARS	LVRLALVQYL	T.SL.LVPHE	TEPV.DWG	[184]
#HORSE_NM_001082494	RGT.VQ.PGR	E.SEGAAHRR	SCAI.DAVTL	WRRLTQV.YQ	D.F.CLVLC.	R.HV.D.G	[184]
#HORSE_NM_001082493	GST..Q.PGR	E.SEEAAHRR	SCVR.N.ARS	LKRLAQVQYL	T.SL.LVPHE	TEPV.DWG	[184]

Figure 1. Amino acid prediction of mammalian β-Lg, deduced by ClustalW excluding sites with missing and ambiguous data and gaps Dots indicated identical amino acids and numbers on the right hand side represent site number.

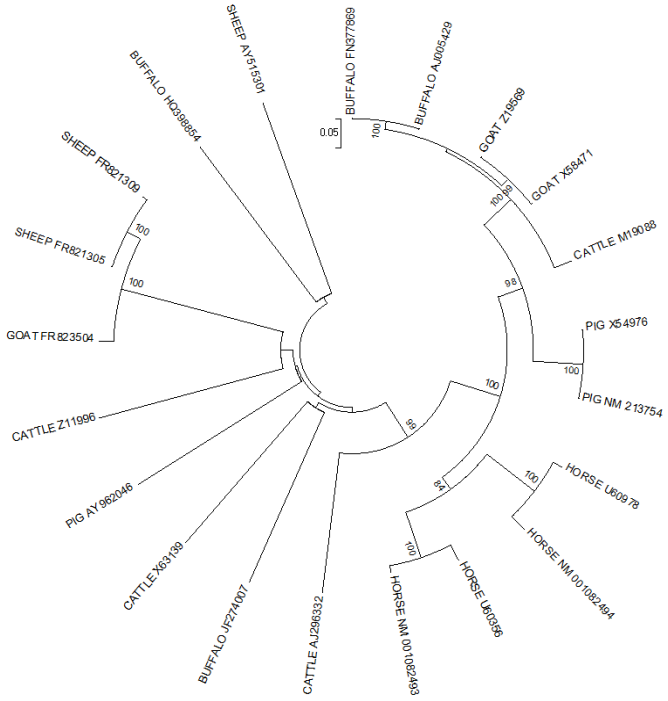

Figure 2. Phylogenetic tree of mammalian β-Lg computed using NJ-method

.

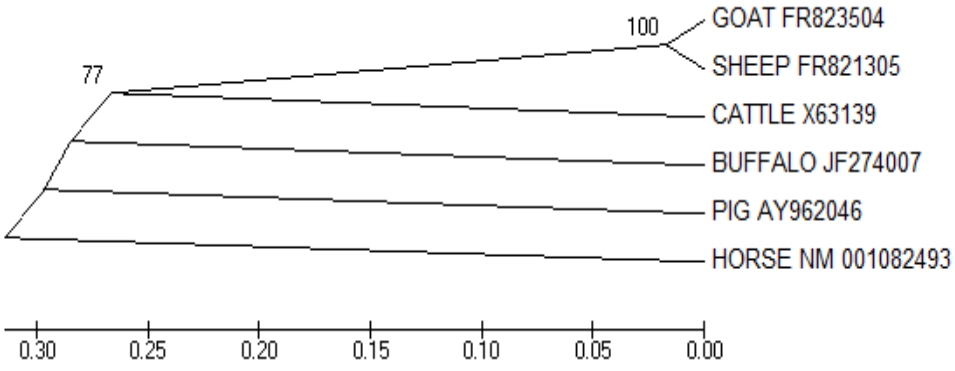

Figure 3. Phylogenetic tree derived from consensus sequences of each of the selected species using the UPGMA method.

Conclusion

In this study, the hypothesis of strict neutrality (d_N=d_S) was rejected for all extant species as allelic sequence evolution was driven by both purifying and positive selection. However, only those of pig and buffalo were driven by positive selection. *In-silico* functional analysis of non-synonymous mutations using PANTHER revealed that, all the 12 amino acid substitutions (10 in cattle and 2 in sheep) did not impair protein function. The phylogeny revealed trans-species evolution, but was species-wise on with consensus sequences.

Kompjuterska analiza gena beta-laktoglobulina kod nekoliko odabranih vrsta sisara

O.M. Momoh, S.T. Vincent, A. Yakubu

Rezime

Cilj ove studije je kompjutersko ispitivanje genetske raznolikosti β-Lg, njihove evolucije i diferencijacije unutar i između određenih vrsta sisara; takođe ispituje prateće efekte polimorfizma na funkcionalnost gena. Ukupno 21 sekvenci β-Lg gena sa odgovarajućim aminokiselinama koje pripadaju 6 vrsta [goveda (4), bivoli (4), ovce (3), koze (3), svinje (3) i konji (4)] su preuzeti iz GenBank (www.ncbi.nlm.nih.gov). Sve sekvence su obrađene do jednake dužine (500bp), i odgovaraju istom regionu. Poravnanje sekvenci, prevod i upoređivanje je urađeno pomoću ClustalW koristeći IUB supstitucije matrice, otvor od 15 i 6.66. Poravnjanje je otkrilo visok polimorfizam sekvenci. Dxy izveden korišćenjem p-distance otkrio je da su kod ovaca i koza postojale najmanje distance od 0.05 sa maksimalnim rastojanjem od 0,65 između koza i konja. Hipoteza o strogoj neutralnosti ($d_N = d_S$) je odbijena jer je evolucija alelske sekvence pokrenuta i vođena kako prečišćavanjem tako i pozitivnom selekcijom. Samo u slučaju svinja i bivola, su vođeni pozitivnom selekcijom. Kompjuterska funkcionalna analiza ne-sinonimnih mutacije korišćenjem PANTHER otkrila je da nijedna od 12 supstitucija amino kiselina (10 kod goveda i 2 kod ovaca) ne narušava funkciju proteina. Prema tome, nijedan analizirani SNP u ovoj studije nema štetan efekat i može se tolerisati od strane odgajivača pri izboru grla za poboljšanje proizvodnje mleka.

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SOURCES OF VARIABILITY OF GROWTH AND BODY DEVELOPMENT TRAITS OF SIMMENTAL BULLS IN PERFORMANCE TEST

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Abstract: To test the variability of traits of Simmental bulls in performance test, data of the Livestock - Veterinary Centres for Reproduction and Artificial Insemination of Velika Plana and Krnjača were used. In the analysis, data on 113 performance tested bulls born from 2008 to 2009 were used. The analysis included two sets of characteristics: body development traits and growth traits. The average body mass of calves entering the test was 195.75 kg, while the body mass at the end of the test was 476.50 kg, average daily gain in the test was 1138.69 g. Average values of body development traits measured at the end of the test, with 12 months of age were: height at withers 127.13 cm, chest circumference 179.42 cm, the chest depth 61.19 cm and body length 151.34 cm. The influence of their sires, the year and the Centre on the variability of traits was studied. The effect of age is present at a high level of statistical significance ($p<0.01$) for all traits that are registered at the end of the test, while the effect of the Centre was present in the variability of body mass at the end of the test, the daily gain in the test and the length of the body. The bulls-sires' influence was demonstrated ($p<0.05$) on the variability in body mass of calves entering the test.

Key words: Simmental breed, bulls, growth traits, body development, variability of traits, performance test

Introduction

Simmental cattle belong to the combined type, which means almost equal economic importance of milk and meat production. It belongs to the big breeds that have the genetic potential for intensive improvement of both components. Therefore, proper selection and selection of young bulls as future breeding bulls

should ensure improvement of traits and improvement of production of milk and meat (*Bogdanović, 2001*).

In assessing the breeding value of performance test bulls is one of the main animal husbandry practices, which determines the genetic improvement of a certain group of properties. Selection based on the results of performance test, is of special importance for traits that are characterized by medium to high heritability values *(Bogdanović, 2001).*

Performance test is used for production traits which can be determined or measured in each individual animal. This process is known as direct test because its application covers control of production traits that are directly measured on animals still in development.

The performance test of Simmental bulls in Serbia officially started to be performed during 1982 in Test station at the Center for Artificial Insemination in Velika Plana. From the very beginning, the adopted test technique was in compliance with all recommendations of the European Zootechnical Federation *(Bogdanović, 2001).*

The study of of growth and body development traits of performance tested Simmental bulls, was topic of research of several researchers in Serbia. The variability of characteristics and influence of individual factors on the variation, values of heritability, phenotypic and genetic correlations of mentuioned traits are stated in the studies of *Perković* (1999), *Romčević,* (1999) and *Bogdanović* (1999, 2001, 2002, 2003, 2006, 2007).

Bearing in mind the importance of Simmental breed in cattle production of Serbia, as well as the lack of research associated with this breed, the aim of this study was to determine the average expression and variation of traits of growth and body development, and then to determine the influence of certain genetic and non-genetic factors on traits measured in performance test.

Material and methods

To test the variability of growth and body development traits of Simmental bulls in performance test, data of the Livestock Veterinary Centres for Reproduction and Artificial Insemination (SVC) from Velika Plana and Krnjača were used. Bulls included in the test are taken to the Centre based on the application of the owner or holder of the bull, from private and state farms. Before bringing the young bulls to the Centre, examination and evaluation are carried out to determine whether they meet the basic requirements to enter the test.

Selected male calves come into the station at the age of about three months, they are placed in quarantine and adjust to conditions of housing and nutrition for at least 30 days, in order to eliminate as much as possible pre-existing effects. After a preparatory period, at the age of 4 months, the test starts and lasts

until the age of one year. Bulls in the test are held in adequate groups, not more than 5 animals in the group, formed in relation to age. Basic forage - alfalfa hay is given at will, while the amount of concentrate is limited by age. During the test, in regular one-month intervals, the body weight and the most important dimensions of the body (withers height, chest circumference, breast depth, width of round, pelvic width and length of the body) are measured. At the end of the test, the average daily gain in the test is calculated and it is a key feature on which the evaluation of bulls in the test is based.

Data on 113 performance tested bulls born from 2008 to 2009 were used in the analysis. In 2008, 72 bulls were in the test while in 2009 41 Simmental bulls were tested. In the LVC Velika Plana, from 2008 to 2009, 91 Simmental bulls finished the test while in the same period in Krnjača 22 bulls were tested. Distribution of performance tested bulls by years and centres is shown in Tables 1 and 2.

Table 1. Distribution of performance tested bulls at centres

AI Centre	V. Plana	Krnjača
No of tested bulls	91	22

Table 2. Distribution of performance tested bulls by years

Year	2008	2009
No of tested bulls	72	41

The analysis included two sets of characteristics: body development traits and growth traits.

Body development traits are represented by the linear dimensions of the body measured at the end of the test, at 12 months of age: height at withers, chest circumference, chest depth and body length.

The following growth traits are included: body weight at the beginning of the test (with 4 months of age), body weight at the end of the test (with 12 months age), average daily gain during the test.

The most attention in the test is directed towards the average daily gain during because it fully reflects the capacity and intensity of the growth of the animal, and therefore its predisposition to a particular form of production.

Statistical analysis of data obtained during the performance test was divided into two parts.

The first part of the analysis included the determination of the basic variation-statistical parameters:

- Arithmetic mean (X),
- Variation range (*Min-Max*),

- Standard deviation (*SD*),
- Coefficient of variation (*CV*).

Descriptive statistics analysis was performed using the statistical program *StatSoft.Inc (2004), Statistica for Windows version 7.*

The second part of the data processing included the identification of various influences on traits variability in performance test. Analysis of the influence of non-genetic and genetic sources of variability was performed by the method of least squares *LSMLMW*. To analyse the influence of non-genetic sources of variability a fixed model with fixed effect of birth and centre is used.

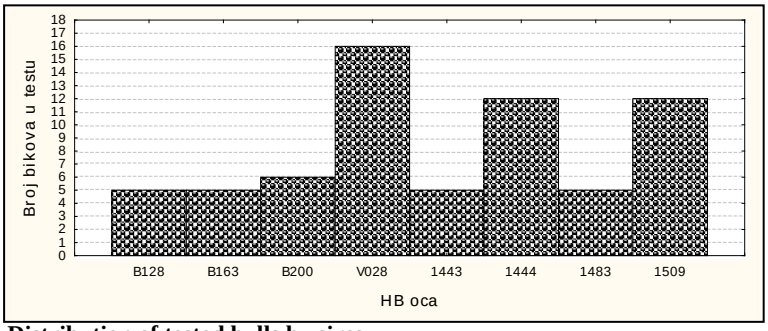
1. Fixed model for analysing the impact of non-genetic sources of variability of traits in performance test:

$$Y_{ijk} = \mu + G_i + C_j + e_{ijk}$$

where:

- Y_{ijk}: studied trait,
- μ: population average for said trait,
- G_i: fixed effect of i-th year of birth of the bull (i=1, 2),
- C_j: fixed effect of j-th centre (j=1, 2),
- e_{ijk}: random error with characteristics N $(0,\sigma^2)$.

To analyse the influence of sires on the variability of traits in the performance test of the basic sample, a sub-sample of 66 bulls originating from 8 sires was formed. For this subsample all sires with 5 and more tested sons were selected. Distribution of tested bulls by fathers is shown in Graph 1.



Graph 1. Distribution of tested bulls by sires

In this part of the analysis the mixed model with random influence of the sire was applied.

2. Mixed model for analysing the impact of sires on variability of traits in performance test:
$Y_{ijkl} = \mu + G_i + C_j + O_k + e_{ijkl}$

where:

- Y_{ijkl}: studied trait,
- μ: population average for said trait,
- G_i: fixed effect of i-th year of birth of the bull (i=1, 2),
- C_j: fixed effect of j-th centre (j=1, 2),
- O_k: random error of k-th sire (k=1,…,8),
- e_{ijkl}: random error with characteristics N $(0,\sigma^2)$.

In countries with developed cattle breeding, performance test is practically no longer performed in the test stations but in the production conditions of the population or is replaced by other methods of selection. Although the number of bulls tested at the centres decreases each year, in Serbia it is still justified given that the progeny test on slaughter traits is not performed.

Results and Discussion

Table 3 presents the descriptive statistical indicators and variability of traits in the performance test of Simmental bulls.

Table 3. Mean values and variability of traits in performance test of Simmental bulls

Trait	*X*	*Min*	*Max*	*SD*	*CV(%)*
Initial body mass, beginning of test, kg	195.75	100	300	36.59	18.69
Body mass at the age of 12 months, kg	476.50	336	685	59.44	12.47
Daily gain in the test, g	1138.69	570	1740	231.65	20.34
Height at withers, cm	127.13	116	136	3.30	2.60
Chest depth, cm	61.19	42	70	4.21	6.88
Chest circumference (girth), cm	179.42	151	210	9.49	5.29
Body length, cm	151.34	125	169	6.41	4.24

Table 4 shows the influence of non-genetic sources of variability of traits in the performance test of Simmental bulls.

The average body mass of calves entering the test was 195.75 kg, which is consistent with the findings of *Bogdanović* (2006). Body mass of young bulls at the beginning of the test is characterized by a wide range of variation. *Perković et al (1999)* state that the average body mass of calves entering the test in Krnjača was 233 kg.

The year and centre showed no statistically significant influence ($p>0.05$) on the variability of body mass at the start of the test which leads to the conclusion that the body mass of young bulls is more influenced by the farm of their origin. Due to the different climatic conditions in which farms are located, feeding and housing that are designated as farm management, body mass at the beginning of the test is more influenced by pre-test factors. The body mass of calves entering the test is heavily influenced by maternal effects and housing/rearing system prior to weaning.

The average body mass of bulls at the end of the test was 476.50 kg, while slightly higher values (515.86 kg) for Simmental bulls tested in the LVC Velika Plana are reported by *Bogdanović* (2006). *Perković et al (1999)* found that the bulls in Krnjača ended the test with a body mass of 509 kg. Body mass at the end of the test varied less in relation to initial body mass of calves entering the test as a consequence of standardized conditions for feeding, housing, etc. Year and centre statistically significantly ($p<0.01$) influenced variation of body mass at the end of the test.

Average daily gain in the test was 1138.69 g with a coefficient of variation of 20.34, which is consistent with the results obtained by *Perković et al (1999).* The high variability ($p<0.01$) was statistically significantly influenced by the year and centre.

Table 4. The influence of non-genetic factors on the variability of traits in the performance test of Simmental bulls, F values (model 1)

Trait	μ	Se	F values of tested effects	
			Year	*AI Centre*
			df1=1	*df1=1*
			df2=110	*df2=110*
Initial body mass, beginning of test, kg	198.18	4.81	2.91^{NZ}	0.05^{NZ}
Body mass at the age of 12 months, kg	492.74	6.44	8.49^{**}	28.52^{**}
Daily gain in the test, g	1171.91	25.39	18.69^{**}	14.54^{**}
Height at withers, cm	126.70	0.40	20.38^{**}	0.02^{NZ}
Chest depth, cm	60.98	0.53	9.75^{**}	0.22^{NZ}
Chest circumference (girth), cm	179.66	1.13	15.04^{**}	3.22^{NZ}
Body length, cm	152.68	0.72	10.75^{**}	19.21^{**}

$p>0.05^{NZ}$, $p<0.05^{*}$, $p<0.01^{**}$

The height of withers and depth of chest at the end of the test were not under significant ($p>0.05$) effect of centre, but the influence of year was significant source of variability ($p<0.01$). The average height to withers at the end of the test was 127.13 cm with a coefficient of variation of 2.60, the average depth of the chest was 61.19 cm with a coefficient of variation of 6.88, which is in line with the results presented in the paper by *Bogdanović (2007).* An average height at the withers of 127.1 cm is reported by *Perković et al. (1999).*

The variability of chest circumference and body length were under statistically significant ($p < 0.01$) of the year, while the variability in the length of the body also included the effect of the centre. The average chest cir4cumference (girth) of Simmental bulls at the end of the performance test was 179.42 cm and the average body length 151.34 cm. Comparing the results of this study with the results obtained by *Perković et al. (1999)*, the bulls at the end of the test had smaller chest size but greater body length compared to bulls tested in Krnjača in the period from 1992 to 1997.

By perceiving the traits of body development and growth in the monitored performance test much lower variability of body development traits can be observed. Given that both sets of characteristics are under the influence of same abiotic factors, it can be concluded that the body development of young bulls is under greater genetic influence.

The effect of the year is present at a high level of statistical significance ($p<0.01$) for all traits that are registered at the end of the test. The impact of the year includes the climatic factors, the schedule and amount of precipitation which is reflected in the quantity and quality of available food.

Although the performance test technique is the same, the influence of centre was statistically significant ($p < 0.01$) on the variability of growth traits measured at the end of the test and the body length. The effect of the centre is reflected in the geographical environment of the station, food quality, conditions in the test, management of the animals, etc.

Table 5. The influence of sires on the variability of traits in the performance test of Simmental bulls, F values (model 2)

Trait	μ	Se	F values of tested effects		
			Sires	*Year*	*AI Centre*
			df1=7	*df1=1*	*df1=1*
			df2=56	*df2=56*	*df2=56*
Initial body mass, beginning of test, kg	192.48	10.46	2.29^{*}	0.01^{NZ}	0.74^{NZ}
Body mass at the age of 12 months, kg	486.79	11.04	1.41^{NZ}	2.71^{NZ}	10.72^{**}
Daily gain in the test, g	1164.48	35.86	0.88^{NZ}	2.62^{NZ}	7.49^{**}
Height at withers, cm	127.09	0.82	1.78^{NZ}	7.04^{**}	0.96^{NZ}
Chest depth, cm	61.22	0.77	1.03^{NZ}	3.29^{NZ}	1.28^{NZ}
Chest circumference (girth), cm	177.68	1.64	0.93^{NZ}	10.71^{**}	0.82^{NZ}
Body length, cm	152.21	1.00	0.58^{NZ}	6.87^{**}	6.56^{**}

$p>0.05^{NZ}$, $p<0.05^{*}$, $p<0.01^{**}$

Table 5 shows the influence of sires on the variability of the traits in the performance test of Simmental bulls.

The effect of the bull sires ($p < 0.05$) was present in regard to the variability in body mass of calves entering the test, while their impact on the variability of traits measured at the end of the test was not significant. The reason is very different conditions on farms of origin of bulls entering the performance test. Lack of influence of sires on the variability of traits measured at the end of the test is explained by the effect of compensatory growth with a standardized feeding conditions, housing and care in the performance test will enable the young bulls, future breeding males, to fully exert additive genetic value, on which the estimates of breeding values are based.

Conclusion

Standardizing of conditions for feeding, housing and care in performance test provides the young bulls, future breeding males, to fully exert additive genetic value, on which the estimates of breeding values are based. In the variability of the monitored traits in the performance test, the effect of year was present at a high level of statistical significance ($p<0.01$) for all traits that are registered at the end of the test, while the AI Centre showed statistically significant ($p < 0.01$) influence on the variability of growth traits measured at the end of the test and the variability of body length. The effect of the bull sires ($p<0.05$), was present on the variability of body mass of calves entering the test, while their impact on the variability of trait measured at the end of the test, showed no statistical significance.

Properties covered by the performance test are characterized by medium to high heritability values indicating that they are hereditary enough to exert selection on them. The results obtained indicate that the traits of growth and body development contained sufficient variability for the successful selection and improvement of the traits of domestic Simmental cattle.

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Izvori varijabilnosti osobina porasta i telesne razvijenosti bikova simentalske rase u performans testu

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Rezime

Za ispitivanje varijabilnosti osobina simentalskih bikova u performans testu iskorišćeni su podaci stočarsko-veterinarskog centra za reprodukciju i veštačko osemenjavanje iz Velike Plane i Krnjače. Za analizu su upotrebljeni podaci o 113 performans testiranih bikova rođenih u periodu od 2008 do 2009 godine. Analizom su obuhvaćene dve grupe osobina: osobine telesne razvijenosti i osobine porasta. Prosečna telesna masa sa kojom su telad ulazila u test iznosila je 195,75 kg, dok je telesna masa na kraju testa 476,50 kg, prosečan dnevni prirast u testu iznosio je 1138,69 g. Prosečne vrednosti osobina telesne razvijenosti merenim na kraju testa, sa 12 meseci uzrasta iznosile su: visina grebena 127.13 cm, obim grudi 179.42 cm, dubina grudi 61.19 cm i dužina trupa 151.34 cm. Analizirani su uticaj očeva, godine i centra na varijabilnost osobina. Efekat godine je prisutan na visokom nivou statističke značajnosti ($p<0,01$) za sve osobine koje se registruju na kraju testa, dok je efekat centra prisutan u varijabilnosti telesne mase na kraju testa, dnevnog prirasta u testu i dužini tela. Bikovi-očevi su ispoljili uticaj ($p<0,05$) na varijabilnost telesne mase sa kojom su telad ulazila u test.

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EFFECT OF *SACCHAROMYCES CEREVISIAE* SUPPLEMENTATION ON HEALTH AND PERFORMANCE OF DAIRY COWS DURING TRANSITION AND EARLY LACTATION PERIOD

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Abstract: Data concerning the effect of probiotics supplementation on many parameters concurrently at the same cows are lacking. Therefore, the objective of this experiment was to investigate the effects of *Saccharomyces cerevisiae* feeding on rumen, blood and milk parameters together in high-producing dairy cattle during the transition and early lactation period. Sixteen clinically healthy Holstein cows were divided into 2 groups: a control group of 6 cows and a probiotics-fed group of 10 cows. Rumen fluid and blood samples were collected 21 days before the expected calving as well as 7, 15, 30, 45 and 60 days-in-milk (DIM). Milk yield for each animal was recorded every 2 weeks. Individual milk samples were collected 15, 30, 45 and 60 DIM. Ruminal pH and rumen ammonia nitrogen were significantly lower, whereas total volatile fatty acids were significantly higher in yeast-fed animals compared with controls throughout the study. Serum concentrations of total proteins and globulins were higher, while albumins were lower in the yeast-treated group. Serum glucose levels were significantly higher in yeast-supplemented animals. Serum triglycerides, high density lipoproteins, and low density lipoproteins concentrations were lower, with cholesterol being significantly lower in the treated group. Milk production and milk fat percentage were higher, whereas milk protein percentage and somatic cell count were decreased in yeast-supplemented cows throughout the study. These results suggest that supplementation of *S. cerevisiae* to dairy cows rations during transition and early lactation period improve their health and milk production parameters.

Key words: blood biochemical parameters, cows, *Saccharomyces cerevisiae*

Introduction

The transition period of a dairy cow is defined as the change from the pregnant, non-lactating state to the non-pregnant, lactating state; it lasts from 3 weeks pre-partum until 3 weeks postpartum (*Goff and Horst, 1997*). It is characterised by numerous changes in physiological, metabolic and endocrine status to accommodate parturition and lactogenesis (*Grummer, 1995*). If nutritional management does not meet these challenges, the transition cow is at high risk of developing a wide range of health problems soon before and, mainly, after parturition *(Bell, 1995)*, like milk fever, fatty liver, ketosis, retained placenta, displaced abomasum, and suppressed immune function (*Goff and Horst, 1997*).

Probiotics are beneficial for animals, affecting their health and production by various mechanisms that are not yet fully understood *(Shriver-Munsch, 2011)*. Feeding yeast (*Saccharomyces cerevisiae*) or its fermentation products during the transition period may counteract some of those challenges by improving appetite, nutrient utilisation and immune function *(Shriver-Munsch, 2011).* Yeast culture used as a dietary supplement for dairy cattle is thought to improve rumen function, and hence milk production and feed efficiency, by stimulating selective growth of rumen bacteria species (*Harrison et al., 1988*).

Inclusion of *S. cerevisiae* in ruminants' diets has been shown to alter the molar proportion of ruminal volatile fatty acids (VFAs) (*Newblod et al., 1990; Dawson, 1993*), reduce rumen ammonia concentration, increase the number of ruminal bacteria and protozoa and alter the flow of the nitrogen (N) fraction to the duodenum (*Dawson, 1993; Williams et al., 1991*). Furthermore, a study by *Kumar et al. (1994)* showed that supplementation of yeast culture as a growth promoter for buffalo calves resulted in increased rumen pH, total bacteria and protozoa culture counts, total VFAs, total N and microbial protein, with reduced rumen ammonia N concentration and improved digestion of cellulose and dry matter (DM) intake. Other researchers have reported that live yeast and yeast culture supplementation may increase feed intake and milk production of dairy cows (*Robinson and Garrett, 1999; Dann et al., 2000*).

However, each published study has investigated the effect of *S. cerevisiae* feeding to dairy cows selectively on a few parameters (either milk yield or milk composition, or either blood biochemical or ruminal parameters) and, therefore, data concerning its effect on many of the parameters together are lacking. The objective of the present study was to investigate the effect of feeding *S. cerevisiae* concurrently on rumen parameters, blood biochemical parameters, milk production,

and milk composition in high-producing dairy cows during the transition and the early lactation period.

Materials and methods

Probiotics (live yeast)

For the study, *Saccharomyces cerevisiae* live yeast culture (Levucell SC 20®, Lallemond co., France) was used as feed-additive. Each gram of Levucell SC 20® provided 20×10^9 CFU/g of *S. cerevisiae* (CNCM 1-1077).

Experimental design, feeding and management

The present study was carried out in a private farm in Ihnasia city, Beni-Suef governorate in Egypt. Sixteen clinically healthy Holstein cows aged 4-5 years old, with an average body weight of 530±22 kg (mean±SE) were used. The animals were randomly allocated into 2 groups that were similar according to parity, body weight and previous mean total milk yield. The first (group A) was consisted of 6 animals, fed on a diet without yeast supplementation and kept as a control group. The second (group B) was consisted of 10 cows, fed on the same diet as group A plus daily in-feed inclusion of 0.5 g/animal of live yeast culture (*Levucell SC 20*®).

Table 1. Ration composition: Ingredient composition (%) of close-up dry period and early lactation diets on a dry matter (DM) basis.

Ingredients (%)	Close-up dry period diet	Early lactation diet
Corn silage	59.35	50.71
Yellow corn (grain)	12.55	19.39
Soya bean meal	12.10	14.17
Beet pulp	10.27	7.46
Alfa-alfa hay	4.57	5.97
Rumen-protected fat	0.00	0.82
Sodium bicarbonate	0.00	0.48
Monocalcium phosphate	0.11	0.36
Sodium chloride	0.09	0.24
Magnesium oxide	0.00	0.12
Mineral mix[a]	0.09	0.12
Vitamin mix[b]	0.05	0.06
Calcium carbonate	0.00	0.05
Calcium chloride	0.27	0.00
Magnesium sulphate	0.55	0.00

[a] Copper sulphate 2800 mg/kg, cobalt carbonate 300 mg/kg, sodium selenite 25 mg/kg, ferrous carbonate 750 mg/kg, magnesium oxide 250 mg/kg, potassium iodide 100 mg/kg and zinc oxide 150 mg/kg.

[b] Vit. A 10,000 IU/kg, vit. D 1,000 IU/kg and vit. E 20 mg/kg.

The animals were housed in a clean and spacious open yard. Cows were milked by an automated milking machine three times daily and fed on a total mixed ration to meet the recommendations of the National Research Council (*NRC, 2001*).They were accustomed to 2 diets, the close-up dry period diet (fed 3 weeks before expected calving up to the day of parturition) and the early lactation diet (fed from parturition up to 60 days-in-milk - DIM). The ingredients and nutrient composition of the 2 diets are shown in Tables 1 and 2.

Table 2. Ration composition: Chemical composition of close-up dry period and early lactation diets on a dry matter (DM) basis.

Chemical composition	Close-up dry period diet	Early lactation diet
NEL[1] (MJ/kg DM)	6.987	7.238
Crude protein (%)	13.9	14.9
Crude fat (%)	2.74	2.82
Crude fiber (%)	18.5	16.5
NDF[2] (%)	39.0	34.4
ADF[3] (%)	23.2	20.7
Calcium (%)	0.43	0.46
Phosphorus (%)	0.44	0.50
Sodium (%)	0.08	0.26
Magnesium (%)	0.21	0.27
Copper (mg/kg DM)	11.4	11.2
Zinc (mg/kg DM)	24.4	25.2

[1] Net Energy for Lactation; [2] Neutral Detergent Fiber; [3] Acid Detergent Fiber

Samplings and analyses

Rumen and blood samples were collected at the commencement of the experiment (21 days before the expected calving date), as well as 7, 15, 30, 45 and 60 DIM. All animals were clinically examined before each sampling.

Rumen fluid

Rumen fluid was collected from all animals using stomach tubing, 4 hours after the morning feeding. At each sampling 100 mL of rumen fluid were collected into a clean, dry flask. Ruminal pH was immediately measured using a portable digital pH meter (350 portable pH meter, JENWAY, Essex, UK). Rumen fluid samples were kept frozen (-20˚C) for analysis. Rumen ammonia nitrogen concentration was determined according to the method proposed by *Conway*

(1974) and total VFAs samples were assayed by steam distillation, according to the method described by *Abou-Akkada and El-Shazly (1964)*.

Blood samples

Blood samples were collected by jugular venipuncture, 2 hours after the morning feeding**.** Samples were centrifuged at 4000 rpm for 15 min to obtain blood sera, which were stored at -20ºC until analysis.

Serum was tested for total proteins (TP) and albumins (ALB) according to *Doumos et al*. (1971), and then serum globulins (GLOB) were calculated. Serum glucose levels were estimated according to *Trinder (1969*). Concentrations of serum total cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) were measured using commercial kits (SPECTRUM DIAGNOSTICS, Obour City, Egypt).

Milk samples

Milk yield of each animal was recorded every 2 weeks. Individual milk samples of each animal were collected for analysis at 15, 30, 45 and 60 DIM. Fat percentage, protein percentage and somatic cell count (SCC) were measured at Animal Reproduction Research Institute using MilkoScan analyser (FOSS ANA MilkoScan FT 120, GERBER INSTRUMENTS, Effretikon, Switzerland), according to the method proposed by *Zecconi et al. (2002)*.

Statistical analysis

Data were statistically analysed using SAS computer software (*SAS, 1985*). The general linear model function was used for analysis of variance (ANOVA). Statistically significant differences between treatment means were measured by least significant difference and means were considered different at ($P < 0.05$) and at ($P < 0.01$).

Results

All 16 animals remained clinically healthy during the whole experimental period. There was not any statistically significant difference between any of the investigated parameters at the commencement of the experiment (21 days before the expected calving) between experimental groups.

Rumen fluid parameters for control and *S. cerevisiae*-fed animals are shown in Table 3. Ruminal pH was significantly lower at 15 ($P<0.05$), 45 ($P<0.01$) and 60 ($P<0.05$) DIM in yeast-fed animals compared to control ones. The levels of

rumen ammonia nitrogen in yeast-treated cows were significantly lower than controls at 15 ($P<0.05$), 30 ($P<0.01$) and 45 ($P<0.01$) DIM. Total VFAs concentrations were significantly higher in treated animals compared to the control cows throughout the experiment.

Table 3. Effect of feeding *S. cerevisiae* during transition and early lactation period on rumen fluid parameters of Holstein dairy cows.

Groups	Rumen fluid parameters	Days from parturition					
		-21d	+ 7d	+ 15d	+ 30d	+ 45d	+ 60d
A (Control cows)	pH	6.86±0.20	7.30±0.15	7.22±0.16**	7.28±0.28	7.3±0.14*	7.38±0.13**
	Ammonia nitrogen (mg/mL)	15.16±0.81	14.0±1.98	15.96±2.9**	16.52±1.53*	16.8±1.98*	13.16±0.76
	Total VFAs[1] (mg/mL)	2.36±0.25	2.4±0.17**	3.1±0.34*	2.16±0.26*	2.04±0.22**	2.16±0.35**
B (Yeast-fed cows)	pH	6.84±0.18	7.21±0.31	7.01±0.15**	7.16±0.34	6.95±0.19*	7.02±0.26**
	Ammonia nitrogen (mg/mL)	14.84±1.18	12.72±1.76	13.1±1.91**	12.9±1.94*	12.9±2.03*	11.48±1.72
	Total VFAs (mg/mL)	2.36±0.21	2.71±0.27**	4.09±0.2*	2.8±0.38*	2.5±0.39**	2.6±0.37**

[1]Volatile Fatty Acids; * Significantly different *(P<0.01);* **Significantly different *(P<0.05)*

As shown in Table 4, serum TP concentrations tended ($P>0.05$) to be higher in the yeast-supplemented group B than those in the control group A, except for the 45th DIM where they were lower. On the contrary, serum ALB values showed a non-significant ($P>0.05$) reduction in the yeast-supplemented group compared with control ones throughout the study. GLOB concentrations tended ($P>0.05$) to be higher in the yeast-supplemented group, except for the 60th DIM where a significant difference ($P<0.01$) was noted.

Table 4. Effect of feeding *S. cerevisiae* during transition and early lactation period on blood serum parameters of Holstein dairy cows.

Groups	Parameters	Days from parturition					
		-21d	+7d	+15d	+30d	+45d	+60d
A (Control cows)	Total proteins (g/L)	70.1±10.12	70. 4±7.3	77.3±10. 4	79.2±12.5	82.0±8.8	82.6±3.3
	Albumins (g/L)	39.5±1.4	40. 1±2.1	41.1±4.1	42.9±4.7	42.9±5.1	41.5±5.1
	Globulins (g/L)	31.5±3.9	30. 3±4.0	36.2±7.8	36.3±5.1	39.1±7.6	41.1±4.5[3]
	Glucose (mmol/L)	3.73±0.37	3.74±0.37	3.76±0.35	3.70±0.14[3]	3.56±0.21[3]	3.72±0.19[3]
	Triglycerides(mmol/L)	0.31±0.05	0.36±0.04	0.33±0.04	0.39±0.03	0.35±0.06	0.26±0.05[4]
	HDL[1] (mmol/L)	2.02±0.24	2.26±0.34	2.64±0.46	2.51±0.35	3.2±0.33[**]	3.40±0.4
	LDL[2] (mmol/L)	0.76±0.17	0.71± 0.11	0.9± 0.1	0.88 ± 0.13	0.84 ± 0.22	0.81± 0.13
	Total cholesterol(mmol/L)	2.51±0.32	2.46±0.31	3.45±0.35	3.95±0.61[3]	4.1±0.31[*]	4.44±0.53[4]
B (Yeast-fed cows)	Total proteins (g/L)	71.4±7.0	72.0±5.5	77.4±8.1	80. 5±11.0	80. 2±11.9	85.9±5.7
	Albumins (g/L)	38.5±4.5	38.3±5.3	39.4±3.6	38.3±6.0	40. 2±4.5	38.9±4.5
	Globulins (g/L)	32.9±4.5	33.7±6.7	38.0±7.3	42.2±11.1	40.0±10. 6	47.0±5.8[3]
	Glucose(mmol/L)	3.72±0.42	3.75±0.14	3.82±0.28	3.93±0.15[3]	3.89±0.27[3]	3.99±0.11[3]
	Triglycerides(mmol/L)	0.31±0.08	0.36±0.07	0.28±0.04	0.39±0.03	0.33±0.06	0.21±0.03[4]
	HDL (mmol/L)	1.92±0.27	2.02±0.27	2.63±0.39	2.21±0.50	2.42±0.54[4]	2.91±0.56
	LDL (mmol/L)	0.66± 0.11	0.71± 0.1	0.74± 0.18	0.74± 0.11	0.75± 0.17	0.80± 0.20
	Total cholesterol(mmol/L)	2.52±0.51	2.23±0.43	3.34±0.42	2.85±0.35[3]	3.04±0.50[3]	3.55±0.71[4]

[1]High Density Lipoproteins; [2] Low Density Lipoproteins; [3] Significantly different *(P<0.01);* [4]Significantly different *(P<0.05)*

Serum glucose levels were significantly higher (P<0.01) in yeast-supplemented animals than controls at 30, 45 and 60 DIM. Concerning the effects of probiotics upon serum lipids, a significant reduction (P<0.05) in the levels of triglycerides was evident at 60 DIM in *S. cerevisiae*-supplemented group. HDL levels were significantly lower (P<0.05) in yeast-fed group at 45 DIM, while for LDL no significant differences were observed between 2 groups during the whole study period. Furthermore, a significant reduction was found for serum cholesterol concentrations in the yeast-fed group compared to the control group at 30 (P<0.01), 45 (P<0.01) and 60 (P<0.05) DIM.

Regarding milk production and milk parameters (Table 5), the results revealed that milk yield was higher in yeast-supplemented group, but it was significantly higher (P<0.01) only at 60 DIM. Milk fat percentage was higher (P>0.05) in yeast-fed group throughout the study. In contrast, a significant reduction (P<0.01) was evident for milk protein percentage at 45 and 60 DIM in

yeast-fed animals. Similarly, SCC was lower in this group, but significantly lower (P<0.01) at 15 DIM.

Table 5. Effect of feeding *S. cerevisiae* during the transition and early lactation periods on milk production and milk parameters of Holstein dairy cows.

Groups	Parameters	Days-in-milk			
		+15d	+30d	+45d	+60d
Group A (Control cows)	Milk yield (kg)	28.86 ±2.35	27.18 ±3.08	29.00 ±2.15	28.42 ±2.51**
	Milk fat %	3.66±0.16	3.73±0.92	3.52±0.52	3.86±0.45
	Milk protein %	2.57±0.30	2.61±0.30	2.78±0.08*	3.02±0.26*
	Somatic cell counts (10^3/mL)	84.00±17.28*	66.0±24.53	41.25±16.46	66.5±18.78
Group B (Yeast-fed cows)	Milk yield (kg)	29.61±2.88	29.56±4.24	31.79±3.17	31.03±2.05**
	Milk fat %	3.67±0.83	3.85±0.95	3.95±0.54	4.11±0.44
	Milk protein %	2.78±0.22	2.43±0.06	2.41±0.12*	2.44±0.07*
	Somatic cell counts (10^3/mL)	58.5±16.22*	46.37±14.09	34.75±15.21	54.5±17.56

* Significantly different *(P<0.01);* ** Significantly different *(P<0.05)*

Discussion

The results showed that rumen pH was lower in yeast-fed animals compared to controls. These results are in accordance with those found by other workers *(Herrick,1971; Khattab et al., 2003).* The reduction in the rumen pH of yeast-fed animals may be attributed to the increase in the concentration of VFAs production *(Hristov et al., 2001; Ghorbanietal., 2002)* and/or the increase in lactate synthesis in probiotics-supplemented animals *(Benjamin 1990).* The fact that the total VFAs concentration in our study were higher in the yeast-fed group throughout the experimental period is supportive for the hypothesis of *Hristov et al. (2001)* and *Ghorbani et al. (2002).* The present results concerning total VFAs are consistent with those obtained by previous *studies (Windschtil, 1991; 1998; Agarwal et al., 2002; Abd El-Tawab, 2007).* The increase of total VFAs concentrations in yeast-supplemented animals may be attributed to decreased methane production and consequent reduction of energy loss, providing thus additional energy for VFAs synthesis *(Williams and Newbold, 1990).*

Concentrations of rumen ammonia nitrogen were significantly lower in the yeast-treated animals. This is in agreement with previously published results *(Harrison et al., 1988; Carro et al., 1992; Erasmus et al., 1992).* The reduction of rumen ammonia nitrogen in yeast-supplemented animals may be attributed to the incorporation of ammonia into microbial proteins rather than a decrease in protein degradation *(Williams and Newbold, 1990),* or it may be due to an inhibitory effect on proteolysis, amino acid production, or ruminal urease activity *(Khattab et al., 2003).*

Concentrations of serum TP and GLOB were non-significantly higher in yeast-supplemented group. Similar results were obtained by other researchers *(Abd El-Tawab, 2007; Helal and Abdel-Rahman, 2010).* The elevation in the levels of serum TP in probiotics-treated animals may be attributed to the fact that yeast supplementation stimulates the rumen microbial protein synthesis, so it elevates the populations and the activity of cellulolytic bacteria in rumen, consequently enhancing the fiber digestion, lactate utilization in the rumen and increase flow of microbial protein from the rumen to duodenum (*Guedes et al., 2008*). Moreover, the elevated levels of GLOB in the *S. cerevisiae*-fed group may be due to the increase of net globulins, as a result of the increase in gamma globulins caused by Kupffer cell proliferation and an increase in the number of plasma cells in the bone marrow *(Benjamin, 1984)*. This hypothesis is supported by other researchers *(Buts et al., 1990),* who found that oral administration of *S. cerevisiae* to growing rats significantly increased IgA and secretory components of immunoglobulins.

Serum ALB values were not significantly different between the 2 groups, although a slight reduction was observed in the yeast-supplemented group. This reduction might be attributed to the increased milk production of these animals *(Benjamin, 1984; Helal and Abdel-Rahman, 2010).* Results of milk production in the present study favour this hypothesis.

The finding that serum glucose levels were significantly higher in yeast-supplemented animals is in agreement with the results of other studies *(Abd El-Tawab, 2007; Kawas et al., 2007; Stanislaw and Przemyslaw, 2009).*The glucose increase in yeast-treated animals may be attributed to increased gluconeogenesis, which raises blood glucose levels in ruminants (*Huntington and Eisemann, 1988*).This explanation is supported by *Antunovic et al. (2005),*who recorded low glucose levels in probiotics-treated lambs after inhibition of gluconeogenesis by insulin, which inhibits phosphorylase and gluconeogenic enzymes. It is worth mentioning that propionate is the major precursor for gluconeogenesis in ruminants and thus increments of rumen propionate production result in an increase of hepatic glucose production *(Reynolds et al., 2003; Stein et al., 2006).* The higher propionic acid production is the cause of the increased glucose levels in *S. cerevisiae*-fed cows *(Nisbet and Martin, 1991).* Several studies demonstrated that feeding *S. cerevisiae* increased the production of acetate, propionate, and total VFA in dairy cows *(Nisbet and Martin, 1991; Piva et al., 1993; Miller-Webster et al., 2002).* Total VFAs were significantly higher for yeast-fed cows in our experiment and this could explain the recorded higher glucose levels in yeast-treated cows.

Serum triglycerides, HDL, LDL and cholesterol were lower in the yeast-fed group compared with the control one. This reduction may be due to an increase in lipid metabolism and utilisation by the cows because of their increased milk production *(Stein et al., 2006)*, which was the case for the group of the yeast-supplemented cows in our study. Moreover, the reduction in cholesterol level could be attributed to the inhibition of the cholesterol synthesis or the direct assimilation

of cholesterols *(Zacconi et al., 1992).* The lipid profile estimated in the current study is in harmony with that reported by other researchers *(Taranto et al., 1998; Begely et al., 2006).*

The higher milk yield recorded for the yeast-supplemented cows in our experiment has also been observed by other workers and may be attributed to an increase in DM intake *(Robinson and Garrett, 1999, Jounay, 2006),* a greater flow of microbial protein and amino acids to the duodenum (*Erasmus et al., 1992*), and the fact that yeast supplementation may act as a source of vitamin B complex *(Abdel-Khalek, 2003; Helal and Abdel-Rahman, 2010).* However, some other researchers *(Erdman and Sharma, 1989; Arambel and Kent, 1990; Kung et al., 1997)* have not found probiotic administration to increase the milk production in cows.

The results for milk fat percentage in the present study are in agreement with those recorded by others *(Oetzel et al., 2007; Hanafy et al., 2009; Metha et al., 2011).* The increase in milk fat percentage in yeast-supplemented animals may be attributed to the increment in total bacterial populations and cellulolytic micro-organisms in the rumen, which improve fiber digestibility and fermentation and, consequently, increase milk fat content *(Doreau and Jounay, 1998; Wang, 2001; Chaucheyras-Daurant et al., 2008).*

Milk protein content increases after probiotic administration according to some researchers *(Abdel-Khalek, 2003; Helal and Abdel-Rahman, 2010)* and decreases according to others *(Lehloenya, et al., 2008).* In the present experiment, a reduction of milk protein percentage was recorded after the first 15 DIM for yeast-fed cows. This reduction could be interpreted on the base of dilution effect of the higher milk production in yeast-fed cows *(Lehloenya et al., 2008).* On the other side, milk protein percentage was higher in yeast-treated cows at 15 DIM. These findings show that the effect of probiotics on milk protein concentration needs further investigation.

SCC in yeast-fed cows was lower compared with that of controls. These results are in agreement with other studies *(Stein et al., 2006; Sretenović et al., 2008).*The reduction of SCC in yeast-treated cows may be attributed to a better health status of their udder *(Sretenović et al., 2008)* or may be due to an improvement of the immune status of the yeast-supplemented cows, as a result of the increase in IgA and secretory components of immunoglobulins *(Buts et al., 1990).* The increased values of globulins recorded in the yeast-treated group in our experiment could support this hypothesis.

Conclusion

Feeding *S. cerevisiae* to dairy cows during the close-up dry and the early lactation period significantly reduced rumen pH and rumen ammonia nitrogen, while significantly increased rumen total VFAs content. Serum glucose was

significantly increased in the yeast-supplemented animals. Serum concentrations of total proteins and globulins were higher, while albumins were lower in the yeast-treated group. Serum lipids were significantly reduced in the yeast-fed group, except for LDL. Finally, milk production and milk fat percentage were higher, whereas milk protein percentage and SCC were lower in the *S. cerevisiae*-supplemented cows throughout the experiment.

CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

Uticaj dodatka *Saccharomyces cerevisiae* u ishrani na zdravlje i proizvodnju krava tokom perioda tranzicije i početka laktacije

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Rezime

Podaci koji bi se odnosili na uticaj suplementacije probiotika na više parametara istovremeno na istim grlima nedostaju u literaturi. Stoga je cilj ovog eksperimenta bio da se ispita uticaj *Saccharomices cerevisiae* u obroku/hrani na parametre rumena, krvi i mleka zajedno u visoko-proizvodnim mlečnim govedima tokom tranzicije i početkom laktacije. Šesnaest klinički zdravih holštajn krava je podeljeno u 2 grupe: kontrolna grupa od 6 krava i grupa od 10 krava hranjenih probiotikom u obroku. Buražna tečnost i uzorci krvi su sakupljeni 21 dan pre očekivanog teljenja, kao i 7, 15, 30, 45 i 60 dana tokom laktacije (days in milk - DIM). Prinos mleka za svaku životinju zabeležen je svake 2 nedelje. Pojedinačni uzorci mleka su prikupljeni 15, 30, 45 i 60 DIM. pH buraga i buražni amonijačni azot su bili značajno niži, dok su ukupne isparljive masne kiseline bile značajno veće kod životinja hranjenih kvascem u poređenju sa kontrolama kroz celu studiju. Serumske koncentracije ukupnih proteina i globulina bile su više, dok su koncentracije albumina bile niže u grupi sa kvascem. Serumski nivoi glukoze bili su značajno viši kod životinja sa dodatkom kvasca. Trigliceridi u serumu, koncentracije lipoproteina visoke i niske gustine bile su niže, sa holesterolom koji je bio znatno niži u tretiranoj grupi. Proizvodnja mleka i procent mlečne masti bili su viši, dok je sadržaj proteina mleka i somatskih ćelija bio niži u krava hranjenih sa dodatkom kvasca kroz celu studiju. Ovi rezultati ukazuju na to da dodatak *S.*

cerevisiae u obrocima muznih krava tokom tranzicije i rane laktacije poboljšava njihovo zdravlje i parametre proizvodnje mleka.

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RESEARCH ON SUBCLINICAL MASTITIS AND ITS ETHIOLOGY IN DIFFERENT BREEDS OF COWS

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Abstract – Detection of subclinical mastitis in five different breeds of cows were done on the territory of a municipality Ključ, through all four year seasons by using the California mastitis test, at the level of the udder's quarter with a certificate of bacteriological findings in order to justify their application in the diagnosis of mastitis. In total, 2150 cows of different breed composition: Simmental 1090, red Holstein 322, Holstein-Friesian 340, montafon 108 and various crossbreeds of 290 in total. After 1978 tested cows, 56.02% of cases had positive reactions to the California mastitis test. The most common positive reaction we found in the milk was in one or two quarters with the intensity of the reaction of one and two plus. The most common causes of mastitis were staphylococci, streptococci and mixed infections. Continuing use of these methods it is possible to timely detect the presence of subclinical mastitis and get satisfactory results in the prevention and treatment of mastitis as well as increasing the quantity and improving the quality of milk.

Key words: subclinical mastitis, California mastitis test, somatic cells count, cow

Introduction

Inflammation of mammary gland or mastitis is response to effects of internal and outside factors. During last several decades mastitis has become very expensive disease in dairy cows (*Bennett et al., 1999; Fourichon et al., 2001; Kelmus et al.,2006*). Researchers had found, there is high risk of developing subclinical mastitis in period of early lactation and high procent of intramammary infections in postpartum period (*De Viegher et al., 2005; Milne et al., 2002; Sol et al., 2002*). Goal of various researches was to put efforts to enhance success of healing against clinical mastitis and to monitor appearance of subclinical mastitis

for obtaining milk of high quality (*Fabre et al., 1999; Fleiss 1981*).

Identification and removal of intramammary infections in period of early lactation is important for economical benefits. Mastitis occurence is according to Barkema et al. (1999) result of herd management, including rearing condition, nutrition and udder management. Udder infections can be expressed as clinical or subclinical mastitis. Clinical mastitis is characteristic because of visible changes in milk with appearance of flakes or beads and with signs of inflammation and pain. Subclinical mastitis is defined as inflammation without clear signs. Types of subclinical mastitis are: disorder of secrition, latent infection and chronical chataral mastitis. Subclinical mastitis is most widespread disease in milk production, where upon one clinical case, comes new 15-40 subclinical cases (*Hillerton, 1998*).

For detection of subclinical mastitis there is array of methods and tests, but most simple and reliable is California Mastitis Test (CMT), (*De Viegher et al., 2005; Dingvell et al., 2003*) as well as somatic cells counting with electronic counters.

Aim of this research was to establish representation of subclinical mastitis in researched area and to reveal their etiology with help of California Mastitis Test and microbiology results of mammary gland secretion from different breeds of cows through all seasons of the year.

Materials and methods of work

Research in field conditions had been conducted in breeds from individual milk producers, as well as in the mini farms, capacity 5 – 15 cows in the area of one municipality.

This experiment have included 2150 cows of different breeds: simmental 1090, red holstein 322, holstein-friesian 340, montafon 108 and various cross breeds 290. Control of udder health have been performed with clinical examination, CMT tests for each quarter and milk sampling for bacteriological findings.

Clinical examination have revealed all changes that occurred in udder as result of acute or chronical inflammation.

After clinical examination, milk have been tested with CMT test from all quarters (SOMA TEST reagent and Test plate by FARM d.o.o.Vrbanovac RH). Examinations have been performed through all seasons of the year, before morning milking. Reagent for CMT test have been mixed with aproximately same amount of milk (1-2 ml) and with easy circle moves of test plate reaction was read after 1-2 minutes. Milk samples rich with somatic cells gave visible changes within few seconds. Presentation of reaction was: negative (-) in cases where we had mixture homogenously blur, suspicius (±) in cases where mixture had flakes and beads that disappear with continuous mixing, positive (+) where present flakes are concentrated in the middle, very positive reaction (++) where during mixing

dense viscous mass separate from clear liquid part and extremely positive reaction (+++) where gelatinous mass is created.

Before milk sampling for microbiology analysis, teats were washed and desinfected with 70% alcohol and milk have been taken into sterile tubes. Every tube have been marked with number of quarter and animal. Samples have been taken from all quarters regardless for CMT findings and sent to laboratory for analysis.

All obtained results have been processed with Chi-quadrat test (*Trinidad et al., 1990*). During processing of results, category of cross breeds have been neglected, because of possibility biasa and for interpretation of results one less number of considered categories was used (df=n-1).

Results

From total of 2150 cows, 1978 were tested with CMT and 1108 cows or 56.02% had positive or suspected reaction and negative 870 or 43.98% (table 1).

Table 1. Results of CMT reactions according to breeds and in total

Breed	Number of CMT tested cows	Positive reaction		Negative reaction	
		number	%	number	%
Simmental	1003	519	51.74	484	48.26
Red holstein	296	195	65.88	101	34.12
Holstein-Friesian	313	200	63.90	113	36.10
Montafon	99	50	50.51	49	49.49
Cross breed	267	144	53.93	123	46.07
Total	**1978**	**1108**	**56.02**	**870**	**43.98**

Within certain breeds procent of CMT positive animals is 50.51-65.88%. In table 1. is visible relative equalization between certain breeds with exception of montafon and Holstein-Friesian cows as well as Montafon and Red holstein. Differences between other breeds are from 1.24% to 3.43%.

From 1108 positive cows, most disorders have been found in milk in one quarter (465 or 41.97%) and at least cows had positive all four quarters (123 or 11.10%). After 4432 examined quarters, suspected and positive reaction we had in 2166 quarters or 48.87% cases. According to seasons of the year most quarters had positive or suspected reaction in winter 50.74%, spring and summer were pretty much equal, while autumn period had least number of positive and suspected quarters 47.45%.

According to breeds (figure 1) the highest percent od disorders in secretion

in one quarter have been noted in Montafon breed (52%) and least in Simmental breed 36.22%, while in other breeds result was pretty much equal. Results with two quarters affected, highest disorder in secretion had Simmental 35.07%, then Montafon 34%, while least was in cross breeds 17.36%.

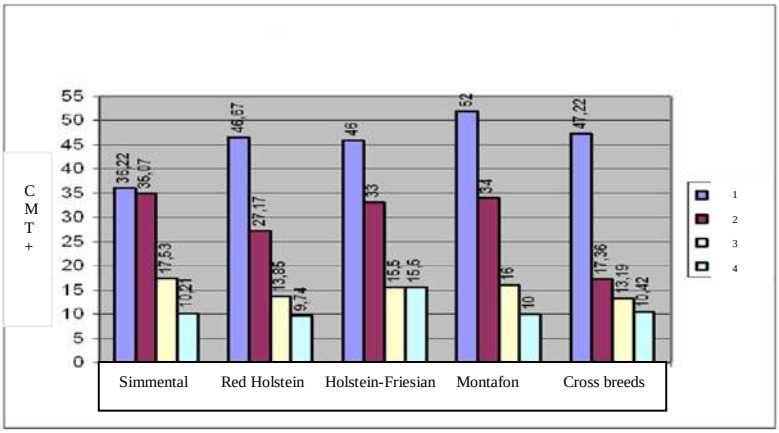

Figure 1. CMT positive results (%) according to number of affected quarters

The highest procent of positive with three quarters was in Simmental 17.53%, while in other breeds it was almost equal. When it comes to all four affected quarters, highest results have been found in Holstein-Friesian 15.5%, while other breeds are mostly equal.

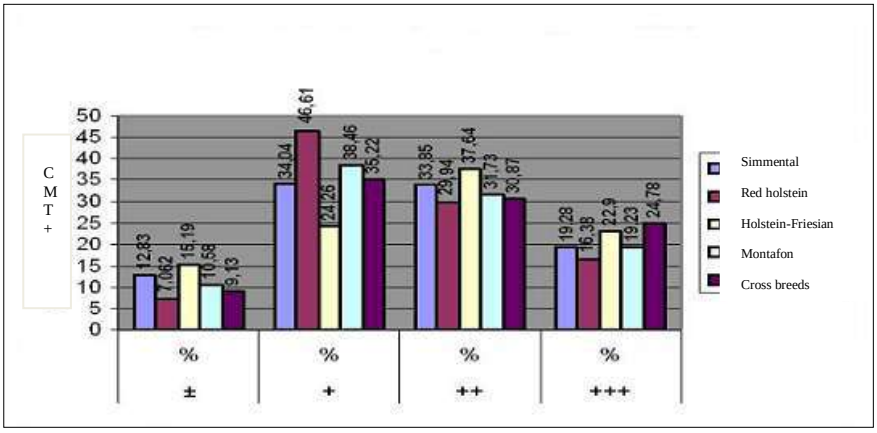

Figure 2. Number of positive quarters (%) according to CMT intensity in exemined breeds

Percent of CMT reaction intensity related to breeds is least for suspicious reactions 7.062% to 15,19% (Figure 2). Most of the cows had one plus 24.26% to 46.61%, two plus 29.94% to 37.64% and with three plus 16.38% to 24.78%.

After analizing 1400 bacteriological results, obtained from 350 cows during research (Figure 2) through seasons of the year, it is clear that during summer we had highest number of bacteriological positive samples. Most common isolates were staphylococci, streptococci, enterobacterias and mixed cultures.

Table 2. Results of bacteriological findings through seasons of the year in %

	Seasons of the year											
	Winter			**Spring**			**Summer**			**Autumn**		
	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Okt	Nov
Negative findings	**83**	**86,1**	**85,4**	**80,2**	**85,3**	**83**	**83,3**	**77,8**	**80,7**	**84,4**	**78,3**	**82,5**
Staphylococcus spp.	5,6	4,9	5	6,7	5,6	5,9	6,2	6,4	5	5,8	8,1	7
S. aureus	3,2	3,4	2,8	2,8	3,5	4,2	3,1	2,9	4	2	4,2	3,9
Streptococcus agalactiae	2,3	0,8	0,8	-	-	1	0,6	1,4	1	0,6	1,2	0,5
Streptococcus uberis	-	-	-	0,9	-	-	0,2	0,9	0,5	0,4	-	0,6
Streptococcus dysgalactiae	0,5	-	-	-	0,9	-	-	0,3	0,5	0,4	-	-
Other streptococci	2,2	-	2,4	1,9	2,7	2,4	2,7	3,4	2,7	1,7	5,1	3,9
Mixed culture (S. aureus, Nonpathogen stafilococci)	2,7	2,3	1,9	4,7	-	3,6	2	2,3	2,9	1,5	3,1	-
Enterobacterias (E colli, Proteus sp,)	0,5	2,5	1,7	2,8	1,4	-	1,6	4,6	2,7	2	-	1,6
Arcanobacterium pyogenes	-	-	-	-	0,6	-	0,2	-	-	0,2	-	-
Number of tested samples	**96**	**136**	**116**	**84**	**104**	**128**	**120**	**164**	**164**	**108**	**84**	**96**
% of positive	**17**	**13,9**	**14,6**	**19,8**	**14,7**	**17**	**16,7**	**22,2**	**19,3**	**15,6**	**21,7**	**17,5**

Results of statistical assessment related to number of quarters positive to CMT and breeds of cows, as well as relationship of number of quarters positive to CMT and season of the year did not fulfilled given criteria of statistical signifficance (table 3). In both cases zero hypothesis have been confirmed, which is about absence of researched relationship, respectively lesser or higher number

of quarters affected with subclinical mastits (CMT+) appers independently of breed or season.

Table 3: Results of statistical testing of relationship of number of quarters positive to CMT and breed of cows and season of the year

Ho	Ha	X^2	p vrijednost
Proportions of cows with one, two, three and four CMT+ udder quarters **are equal** in all breeds i.e. **there is no relationship** in number of CMT+ quarters with breed.	Proportions of cows with one, two, three and four CMT+ udder quarters **are not equal** in all breeds i.e. **there is relationship** in number of CMT+ quarters with breed.	12,6	0,181
Proportions of cows with one, two, three and four CMT+ udder quarters **are equal** for all seasons i.e.**there is no relationship** in number of CMT+ quarters and season.	Proportions of cows with one, two, three and four CMT+ udder quarters **sre not equal** for all seasons i.e. **there is relationship** in number of CMT+ quarters and season.	1,2	0,999

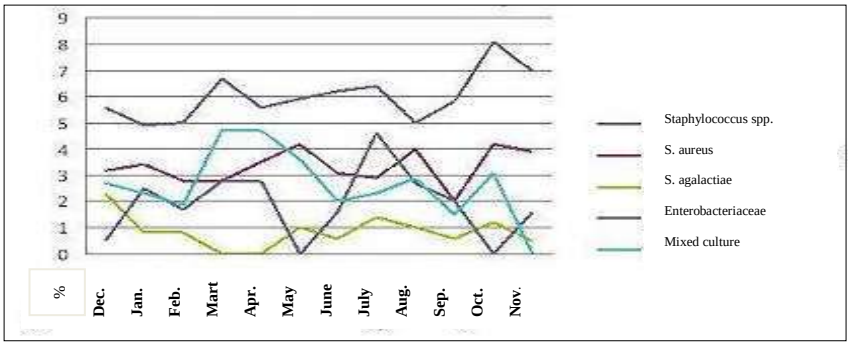

Figure 3. Percentage of different etiological agents that caused subclinical mastitis during the year

Statistical significance in representation of different etiological causes of mastitis (Figure 3) during the year is not established, although mixed cultures were more prevalent in spring season, while enterobacterias in summer season, especially in July. Nonpathogenic staphylococcus were more prevalent in October and pathogenic staphylococcus in May, August and October.

Discussion

Previous efforts in eradication of mastitis and control of udder's health status are based on detection of animals with disorders in milk secretion and identification of causal agents, that cause such conditions. To prevent and reduce udder infections with pathogenic bacterias from surroundings, it is necessary to take care for complete management in milk production (*Compton et al., 2007; Ferguson et al., 2007; Kelly 2002*). Mastitis is usually characterised with increase of SCC in milk. Every increase should be considered as abnormal and point to inflammatory condition (*Oliver et al. 1992*), which is confirmed by our results of CMT and bacteriological analyse. CMT represents suitable test for herd investigation and detection of subclinical mastitis caused by mastitis agents in 84% of cases. Bacteriological findings show that increase in CMT reaction, increase probability of infection (*Oliver et al. 1992*), while 25% of infection is present in negative CMT reaction, 50% in suspected CMT reaction, 75% in positive CMT reaction, 90% in very positive CMT reaction and 90-100% in pronouncedly positive reaction, which is in agreement with our research.

Ratio of CMT congruence and bacteriological findings is 70-80%, depending of causal agent (*Pyrola 2003*), while *Bastan et al.* (*2008*) gives information about congruence of 85%. As most often isolated causal agents more authors allegate (*Bradley 2002; Dingvell et al., 2003; Fatur et al., 2000; Kossabiati and Esslemont 1997; Sanford et al., 2006; Schukken et al., 1989*) staphylococcae and streptococcae. Our research are matched with research of mentioned authors, but among mentioned causal agents we have noted mixed infection in different months of seasons in representation of 1,9 % to 3,6% of cases.

Conclusions

Based on milk analyse with CMT and bacteriological examination we can conclude:

1. CMT represents valuable diagnostic method in detection of cows with disorder of secretion without clinical signs of disease.
2. After examination of 4432 quarters, positive reaction to CMT had 2166 or 48.87 %.
3. Disorders in mammary gland secretion, detected by CMT, depending of breed, ranged from 51.74% to 65.88% of cows.
4. According to season, the most quarters reacted in winter season, spring and summer season were equable, while autumn season is characterised with lowest number of suspected and positive quarters.
5. Most often isolated etiological agents were staphylococcae, streptococcae, enterobacteriaceae and mixed infections.

6. Percentage of subclinical mastitis is high in researched area and in next period, it is imperative to approach more organised in protection of udder health status through mastitis preventive care and education of farmers.

Istraživanje supkliničkih mastitisa i njihovih uzročnika kod različitih rasa krava

N. Varatanović, B. Čengić, E. Imširević

Rezime

Otkrivanje supkliničkih mastitisa kod pet različitih rasa krava, vršili smo na području jedne opštine kroz sva četiri godišnja doba pomoću Kalifornija mastitis testa, na nivou četvrti uz potvrdu bakteriološkim nalazom u cilju opravdanosti njihove primene u dijagnostici mastitisa. Ukupno je pregledano 2150 krava različitih rasa: simentalac 1090, crveni holstein 322, holstein frizijsko 340, montafonac 108 i ukrštenih životinja 290 grla. Od 1978 testiranih krava u 56,02% slučajeva utvrđena je pozitivna reakcija na Kalifornija mastitis test. Najčešće je pozitivna reakcija ustanovljena u mleku jedne i dve četvrti sa intenzitetom reakcije od jednog i dva plusa, a najzastupljeniji uzročnici mastitisa su bili stafilokok, streptokok i mešane infekcije. Kontinuiranom upotrebom ovih metoda moguće je blagovremeno otkriti prisutnost supkliničkih mastitisa i tako dobiti zadovoljavajuće rezultate u prevenciji i terapiji mastitisa kao i povećanju količine i poboljšanju kvaliteta mleka.

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COMPARATIVE TESTING OF SLAUGHTER TRAITS AND MEAT QUALITY OF MALE AND FEMALE SIMMENTAL CATTLE

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Abstract: The paper presents the results of comparative testing of slaughter traits and meat quality of male (A) and female (B) young cattle of domestic Simmental breed. The sample included a total of 30 heads, 15 in each group. Cattle were slaughtered at the same age with an average mass of about 660 kg in the group (A), and about 500 kg in the group (B). The study results show that cattle of group (A) achieved statistically ($p<0.001$) significantly higher share of pre slaughter mass and mass of warm carcass sides while female cattle achieved statistically ($p<0.01$) significantly higher share of kidney fat. The share of tissues in the three rib cut showed statistical differences between the groups, in the share of the *M. longissimus dorsi* that was statistically ($p<0.05$) significantly higher in male cattle and the share of fat was statistically ($p<0.01$) significantly higher in group (B). The chemical composition of *M. longissimus dorsi* statistically ($p<0.05$) differed significantly in the share of water which was higher in male cattle while the cattle of group (B) had statistically ($p<0.01$) significantly higher share of lipids. As for the technological quality, cooking loss of *M. longissimus dorsi* was statistically ($p<0.01$) significantly higher in young cattle of group (A), while the tenderness/softness of *M. longissimus dorsi* ($p<0.01$) was significantly better in young cattle of group (B). Sensory characteristics of *M. longissimus dorsi* differed statistically ($p<0.001$) significantly in the tenderness of the meat that was better in young cattle of group (B). Male Simmental cattle had better slaughter performance and meat quality characteristics, except tenderness of meat which was better in female cattle.

Key words: slaughter traits, meat quality, *M. longissimus dorsi*

Introduction

In recent years, improved techniques for beef production have considerably increased the production of calves, offering not only a higher number of males for

slaughter, but also increasing the number of replacement heifers, giving the producer the option to be more rigid for selection pressure on the cow herd, and to the packing plants, a greater offer of cull females *(Vaz et al., 2002).*

Beef meat is characterized by good nutritional value, which sets it apart from other types of meat and makes it very esteemed food. The importance of meat in human nutrition is well known and it is considered the indispensable and the best-quality component of proper and well balanced diet (*Petričević et al.*, 2015).

As far as the quality of beef is concerned, special significance is given to breed, feeding method and sex of animals intended for slaughter (*Bures et al., 2006*). In modern society, consumer acceptance of beef depends on two major aspects of meat quality, nutritional quality which is objective and 'eating' quality as perceived by the consumer – flavor, juiciness, tenderness and color– which is highly subjective (*Venkata et al., 2015*). Major factors which improve the appearance of meat consist of color, retention of fluid and fat content of meat or marbling. Tenderness of meat is affected by various factors like feeding regime, pre-slaughter treatment, slaughtering procedures, post mortem cooling, electrical stimulation and ageing of meat, age and sex of the slaughtered animal, and genetic makeup of the animals being consumed for meat production (*Strydom et al. 2000; Bonfatti et al. 2013*).

The share of tissues and meat, in retail cuts, define their quality, value and market demand. Information on the content of tissue in main carcass parts that are available in the literature are mainly related to a particular anatomical region of the carcass, with large variations in regard to the cutting procedures of carcasses and heterogeneity of material used in the study (breed, gender, age, level of fattening, etc.) (*M. Petričević et al., 2011*).

It appears that bulls and heifers of the same breed entering the finishing period at the same age and fed the same diet need different times to reach the optimum endpoint. The results of such comparison are, however, limited in the literature *(D. Bureš and L. Bartoň, 2012)*.

Sex of the cattle greatly affects the texture of beef, which is due to the presence of intra-muscular fat content and smaller muscle fiber diameter in cows, making the meat tenderer than bulls *(Church and Wood, 1992).*

Materials and Methods

The study was conducted on male and female fattening young cattle – two groups were formed group A (n= 15) male cattle and group B (n=15) female cattle. Fattening was carried out on the experimental cattle farm of the Institute for Animal Husbandry, Belgrade-Zemun. The cattle were fed diet with whole plant silage, hay and concentrate. Both groups of cattle were of same age. Day before slaughtering cattle received no food, only water. Animals were weighed immediately before slaughter, and then slaughtered according to standard

commercial procedures, in the experimental slaughterhouse, and chemical analysis performed in the Laboratory of the Institute for Animal Husbandry. Three rib cut 9-10-11 rib was separated from the left chilled carcass side, cut at the cranial edge of the 9th and 11th rib and cut parallel to the spinal column. On scale with an accuracy of 0.001 kg, muscle mass (especially measured *M. longissimus dorsi*), fat and connective tissue and bone were measured. The chemical composition of the *M. longissimus dorsi* sample was performed: the water content - the method of drying the sample at 103±2 ° C (SRPS ISO 1442, 1998), the fat content - extraction method by Soxhlet (SRPS ISO 1444, 1998), the amount of mineral matter (ash) - by annealing the sample at 550 ± 25°C (ISO 936, 1999) and protein content - Kjeldahl method according to (ISO 937, 1992).

Technological properties of the sample *M. longissimus dorsi*, namely: cooking loss was determined on the basis of the mass difference of the piece of meat (size: 3 x 4 x 1.5 cm and a mass of about 70 grams) before and after cooking in distilled water (where the ratio of meat and water was 1: 2) in a closed glass vessel (at 100°C for 10 minutes) and expressed as the percentage relative to the mass of the sample prior to cooking (*Official Gazette of SFRY no. 2/85, 12/85 and 24/86*); roasting loss was determined on the basis of the difference in mass of the pieces of meat before and after cooking; the cut of *M. longissimus dorsi* muscle, which was transversely cut to provide the direction of muscle fibers, weighing 150 ± 1 g was wrapped in aluminum foil and baked for 25 minutes at 250°C. Subsequently, it was extracted from the foil and immediately measured. The softness (tenderness) of meat was determined using consistency meter by *Volodkevich (1938)* by cutting pieces of meat transversely to the direction of the muscle fibers. Determination of total pigments according to Horsney (*Bunning and Hamm, 1970*) and instrumental color measurement was done using Chroma Meter CR-400 (Minolta, Japan), which had been previously calibrated in relation to a standard white surface (illumination D65, viewing angle 2° and the opening of the probe 8 mm) on fresh meat samples (24 hours *post-mortem*). Samples of meat were cut off and left 30 min in air to stabilize colour. Colour values are represented in the CIE L*a*b* system (*CIE 1976)*, where L* is a measure indicating lightness of meat. Three readings for each sample of meat were carried out and their average value were used for statistical data processing. The pH value of the meat 24 hours *post mortem* was measured using the pH meter with combined stab electrode Hanna HI 83141 (Hanna Instruments, USA). The scores of taste, aroma, juiciness/succulence and tenderness of the meat were determined after cooking and after roasting. Seven semi trained assessors were included in sensory evaluation. The quantitative descriptive scale of 5 points was used for each evaluated parameter: marbling: 1-very bad marbling, 2- bad marbling, 3-neither good nor bad marbling, 4-good marbling, 5-very good marbling; taste and odor: 1-very bad, 2-bad, 3-neither good nor bad, 4-good, 5-very good; Softness/tenderness: 1-very

firm, 2-firm, 3-neither firm nor soft, 4 soft, 5-very soft; juiciness/succulence: 1-very dry, 2-dry, 3-neither dry nor succulent, 4-succulent, 5-very succulent.

The obtained data were analyzed using analysis of variance in single factorial experiment (One-way ANOVA) by SPSS Statistics 20. The statistical significance of differences between mean values was determined by t-test.

Results and Discussion

Table 1 shows the average slaughter values of the studied beef cattle. As expected, male cattle were statistically ($p<0.001$) significantly heavier than the female cattle at the end of the fattening period. Warm carcass mass was statistically ($p<0.001$) significantly higher in male cattle compared to group (B). Similar results were obtained in in the research of *Bureš and Bartoň (2012)* who state the warm carcass mass of male beef cattle of 388.2 kg and 299.6 kg of female beef cattle.

The share of kidney fat was statistically ($p<0.001$) significantly higher in young cattle of group (B). *Petričević et al. (2013)* suggest that the share of kidney fat was 0.80% in male beef cattle of average pre slaughter body mass of 615.4 kg, which is similar to results obtained in this experiment. *Bartoň et al. (2006)* in their research report the share of kidney fat 1.40% for male cattle of Simmental breed.

Table 1. Average values of slaughter traits of beef cattle

Indicator	A	B	t-test
Pre-slaughter mass (kg)	660.91±66.85	504.88 ± 31.93	***
Warm carcass mass (kg)	387.05± 41.37	292.21± 15.86	***
Warm carcass dressing percentage (%)	58.50 ± 0.73	57.92 ± 1.25	ns
Kidney fat (tallow) (%)	0.68 ± 0.30	1.97 ± 0.65	***

ns – not significant
*** significant at the level of ($p<0.001$)

The share of different tissues in three rib cut is shown in Table 2. Share of *M. longissimus dorsi* differed significantly between groups ($p<0.05$). The higher share of *M. longissimus dorsi* by 4.81% was recorded in male cattle compared to the group (B). A statistically significant difference was found in the share of fat tissue ($p<0.01$), which was higher in young cattle of group (B). *Aleksić et al. (2005)* in their research report the proportion of fatty tissue of three rib cut of 8.34% for male cattle of Simmental breed.

Taking into consideration the higher share of the muscle tissue and lower share of fat tissue the advantage is on the side of young cattle of group (A).

Table 2. Share of tissues in three rib cut

Item	A	B	t-test
Three rib cut (%)			
M. longissimus dorsi	34.77 ± 2.70	29.96 ± 6.53	*
Remaining muscle tissue	29.86 ± 4.66	32.06 ± 4.03	ns
Fat tissue	13.25 ± 4.02	19.93 ± 5.25	**
Binding tissue	0.98 ± 0.36	0.84 ± 0.42	ns
Bones	20.51 ± 2.73	16.96 ± 4.65	ns

ns – not significant
* significant at the level of ($p<0.05$)
** significant at the level of ($p<0.01$)

The chemical composition of *M. longissimus dorsi* is shown in Table 3. Statistically ($p<0.05$), significant difference was found in the water content that was higher in male cattle and lipid content, which was statistically ($p<0.01$) significantly higher in beef cattle the group (B). *Štoković et al. (2013)* report the water content of 75.16% and lipid 3.28% for male young cattle of domestic Simmental breed. *Kelava et al. (2013)* suggest that the share of lipid of 1.79% for male cattle, which is similar to results in the present study. In the study of *Petričević et al. (2013)* the water content of 75.29% and lipid content of 1.57% are reported for male cattle of Simmental breed.

Table 3. Chemical composition of *M. longissimus dorsi*

Item	A	B	t-test
Water, (%)	75.04 ± 1.00	73.54 ± 1.36	*
Lipid, (%)	1.46 ± 0.64	3.33 ± 1.76	**
Ash, (%)	1.11 ± 0.04	1.07 ± 0.08	ns
Protein, (%)	22.38 ± 0.75	22.04 ± 1.23	ns

ns – not significant
* significant at the level of ($p<0.05$)
** significant at the level of ($p<0.01$)

Table 4 shows the technological characteristics of *M. longissimus dorsi*. Statistically ($p<0.01$) significant difference was found in the cooking loss, which was greater in male cattle male cattle and softness/tenderness of *M. longissimus dorsi*, which was more favorable in group (B).

In their study *Petričević et al. (2013)* report the value of the cooking loss of meat tenderness of 37.10% and 9.30%, respectively, for male young cattle of domestic Simmental breed and the results of research of *Aleksić et al. (2013)* show the cooking loss 34.21%.

Table 4. Technological characteristics/properties of *M. longissimus dorsi*

Item	A	B	t-test
Cooking loss, %	42.92 ± 1.81	26.64 ± 14.98	**
Roasting loss, %	38.76 ± 1.71	38.81 ± 2.37	ns
Colour (L*)	38.78 ± 1.93	38.44 ± 2.73	ns
Tenderness	14.09 ± 2.14	9.77 ± 3.86	**
pH_{24}	5.53 ± 0.06	5.52 ± 0.05	ns

ns – not significant
** significant at the level of ($p<0.01$)

The sensory characteristics of cooked meat are shown in Table 5. Tenderness and flavor have been identified as the most important attributes that determine eating quality of Europeans *(Glitsch, 2000)*. Tenderness is one of the major criteria that contributes most to eating satisfaction and consumers are willing to pay more for tender beef *(Wheeler and Koohmaraie, 1994; Chambaz et al., 2003)*.

Based on the results of sensory evaluation of *M. longissimus dorsi*, softness/tenderness of meat in female cattle was statistically ($p<0.001$) significantly better. Differences in sensory characteristics can be attributed to a different content of intramuscular fat (*Christensen et al., 2011)*.

Table 5. Sensory properties of *M. longissimus dorsi*

Item	A	B	t-test
Cooked meat			
Aroma	5.00 ± 0.00	4.81 ± 0.37	ns
Taste	4.86 ± 0.23	4.68 ± 0.59	ns
Tenderness	3.00 ± 0.59	4.37 ± 0.58	***
Succulence	3.45 ± 0.42	4.06 ± 1.05	ns

ns – not significant
*** significant at the level of ($p<0.001$)

Conclusion

Based on the results of the test can be concluded:

- male cattle achieved statistically significantly ($p<0.001$) greater pre slaughter body mass and mass of warm carcass and significantly ($p<0.001$) lower share of kidney fat;

- male cattle achieved statistically significantly ($p<0.01$) higher share of *M. longissimus dorsi* and significantly ($p<0.05$) lower share of fat tissue in three rib cut;

- male cattle had statistically significantly ($p<0.05$) more water in the *M. longissimus dorsi* and significantly ($p<0.01$) lower lipid content;
- male cattle had statistically significantly ($p<0.01$) greater cooking loss, while the female cattle had significantly ($p<0.01$) better tenderness of meat;
- sensory properties of meat differed significantly ($p<0.001$) only in regard to meat tenderness that was better in young cattle of group (B), i.e. female cattle had more tender meat.

Based on this we can conclude that male cattle of domestic Simmental breed have better slaughter traits and meat quality characteristics except tenderness of meat which is better in female beef cattle.

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Uporedno ispitivanje klaničnih osobina i kvaliteta mesa muške i ženske junadi simentalske rase

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Rezime

U radu su prikazani rezultati uporednog ispitivanja klaničnih osobina i kvaliteta mesa muške (A) i ženske (B) junadi domaće simentalske rase. Uzorkom je obuhvaćeno ukupno 30 grla, po 15 u svakoj grupi. Junad su zaklana u istom uzrastu sa prosečnom težinom u grupi (A) oko 660 kg i grupi (B) oko 500 kg. Dobijeni rezultati istraživanja pokazuju da su junad grupe (A) ostvarila statistički ($p<0.001$) značajno veći udeo mase pred klanje i mase toplih polutki dok su junadi grupe (B) ostvarila statistički ($p<0.001$) značajno veći udeo bubrežnog loja. Udeo tkiva u trorebarnom isečku se statistički razlikovao među grupama i to u udelu *M. longissimus dorsi* koji je bio statistički ($p<0.05$) značajno veći kod junadi grupe (A) i udelu masnog tkiva koji je bio statistički ($p<0.01$) značajno veći u grupi (B). Hemijski sastav *M. longissimus dorsi* se statistički ($p<0.05$) značajno razlikovao u

udelu vode koji je bio veći kod junadi grupe (A) dok su junad grupe (B) imala statistički ($p<0.01$) značajno veći udeo masti. Što se tiče tehnološkog kvaliteta, kalo kuvanja *M. longissimus dorsi* je statistički ($p<0.01$) značajno bio veći kod junadi grupe (A) dok je mekoća *M. longissimus dorsi* ($p<0.01$) značajno bila bolja kod junadi grupe (B). Senzorne karakteristike *M. longissimus dorsi* su se statistički ($p<0.001$) značajno razlikovale u mekoći mesa koja je bila bolja kod junadi grupe (B). Muška junad domaće simentalske rase imala su bolje klanične osibine i karakteristike kvaliteta mesa sem mekoće mesa koja je bolja kod ženske junadi.

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TYPOLOGY OF DAIRY FARMING SYSTEMS IN THE MEDITERRANEAN BASIN (CASE OF ALGERIA)

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Abstract: Characterization of breeding dairy cattle systems from the Mediterranean basin was conducted on 16 farms in the north center region of Algeria through a survey. Results are highly variable both structurally and in techno- economic management terms. The principal component analysis and clusters analysis have identified four groups of farms that differ in feeding strategies. The first group contains four farms that promote the use of forages (61.8% of the total dry matter (DM) intake). The costs are above the general averages (cost of production: 38.4 DA / liter ≈ 0.34 € and cost of food in total production costs = 71.8%). The average annual productivity is about 4328.6 kg. Five farms of group 2 are characterized by milk yields below average (4146.5 kg). The concentrates represent only 39.3% of total DM intake. The cost of production (37.1 DA ≈ 0.33 €/ liter of milk) and food costs are the lowest (65.17% of total production costs). The third group contains 5 farms dominated by profitable farms (4833.4 kg) and the lower cost of production (35.2 DA ≈ 0.31 €). A relatively high proportion of DM is provided by forages (53.6%). Food accounts for 69.2% of the total production cost. The fourth group consists of two farms whose main characteristic is the total absence of forage production. This is associated with a significant contribution of concentrates in the global feed balance (48.8% of total DM intake). These concentrated foods were poorly converted into milk as recorded yields are the lowest (3561.2 kg). Production costs are highest (45.1 DA ≈ 0.40 €) and relation price of food/total cost of production is very high (79.3%). So there are areas for improvement *via* land restructuring and the adoption of healthy feeding practices in order to ensure the profitability and sustainability of farms identified in this study.

Key words. Production system - food - milk yields - production costs - principal component analysis -clusters analysis.

Introduction

Milk is an important food in the Algerian consumer tradition, this is due to its nutritional value, its substitution with red and white meats relatively expensive and mainly related to the support of consumer prices by the State. At independence, the dairy sector, industrially almost nonexistent, based mainly on some craft workshops of milk derivatives production and dairy units in the center (Algiers), East (Constantine) and west (Oran) of the country. Dairy herd consisted of two local cattle breeds conducted in extensive conditions. These cows had been crossed with previously introduced breeds. The problem of food insecurity and its negative effects on the national economy forced the state as early as 1990 to reflect on a series of upgrade policies of the local milk production in order to promote self- sufficiency (*Belhadia et al., 2009)*. Today, with a herd estimated at 1.9 million heads of cattle, including nearly one million dairy cows, domestic increasing demand for milk is not yet satisfied. The average consumption was estimated at 147 liters/capita/year in 2013, which ranks Algeria as a country of major consumption of milk when compared to Tunisia (83 liters), or Morocco (64 liters) (*Kacimi, 2013)*. Our country imports milk powder to fill the gap, which costs approximately 769 million Dollars (M.A.D.R, 2013). These imports were a major constraint on the development of local production and collection of raw milk (*Srairi et al., 2013*). Efforts are being made by the government to encourage the development of this sector. Thus, national milk production was estimated at more than 3 billion liters during 2012/2013, an increase of 7.6% compared to the previous year. Dairy farming remains a kind of speculation that is difficult to manage given the diversity of parameters that are linked to it. Forage crops are far from meeting the food needs of the national herd in quantity and quality. In fact, the coverage rate is between 75 and 80% (M.A.R.D, 2012). To our knowledge, the Algerian bibliography is lacking data on the actual operation and the level of profitability of the dairy farms in a context of reduced public institutions of control and monitoring functions of the national herd performance. The purpose of this manuscript is to describe dairy cattle farms in the mid-northern region of Algeria through: (i) the characterization of the producers involved in milk production, (ii) analysis of different practices and strategies in place to manage the units surveyed and finally, (iii) identification of constraints and potentialities of current systems.

Materials and methods

Characteristics of the study area

It includes 5 regions, namely: Algiers, Blida, Boumerdes, Bouira and Tizi Ouzou. It covers an area of 1248400 ha or 0.52% of the total area of the country. In 2012, it accounted for around 11% of national milk production. The study area is

characterized by a temperate Mediterranean climate. Summers are hot and winters are rainy, sometimes snowy. The average annual rainfall is between 500 and 800 mm. Most rainfall is concentrated in the period from early October to late March. The temperatures are moderated by the maritime proximity and vary from 11°C in winter to 28°C in summer.

Data collection

The study was conducted by survey in 2013. Sixteen farms were selected to represent different conditions of milk production. The selection criteria are based on a minimum of 10 dairy cows per farm and the acceptability of the farmer to participate in this study. Data collected focused on the operator (age, education level and seniority in the practice of dairy cattle), the structural parameters of operations (agricultural land, cattle and equipment), management settings (feeding, production and reproduction of dairy cows) and economic burdens. The production cost of a liter of milk includes all food costs, labour, veterinarian care, artificial inseminations (if made) and even the litter and fuel. The estimation of rations was based on the quantities of food and concentrates in the ration distributed to the dairy cows. Nutrient intakes were determined according to data given by INRA (2007).

Statistical procedure

A descriptive analysis was performed for the evaluation of averages, standard deviations, minimum and maximum of the various parameters chosen. A typology of farms was established through the use of multivariate statistical analyzes: principal component analysis (PCA) and a cluster analysis. Statistical analyzes of data were performed using the Statistica 8.0 software (2008). For each farm, 10 variables were selected for characterization.

Results

Operators presentation

Through descriptive statistics performed on the data, it appears that 56% of farmers (n=9) were over 40 years of age. The level of education is considered low in 69% of cases since the farmers barely exceeded the primary level. Two farmers of our sample are private milk collectors. The number of years of service in the practice of dairy farming exceeds 10 years in 10 farms. Labour used is mostly typical employee in 62% of farms visited; it is used mainly in tasks related to breeding. Milking is done manually in farm due to the unavailability of a milking machine. The milk is kept in aluminum tanks for refrigeration in 12 farms, the rest uses buckets which they cool in a fridge.

Characteristics of farms studied

The parameters that characterize the surveyed barns are illustrated in table 1. The studied farms represent 138 ha of forage land. Significant differences are noted between the different parameters studied. Surveyed dairy farms are characterized by an average forage land of 8.6 ha per farm. Over 86% of farmers use their entire useful agricultural land for forage crops, mainly dry forages such as oats and barley and green fodder as clover, sorghum, corn and alfalfa. The study included a total of 365 heads of dairy cows with an average of 22.8 cows per farm and an average density of 2.7 cows/ha of forage. These farms showed a clear dominance of imported breeds (Holstein and Montbeliarde). The remainder (approximately 6% of cows) is genetic crossover. Stabling is hampered in all farms visited. The base feed consists essentially of oat hay and straw. Grazing is practiced on natural grass lands in order to enhance re – growth and in cereal fallow after the harvest. Silage is distributed in 3 farms only, mainly corn and sorghum silage. Concentrates are represented by industrial compound feed specially made for dairy cows. They are distributed in various quantities from a farm to another, from 5.5 to 10 kg per cow per day. These concentrates are 29 to 53% of total dry matter intake, with an average of 42.4 and between 0.34 and 0.56 Milk Forage Units (UFL) per kg of milk produced. The annual milk yields per cow in these farms fluctuate between 3053.4 and 6551.5 kg with an average of 4333.5 kg. The average production cost of a liter of milk in this study is 37.82 DA. The results showed that between 53.2 and 82.7% of the cost of production of one liter of raw milk was due to food. Artificial insemination was practiced in about 69% of farms and the average interval between two calvings is 452.1 ±31.7 days.

Table 1. Characteristics of farms surveyed (*n* =16).

Parameters	Minimum	Mean ± standard deviation	Maximum
Usefull Agricultural Land (ha)	0	9.3±7.7	27
Forage Land (ha)	0	8.6±8.1	27
Density: cow / ha of forage	-	2.7±1.8	6
Number of cows	10	22.8±19.2	78
Milk yield (Kg/cow/year)	3053.4	4333.5±961.3	6551.5
Energy from concentrates /kg of milk (UFL)	0.34	0.44 ±0.06	0.56
Share of concentrates in a total ration (% total dry matter intake)	29.2	42.4±5.9	53.25
Interval calving-calving (days)	407.2	452.1±31.7	505.7
Production cost of 1 liter of milk (DA)	30.4	37.8±5.1	45.2
Food costs/ total costs (%)	53.2	69.9±7.0	82.7

1 DA = 0.090 €. (DA: Dinar Algérien, €: Euro).

Typology of farms according to their characteristics

The survey identified four types of livestock from a principal component analysis performed on 10 structural and techno-economic variables. The first three factors in the analysis that explain 68% of total variability are taken into account. The first factor explains 30.3% of total variability, the second (22.8%) and the third (15.3%) as indicated in table 2. Only two correlations to the axis 3 were important for milk yield parameters (r =0.30) and the cost of food (r = -0.49) as <0.5.

Table 2. Results of principal component analysis (PCA) and axis of variation determined.

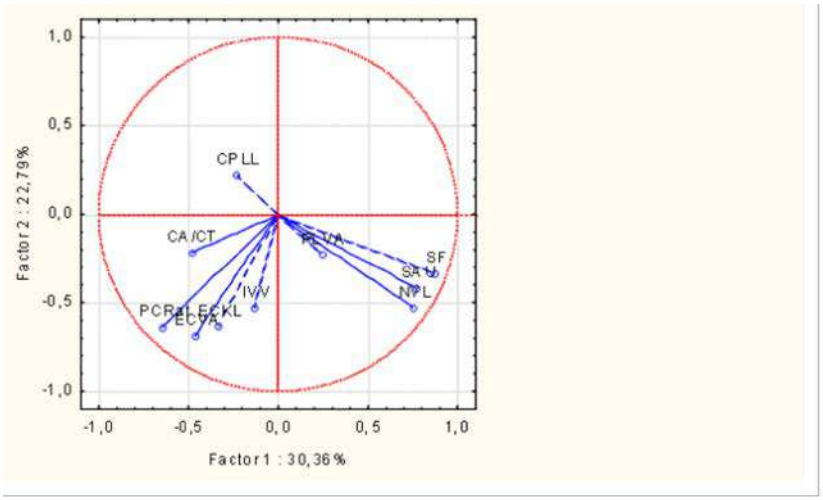
Axis	Variables	Correlation of variable to axis	Total variability (%)	Cumulative variation (%)
Axis 1	- Useful Agricultural Land (SAU) - Forage Land (SF) - Number of cows (NVL)	0.76 0.87 0.75	30.3%	**30.3%**
Axis 2	- Energy from concentrates /cow / year) (ECVA) - Energy from concentrates / kg of milk (ECKL) - Share of concentrates in a total ration (PCRat)	-0.68 -0.63 -0.63	22.8%	**53.1%**
Axis 3	- Milk yield per cow per year (RLVA) - Interval calving-calving (IVV) - Production cost of a liter of milk (CPLL) - Food costs/ total costs (CA/CT)	0.30 0.51 -0.75 -0.49	15.3%	**68.4%**

The correlation between selected variables and the main factors (PF) indicates that the variables that strongly influence PF1, PF2 and PF3 are the areas and the number of dairy cows respectively: (PF1=structure parameters); the energy of concentrates/cow/year and per kg of milk produced and the proportion of concentrate in total dry matter intake (PF2=variables related to food strategy); calving interval, yields, cost of production and cost of food (PF3=variables related to management settings of reproduction, milk production and the production economics). However the correlation determined by the axes 1 and 2 between the master plan and the variables appears in graph 1.

Discussion

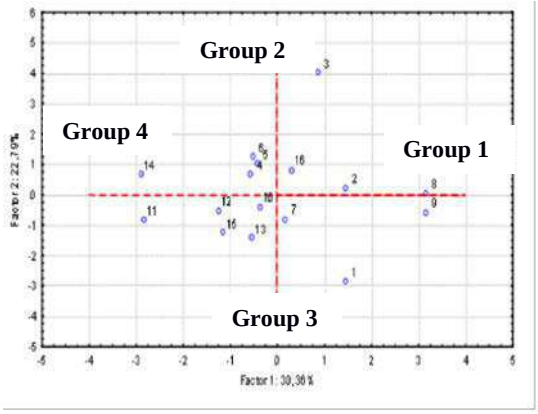
According to the results obtained for the farmers in the north-center region of Algeria, the collection means (cooling tank) exist among older farmers (r= 0.47), with lowest instruction level (r= -0.59) and oldest in the practice of dairy cattle (r=

0.25) with $p<0.05$. The concentrates in our study represent an average of 42.4 ± 5.9 % of total DM intake and 0.44 ± 0.06 UFL per kg of milk produced.



Graph1. Representation of structural and techno- economic variables of dairy farms on axes 1 and 2 determined by the Principal Component Analysis.

Four groups of farms were selected (graph 2) following the cluster analysis.



Graph 2. Graphical representation of farmers groups (determined by the axes 1 and 2).

In a previous study of *Madani et al.* (2004) in semi-arid region of Algeria, similar intervals to ours were highlighted. The amount of concentrates represent between 42 and 53% of DM intake and between 0.32 and 0.53 UFL per kg of milk. While varying mean values between 40.9 and 70.5% of the total DM with an

average of 56 ±7.42% were reported by *Ghozlane et al.* (2009) in the farms of Eastern Algeria (Constantine). The result was higher (73.1%) in intensive farms in Morocco (*Srairi and Kessab*, 1998). While in France, on the tropical island of Réunion, where breeders lack of fodder, there they are forced to distribute significant amounts of concentrate which represent on average 55% of the dry matter intake (Bony *et al.*, 2005). A significant change in milk yields is observed in 7 farms (CV>20%). This variability between farms and within the same farm is mainly related to the animal itself (race, stage of lactation, lactation rank and different lactation lengths) (*Millogo et al.*, 2008). A higher average milk yield (4884 kg) was found in the farms in the region of Médéa in Algeria (*Kaouche et al*, 2012). The production cost of a liter of milk was exceeding the sale price to dairies at 11 breeders. This selling price was set in 2012 at 34 DA a liter of milk grading 34 g of fat. Note that the difference between the sale price and the production cost is compensated by the help of the State estimated at 12 DA for each liter of milk produced by the farmer and 1 DA additional for each gram of fat beyond 34 g/liter.

These high production prices observed mainly on farms where the concentrate was used at a rate higher than 53% of total dry matter intake by cows. The results showed that nearly 70% of the cost of production of one liter of raw milk is allocated to food. *Ghozlane et al.* (2009) reported even a higher ratio (80%). The interval between two calvings was variable in the present study from one farm to another and within the same farm. Indeed, the difference introduce wide variations (CV =23%) in a single production unit. It exceeds the economic targets for 12 months on all farms visited. One of the factors commonly put forward to explain these delays is the conduct of reproduction with a lengthening of the interval between calving and fertilizing insemination. However, lengthening interval between two calvings from 4 to 6 months compared to the standards with an average of 420 days in nearly 83% of farms was also noted by *Kaouche et al.* (2012). The control of reproduction is a determining factor in the economy of a farm. Indeed, the presence of animals that do not reproduce increases expenses for the farmer and prevent the renewal of the herd. Improvements in farming practices, including food, have a positive effect on calving intervals (*Compère and Dupont*, 2005). Of all the variables studied, the cow's number in our study sample appears to be strongly associated (R^2= 0.60) to the size of the forage land (p<0.01) but weakly correlated (R^2=0.03) to milk yield. This is related to poor livestock management. However, energies ECVA and ECKL were determined to be correlated with PCRat, with respectively (R^2= 0.56), p<0.01 and (R^2= 0.30) with p<0.05.This demonstrates the high share of energy from concentrates in the total energy balance. The various parameters characterizing the 4 groups of farms identified from the cluster analysis are shown in table 3.

Table 3. Characteristics of the groups of farms identified.

Groups of farms **Numbers**	Group 1 **(*n*=4)**	Group 2 **(*n*=5)**	Group 3 **(*n*=5)**	Group 4 **(*n*=2)**
Useful Agricultural Area (ha)	21	3.8	7.2	5
Forage Area (ha)	21	3.6	7.2	0
Number of dairy cows	47	12.6	18.8	10
Milk Yield per cow per year (kg)	4328.6	4146.5	4833.4	3561.2
Energy from concentrates/kg of milk produced (UFL)	0.43	0.41	0.49	0.45
Share of concentrate in total ration (% DMI)	38.2	39.3	46.4	48.8
Production Cost of 1 liter of milk (DA)	38.4	37.1	35.2	45.1
Food cost/total cost (%)	71.8	65.1	69.2	79.3

DMI : Dry Matter Intake.

The first typology group "large farms tend to forage", contains four farms with useful agricultural land completely used for forage crops (21 ha). This means that this group of farmers promotes the exploitation of fodder compared to concentrate in the energy balance of the cows (the largest share of fodder compared to other groups and compared to the average: 61.8% of total dry matter). However, the economic burdens remain negative and higher than the overall averages (production cost=38.4 DA and food cost=71.83% of the total cost). This may be due to management difficulty of the important number of cows in this group (*n* = 47). Indeed, these large herds require much more food, labour and care, which increases farmers spending. The average annual productivity is high, on the order of 4328.6 kg, it is almost equal to the average (4333.5 kg). This is a group of breeders that characterizes the beginning of specialization in the field of bovine milk production. The five farms in group 2, "Small farms with limited resources", are characterized by milk yields below average (4146.5 kg). Concentrates represent only 39.3% of DMI. Indeed, this group of breeders records low and below average rates production costs (37.1 DA/liter of milk) and minimum food expenses (65.2%). But, he holds an effective reduced cattle (12.6 heads) and low forage area (3.6 ha), this type of farmers suffer from a lack of financial means to supply concentrates to improve yields. The third group “specialized farms”, with 5 farms, dominated by profitable units with the highest average yield (4833.4 kg) and the lowest cost of production (35.2 DA/liter). A relatively high proportion of dry matter intake is provided by concentrates used intensively (46.4%) but effectively valued. This means that a good feed management is practiced in these farms to cover the needs of the animals. This is the group of farms considered as leaders in milk production. The cost of food is fairly low compared to other groups and slightly below average (69.2%). In contrast, it was the 4th group containing 2 farms, "Without land holdings", whose main characteristics are the complete lack of forage production and a herd size of 10 cows on each farm. This is associated with significant concentrates contribution in the global energy balance (48.8% of DMI).

These concentrates were poorly converted into milk as yields were recorded as the lowest (3561.2 kg). This use of the massive purchase of food (forage and concentrates) have added to the costs of these operators, production cost of a liter of milk highest (45.1 DA) and a very important food prices (79.3% of total). This category typically represents smallholder's dairy farms. The results of this study confirm the importance of the effect of diet on the diversity of farming systems in the study area. This is consistent with results of the literature (*Srairi and Lyoubi, 2003; Millogo et al., 2008; Gabbi et al., 2013*). The indicators that define axis 2 (food strategies) and axis 3 (parameters related to production economics), coincide with those found in Morocco (*Srairi and Lyoubi*, 2003), while the food management in the study of *Gabbi et al. (2013*) have a correlation with axis 1.

Conclusion

The analyses of all structural and techno-economic characteristics of surveyed farms shows that there is not in our sample an ideal type of farming that would bring together the contributions reasoned forage/concentrate in total DMI (group 2) so that the animals can reach their maximum production potential (group3), an optimal amount of energy from concentrates annually (groups 1 and 3), minimum cost of producing a liter of milk (group 3) with the least food expenses (group 2). In general, groups 1, 2 and 3 totaling 14 of the 16 farmers surveyed, all show higher milk yields than 4000 kg, despite the different dry matter provided by fodder from one group to another (61.8, 60.7 and 53.6%). On the other hand, one group with 2 units suffers from low yields (3157.3 kg), may be due to the excessive use of concentrates that are not valued in their entirety, associated with massive purchases of fodder that are poorly exploited in the absence of rationing and food formulations, which led to heavy spending. Fodder in this study can barely meet the maintenance needs of the animals as on average 0.44 UFL are provided by concentrates for the production of one kg of milk. The performance of animals also are low, although 94% of the cows are imported and therefore of high genetic merit. However, the constraints related to breeding are numerous and their exercise will require an arsenal of human and financial resources. These constraints include in the first place a food factor which represents the major handicap of the entire dairy production industry. So there are areas for improvement through the restructuring of land in order to size the farms with sufficient forage area, recovery of pastures, modification of harvesting techniques and conservation of forages (haymaking, especially silage) in order to compensate for off peak periods and the spreading of good feeding practices (food rationing and formulations) in order to ensure the profitability and sustainability of farms identified in this study.

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Tipologija sistema u proizvodnji mleka u mediteranskom basenu

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Rezime

Karakterizacija sistema uzgoja muznih krava iz mediteranskog basena je sprovedeno na 16 farmi u severnom centru regiona Alžira. Rezultati su veoma varijabilni i strukturno i tehnološki u smislu ekonomskog upravljanja. Analiza PCA i klastera je identifikovala četiri grupe farmi koje se razlikuju u strategijama ishrane. Prva grupa sadrži četiri farme koje promovišu korišćenje kabaste hrane (61,8% od ukupnog unosa suve materije). Troškovi su iznad opštih proseka (troškovi proizvodnje: 38.4 DA/litar ≈ 0.34 € i troškovi hrane u ukupnim troškovima = 71,8%). Prosečna godišnja produktivnost je oko 4328,6 kg. Pet farmi grupe 2 odlikuju prinos mleka ispod proseka (4146.5 kg). Koncentrati predstavljaju samo 39,3% od ukupnog unosa SM. Cena proizvodnje (37,1 DA ≈ 0,33 € / litar mleka) i troškovi hrane su najniže (65,17% od ukupnog broja). Treća grupa sadrži 5 farmi gde dominiraju profitabilne farme (4833,4 kg) i niži troškovi proizvodnje (35.2 DA ≈ 0.31 €). Relativno visok procenat SM obezbeđuje se iz kabaste hrane (53,6%). Hrana čini 69,2% od ukupnih troškova proizvodnje. Četvrta grupa se sastoji od dve farme čija je glavna karakteristika potpuno odsustvo krmne proizvodnje. Ovo je povezano sa značajnim doprinosom koncentrata u globalnoj ravnoteži hrane (48,8% ukupnog suvog unosa materije). Ova koncentrovana hraniva se loše konvertuju u mleko, što pokazuju mali prinosi (3561,2 kg). Troškovi proizvodnje su najviši (45,1 DA ≈ 0,40 €) i veoma visok odnos cena hrane/ukupnih troškovi proizvodnje (79,3%). Dakle, postoje oblasti za unapređenje putem restrukturiranja zemljišta i usvajanje zdravih praksi ishrane kako bi se osigurala održivost i profitabilnost farmi identifikovanih u ovoj studiji.

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EJACULATE PROPERTIES AND REPRODUCTIVE EFFICIENCY OF LARGE WHITE BOARS DURING EXPLOITATION

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Original scientific paper

Abstract: The main objective of this study was to assess the variability of ejaculate properties: volume of ejaculate (VOL, ml), sperm concentration (CON, spermatozoa/ml), total sperm count (NT) and the number of doses produced (NPD) per ejaculate under the influence of season, boar and the interval between two mounts. Reproductive efficiency of boars was analysed based on the farrowing rate (FR). The study included 341 ejaculates from seven Large White boars. Impact assessment was carried out by applying the GLM procedure of the statistical package SAS 9.1.3. The average values of VOL, CON, NT, NPD and FR were: 244.28 ml, 203.77x10^6 spermatozoa/ml, 43.48x10^9 spermatozoa, 17.39 doses and 67.58%. Ejaculate properties varied under the influence of season ($P<0.01$, $P<0.001$). The interval between two mounts did not affect only the variability of CON. The difference between the best and worst boars was 91.99x10^6 spermatozoa/ml of ejaculate ($P<0.001$). During summer VOL ejaculate was the lowest (191.36 ml). The highest NPD (19.04 doses) was produced during the autumn months, and during the same period the highest concentration of sperm was recorded (242.16x10^6 spermatozoa/ml). Regardless of the differences in the farrowing rate between boars not being statistically significant ($P=0.0882$), it is necessary to perform the ranking and promptly identify boars with farrowing rate below average.

Key words: boar, ejaculate, farrowing rate, rank, season

Introduction

Modern pig production is based on highly productive breeds with the use of modern technical and technological solutions and biotechnological methods in

breeding and reproduction. The success of artificial insemination depends largely on quantitative and qualitative properties of the ejaculate.

In the populations of pigs where a continuous selection is conducted, there is a tendency for better exploitation of boars, good production performance, which is reflected in obtaining the greatest possible number of doses per ejaculate of optimal fertile capability (*Savić et al., 2013a*). The volume of ejaculate, sperm concentration and sperm motility are characteristics/properties which determine the number of doses per ejaculate, fertility of doses and the number of sows that can be inseminated.

Variability of sperm properties is influenced by various genetic and non-genetic factors. Properties of the ejaculate vary under the influence of boars *(Savić et al., 2013b),* breed (*Okere et al., 2005; Wolf and Smital, 2009a*), season (*Kondracki et al., 2009; Kunowska-Slósarz and Makowska, 2011*), intensity of use or interval between two mounts (*Frangež et al., 2005; Wolf and Smital, 2009a; Smital, 2010*) and other factors.

Research by *Young et al. (2010)* shows that there are differences in farrowing rate between the 30 herds analyzed (54.7-92.4%), and the cause of such broad range is consequence of different procedures and technological operations carried out in herds. In the pig production, reproductive performance is assessed based on the farrowing rate, and the number of live born piglets, however, in the commercial sector boars of superior and inferior fertility are used (*Flowers, 2013*).

The aim of this study was to evaluate variability of ejaculate and reproductive efficiency of Large White boars during use in reproduction.

Material and Methods

The research was conducted on a reproduction farm, in the period from 2011 to 2012. Total of 341 ejaculates of 7 Large White boars were analyzed during two years of use in reproduction. In addition to the evaluation of ejaculate, also the reproductive efficiency boars was tested.

Sows were inseminated twice, and from a total of 919 matings, 602 farrowings were realized. Of the total number of analyzed ejaculates, 40 (11.73%) were unusable, so they were not evaluated. Reasons for ejaculates being considered unusable were different: dead sperm in the ejaculate, sperm motility subjectively estimated to be below 70%, the presence of impurities, etc.

Boars were housed in a separate facility, in boxes measuring 2×4 m, with partly slated concrete floor. The microclimate conditions in facility for boars were under automatic control. The animals were fed a balanced feed mixture, and fresh water was available to them *ad libitum.*

The impact of season was examined as the impact of a class: 1 (December, January, February), 2 (March, April, May), 3 (June, July, August) and 4

(September, October, November). The average interval between two mounts was 8.80 days. The research included: the volume of ejaculate (VOL, ml), sperm concentration (CON, $x10^6$ spermatozoa/ml), the total number of spermatozoa in the ejaculate (NT, $x10^9$ spermatozoa) and the number of doses produced (NPD). Reproductive efficiency of boars was investigated based on the farrowing rate (FR, %), which is calculated as the relative ratio between the number of farrowed sows in relation to the number of inseminated sows.

Taking of the ejaculate was performed using standard manual method, the introduction of mobile phantoms into boxes where boars are housed. The ejaculate volume was measured by graduated cylinder, with an accuracy of ±2 ml. The concentration of native sperm was evaluated by application of the photo-colorimeter. Dosages for insemination were standardized to a volume of 100 ml and 2.5 billion spermatozoa per dose. The total number of spermatozoa in the ejaculate was obtained by multiplying the sperm concentration by the volume of ejaculate.

Impact assessment was carried out by applying the General Linear Model procedure of the statistical package SAS 9.1.3 *(SAS Inst. Inc., 2002-2003)*, using the following model:

$$y_{ijk} = \mu + B_i + S_j + b(x_{ijk} - \bar{x}) + e_{ijk},$$

where: y_{ijk}- analysed property of the ejaculate, μ- general population average, B_i- the impact of boar (i=1,2,3,4,5,6,7), S_j- the impact of season (j=1,2,3,4), $b(x_{ijk}-\bar{x})$- linear regression impact of the interval between two consecutive mounts and e_{ijk}- random error.

The boars were ranked based on the value of the farrowing rate, by forming two groups of boars (with the farrowing rate above and below the general average). Comparison of Least Square Means (LSMeans) values of ejaculate properties and mean values of the farrowing rate was performed by t-test.

Results and Discussion

The basic statistical parameters are shown in Table 1. With regard to the values of the standard deviation, the ejaculate volume and sperm concentration exhibit a greatest variation with respect to other fertility traits.

Table 1. Basic statistical parameters of fertility traits

Fertility traits	Mean	SD
Volume of ejaculate (ml)	244.28	115.37
Concentration of sperm (x10^6 spermatozoa/ml)	203.77	97.11
Number of total spermatozoa (x10^9 spermatozoa)	43.48	18.57
Number of produced doses	17.39	7.43
Farrowing rate (%)	67.58	9.33

The influence of studied factors on the variability of the ejaculate properties is presented in Table 2. All the analysed properties of ejaculate varied under the influence of the season. In addition to micro-climate indicators, the impact of the season should be viewed through the duration of the photoperiod during the year, and expressed significance of the influences indicates to the need and obligation to include this factor in the models in the analysis of properties of ejaculate.

The results of this study are contrary to the study of *Savić et al. (2013b)* in which the ejaculate volume varied under the influence of boars, while the influence of the interval between two mounts was not significant. The effect of season on ejaculate properties identified in our study is in concordance with the findings of *Kondracki et al. (2009).*

Table 2. Effect of factors on variability of ejaculate traits

Ejaculate traits	Effect		
	Boars	Season	Interval between two consecutive mounts
Volume of ejaculate (ml)	ns	***	***
Concentration of sperm (x10^6 spermatozoa/ml)	***	***	ns
Number of total spermatozoa (x10^9 spermatozoa)	ns	**	*
Number of produced doses	ns	**	*

Significance: ns- not significant, *- P<0.05, **- P<0.01, ***- P<0.001

The differences between boars were recorded only in sperm concentration (Table 3). The difference between the best (ID number 1) and the worst boar (ID number 5) was 91.99x10^6 spermatozoa per millilitre of ejaculate. Since the animals were raised in the same facility, under the same conditions, these differences in phenotypic values of ejaculate concentration, are influenced by the individual characteristics of boars.

Table 3. Least square means (LSMeans) of ejaculate traits by boars

ID number of boar	LSMeans of ejaculate traits			
	VOL	CON	NT	NPD
1	217.31	242.24^{A}	47.47	18.99
2	229.98	$233.61^{a,Aa}$	42.83	17.13
3	216.16	$222.08^{a,Aa,c}$	43.97	17.59
4	264.68	$179.78^{B,b}$	43.75	17.50
5	279.02	$150.25^{B,Bb}$	33.87	13.55
6	244.63	$217.27^{a,Aa,e}$	43.77	17.51
7	240.70	$182.79^{B,d}$	40.13	16.05

VOL- volume of ejaculate (ml), CON- concentration of sperm ($x10^6$ spermatozoa/ml), NT- number of total spermatozoa ($x10^9$ spermatozoa), NPD- number of produced doses; Significance: ab, cd and de- P<0.05; AaBb- P<0.01; AB- P<0.001.

Comparison of LSM mean values for observed traits of ejaculate between seasons is presented in Table 4. During the summer (season 3) ejaculate VOL was the lowest, and the difference compared to the season 2 was -123.06 ml. During the summer, sperm concentration was above average, but the NT and NPD were below average, as a consequence of the low volume of ejaculate. Regardless of the fact that the boars were housed in controlled environment conditions, it is possible that higher temperatures during the summer months demonstrated a negative impact on quantitative sperm production. The highest NPD was produced during the autumn months (season 4), and during the same period the highest concentration of sperm was recorded. In comparison to season 2, during the autumn, the sperm concentration was higher by $86.23x10^6$ spermatozoa/ml. The reason for the highest concentration of sperm, the total number of spermatozoa in the ejaculate, and the number of doses during the autumn can be the stimulating effect of shortening of the photoperiod on neuro-humoral mechanism of sperm production.

Table 4. Least square means (LSMeans) of ejaculate traits by season

Season	Ejaculate traits			
	VOL	CON	NT	NPD
1 (December-February)	$234.04^{A,a}$	180.42^{A}	$37.49^{a,Aa}$	$15.00^{a,Aa}$
2 (March-May)	314.42^{B}	155.93^{A}	44.87^{b}	17.95^{b}
3 (June-August)	$191.36^{A,b}$	237.51^{B}	$39.06^{a,Aa}$	$15.62^{a,Aa}$
4 (September-November)	227.30^{A}	242.16^{B}	47.60^{Bb}	19.04^{Bb}
Population average	241.78	204.01	42.26	16.91

VOL- volume of ejaculate (ml), CON- concentration of sperm ($x10^6$ spermatozoa/ml), NT- number of total spermatozoa ($x10^9$ spermatozoa), NPD- number of produced doses; Significance: ab- P<0.05; AaBb- P<0.01; AB- P<0.001.

During the spring season the highest volume of ejaculate was determined (314.42 ml), as a result of negative correlation between volume and density and the

lowest concentration of sperm ($155.93x10^6$ spermatozoa/ml). The nature of this correlation is reported in many studies (*Wolf and Smital, 2009a; Wolf and Smital, 2009b; Kunowska-Slósarz and Makowska, 2011).* The results of our study are partly similar to the study of *Tomiyama et al. (2008),* who have, during the spring months, determined the highest volume and the lowest concentration, whereas during the autumn period, the volume of ejaculate was the lowest, with a maximum concentration of spermatozoa. Partial similarity exists also with the research of *Stančić et al. (2012)* who have recorded, during the period June-August and September-November, the average volume of ejaculate (213 and 232 ml) and the concentration of spermatozoa ($220x10^6$ and 210x106 spermatozoa/ml), while higher values were recorded in the period from December to February (293 ml, $319x10^6$ spermatozoa/ml) and March-May (285 ml, $284x10^6$ spermatozoa/ml). Contrary to the findings of the present study, *Okere et al. (2005), Kondracki et al. (2009), Wolf and Smital (2009a),* in the spring period, have determined the minimum volume of ejaculate from the tested breeds. This research is not consistent with the results of *Kunowska-Slósarz and Makowska (2011),* who have found, in the cold season (October and November), the highest volume and lowest density of ejaculate. Partial similarity exists with the research of *Rutten et al. (2000)* in which the lowest number of standardized doses is obtained from the semen in the summer period (26.3), and the highest in autumn (28.7), however, in our study, less doses per ejaculate were obtained.

Table 5 shows the ranking of boars according to the value of farrowing rate during the reproductive exploitation. The variation of the farrowing rate between boars ranged from 59.31 to 85.37%, which indicates a large difference in performance during use. Reproductive efficiency depends on several factors: boars, breeding season, sows with which boars are mated, weaning-oestrus interval, methods of insemination, sperm capacitive capabilities, etc.

Table 5. Rank of boar by farrowing rate

Rank of boar (ID number of boar)	Farrowing rate (%)	Group	P value
1 (5)	85.37	I	0.0882
2 (7)	72.22		
3 (4)	69.54		
4 (3)	66.30	II	
5 (2)	60.32		
6 (6)	60.00		
7 (1)	59.31		

Group I/II- Farrowing rate above/below the average (67.58%)

The difference in farrowing rate between the best (rank 1, ID number 5) and the worst boar (rank 7, ID number 1) was 26.06%. Regardless of the fact that the differences in the farrowing rate between boars were not statistically significant

(P=0.0882), it is necessary to rank and promptly identify boars with farrowing rate below average.

A broad interval of variation was found in the research of *Park (2013)* in which the farrowing rate on pig farms with pigs of Yorkshire genotype varied in the range 63.8-91.6%. The differences in the farrowing rate between boars are also due to the individual characteristics, so our research is in accordance with the results of *Ruiz-Sánchez et al. (2006)* who have found that the farrowing rates between boars range from 71 to 98%. The concordance is also present with the results of study by *Didion et al. (2009).* These researchers have determined the variation of farrowing rate in the range 38.9-82.7%, between 18 investigated boars.

Conclusion

The ejaculate properties vary under the influence of the season and the interval between two mounts. The differences in the concentration of sperm are influenced by the individual characteristics of boars. The mechanism of sperm production was strongly influenced by the season, primarily the negative impact of high summer temperatures and a possible stimulating effect of shortening of the photoperiod during the autumn. Control of productivity and ranking of boars based on the farrowing rate should be continuously carried out, regardless of the fact that in this experiment no statistically significant variation in the farrowing rate was determined between boars.

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Osobine ejakulata i reproduktivna efikasnost nerasta velikog jorkšira tokom iskorišćavanja

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Rezime

Osnovni cilj istraživanja bio je da se oceni varijabilnost osobina ejakulata: volumen ejakulata(VOL, ml), koncentracija sperme (CON, spermatozoida/ml), ukupan broj spermatozoida (NT) i broj proizvedenih doza (NPD) po ejakulatu pod uticajem nerasta, sezone i intervala izmedju dva skoka. Reproduktivna efikasnost

nerasta analizirana je na osnovu procenta prašenja (FR). Istraživanjem je bio obuhvaćen 341 ejakulat sedam nerasta velikog jorkšira. Ocena uticaja je izvršena primenom GLM procedure u statističkom paketu SAS 9.1.3. Prosečne vrednosti VOL, CON, NT, NPD i FR bile su: 244,28 ml, $203{,}77 \times 10^6$ spermatozoida/ml, $43{,}48 \times 10^9$ spermatozoida, 17,39 doza i 67,58%. Osobine ejakulata varirale su pod uticajem sezone ($P<0{,}01$; $P<0{,}001$). Interval između dva skoka nije uticao jedino na varijabilnost CON. Razlika izmedju najboljeg i najlošijeg nerasta bila je $91{,}99 \times 10^6$ spermatozoida/ml ejakulata ($P<0{,}001$). Tokom letnjih meseci VOL ejakulata bio je najmanji (191,36 ml). Najveći NPD (19,04 doza) proizveden je tokom jesenjih meseci, a tokom istog perioda bila je i najveća koncentracija sperme ($242{,}16 \times 10^6$ spermatozoida/ml). Bez obzira što razlike u procentu prašenja između nerasta nisu bile statistički značajne ($P=0{,}0882$), potrebno je vršiti rangiranje i pravovremeno identifikovati neraste sa procentom prašenja ispod proseka.

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EFFICIENCY OF PLANT PROTEASES BROMELAIN AND PAPAIN ON TURKEY MEAT TENDERNESS

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Original scientific paper

Abstract: The main subject of study is the effect the plant proteases bromelain and papain exert on turkey meat tenderness. Experiments are conducted with samples of raw meat in 3 different concentration levels of the enzyme solutions (50U/ml 100U/ml and 200 U/ml) and in 3 different time periods (duration) of treatment (24 h, 48 h, 72h). An increase in enzyme concentration and treatment duration results in a higher degree of protein hydrolysis in the turkey meat. The optimal conditions for hydrolysis with minimal loss of protein and highest retention of organoleptic qualities of the meat samples are established.

Key words: tenderizing, turkey meat, bromelain, papin

Introduction

Tenderness belongs to the most important meat quality traits. There are several factors that determine meat tenderness: sarcomere length, myofibril integrity and connective tissue integrity. The latter one determines the quality of background toughness. (*Chen et al., 2006*) There are two different components to meat toughness: *actomyosin toughness* and *background toughness*. Actomyosin toughness is attributed to myofibrillar proteins, whereas background toughness is due to connective tissue presence.

In the recent years interest is growing in the development of better methods to produce meat with improved tenderness whilst preserving its nutritional qualities. (*Koohmaraie, 1996*) Various physical and chemical methods have been developed to improve meat tenderness. (*Qihe et al., 2006*) These methods attempt to reduce the amount of connective tissue in the meat without that resulting in the breakdown of myofibrillar proteins.

Such meat tendernization methods include muscle stretching and electrical stimulation. The basic methods used in meat tendernization are marination and ion/organic acid injections. The aforementioned physical and chemical methods improve meat tenderness with little effectiveness.

Another interesting method in meat tenderization is the application of exogenous enzymes of vegetable, bacterial or fungal origin. It is used to examine

the alteration of the structure of the connective tissue and the integrity of the myofibrils.

The same enzymes can be used not only with beef, pork and poultry, but also with many marine products. Most of these enzymes are vegetable proteases. (*Ashie et al., 2002; Minh et al., 2012.)* In recent years, from all the exogenous proteolytic enzymes used in meat tenderization, the cysteine proteases have attracted considerable interest, in particular, vegetable cysteine proteases. Some of them have long been used in cooking. (*Sullivan and Calkin, 2010*).

The main objective of this project is to study the effect the two trade vegetable enzyme proteases (papain and bromelain) have on raw turkey meat and their capacity to hydrolyze protein complexes, present in the meat and its connective tissue.

Materials and Methods

Materials: Meat – turkey drumsticks (*Meleagris gallopavo*); Enzyme solutions – papain (Merck), bromelain (Merck).

Methods

Enzymatic processing of turkey meat samples – The meat samples are treated with bromelain or papain with alternating enzyme concentration and duration of the process.

Enzyme solutions - Both enzyme solutions are with the following caseinolytic activity – I (50U/ml), II (100U/ml), III (200U/ml). The enzymes are dissolved in a solvent containing 0,9% NaCl, sodium hydrogen carbonate and citric acid. The active acidity of the enzyme solutions is pH 6,30.

Measuring the Water Retention Capacities (MRC) - Meat samples of 3-5 g are wiped with filter paper to remove surface water and to weigh accurately in milligrams. This value is noted as raw met weight (starting weight). The samples are then treated with bromelain and papain solutions at 4°C for 24, 48 and 72 hrs. Then, the surface water is removed with filter paper. Alongside the samples, controls are assigned every full hour of treatment, in which the meat is placed inside enzyme-free marinate. The processed meat is weighted and is assigned value after enzyme treatment (final weight). A water retention percentage is determined.

Enzymatic activity - The caseinolytic activity of the proteases papain and bromelain is measured by the substrate casein in a 50mM Tris/ HCl boofer at pH 8.0 with 1mM $CaCl_2$, in accordance with *Chen's, Zhang et al. 2003's* method. One unit of enzyme activity is defined as the amount of enzyme needed to release 1 μg tyrosine from caseine for 1 minute.

Quantity assessment of free amino acids – ninhydrin test

The concentration of free amino acids in the dissolved fractions after enzyme hydrolysis is assessed by a ninhydrin test. *(Murariu et al., 2003*)

Collagen and elastin hydrolysis experiment in the already utilized enzyme solutions - 2.0 g connective tissue samples from the turkey drumsticks are inundated with 40 ml of the respective enzyme solution and incubated at room temperature for 24-96 hours. The experimental samples are then removed from the solution, leached with distilled water and lyophilized. Lastly, they undergo electrophoretic analysis.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE).

SDS–PAGE was preformed with the method outlined by *Laemmli (1970).*

Polyacrylamide gel - 6% stacking and 10 % separating gel:

Electrolyte buffer: Tris – glycine, pH 8,5 with 0,1 % SDS;

Statistical analysis - All data are presented as means ±SD (standard deviation) for at least three replications for each prepared sample. Statistical analysis was performed using two-sample t-test. The results are considered to be significant when $P<0,05$. All statistical analyses were performed using Excel 2013.

Results and Discussion

Turkey drumstick meat undergoes enzyme catalysis. The main objective is to study the effect the enzymes bromelain and papain exert on raw meat samples. In the conducted experiments 3 different enzyme solutions were used (50 U/ml, 100 U/ml and 200 U/ml). The experiments varied in their duration – 24h, 48h, 72h. Water retention capacity changes in the meat samples are recorded. The diagrams below indicate the varied rates in water retention capacity.

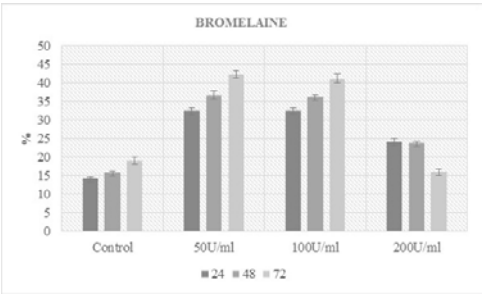

Figure 1. Water retention capacity after bromelain processing (±SD)

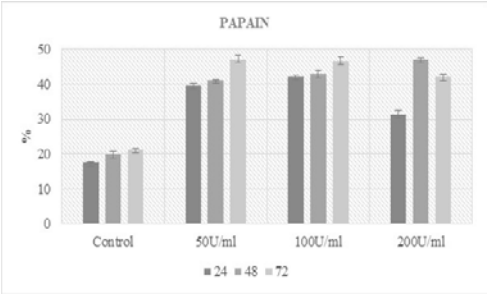

Figure 2. Water retention capacity after papain processing (±SD)

The obtained results establish that both parameters (enzyme concentration and processing duration) affect water retention capacity. Both bromelain and papain variants indicate higher water retention rate.

Bromelain and papain are vegetable cysteine endopeptidases. The improved meat tenderization with vegetable cysteine proteases is due to the higher breakdown of myofibril proteins and the disruption of the muscular fibril structure in the experimental samples compared to the control ones *(Jorgova et al.,1989).*

Due to partial enzyme hydrolysis of the meat proteins a higher affinity for water molecules is established. The augmented hydrophilicity is determined by the many hydrophilic groups (hydroxilic – OH, carboxilic –COO- amino – NH-3, thiolic – SH, amide – $CONH_2$), which are released at the surface of the protein molecules. Consequentially, in the processing with solutions of 50 U/ml and 100 U/ml caseinolytic activity, the water retention rate is higher. In hydrolysis with 200 U/ml, the more time is elapsed during treatment, the lower the water retention rate is, observed mostly in the use of bromelain. This is caused by the higher rate of hydrolysis of the meat proteins, respectively by the gelatinization of the samples and the release of terminal peptides and amino acids.

In order to assess the rate of full hydrolysis the enzymes induce in the solutions a test was conducted to determine the quantity of free amino acids in the reactive liquid. The analysis was done only for the samples with the longest duration in the enzyme solution (72 hrs). The obtained results are indicated below in table 1.

Statistically significant difference is found in the amino acid concentration between the control and experimental variants.

The highest concentration is noted in the reactive liquid containing 200U/ml caseinolytic activity of the respective enzyme. These results correlate with the water retention rate values. The higher the concentration of the enzyme is, the higher the chance of complete hydrolysis to occur in the meat proteins, deteriorating the appearance and taste of the meat.

Table 1. Free amino acid content in the reactive liquid after enzyme hydrolysis (±SD)

Variants	Concentration mg/ml		Significance[1]	Concentration mg/ml		Significance[1]
	Bromelain	Control		Papain	Control	
I	1,262±0,017	0,775± 0,082	**	1,218±0,172	0,728±0,116	*
II	1,210±0,028	0,867±0,004	***	1,335±0,097	0,856±0,088	*
III	1,490±0,021	0,772±0,036	***	1,577±0,576	0,856±0,045	*

[1] Significantly different from the control group at: *p < 0,05; ***p < 0,01; ***p < 0,001.

In the following visual materials (fig. 3 and 4) it is clear that the meat samples processed with 50 and 100 U/ml bromelain and papain for 24 hrs retain their colour and fresh look. On the outside the structure of the experimental variants is similar to the control ones and the muscle fibers are intact. The 48hr samples and, exceedingly most of all, the 72hr samples show muscle fiber deformation and breakdown, the colour fades and the surface turns mucous. The dissociation of the muscle fibers at such high rate is to be avoided since the outward appearance of the product is of major importance to the consumers.

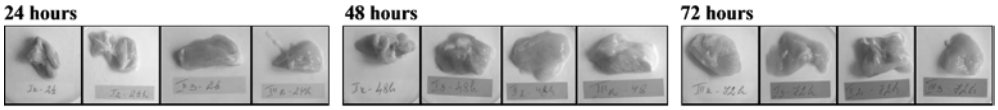

Figure 3. Control and experimental variants processed with bromelain

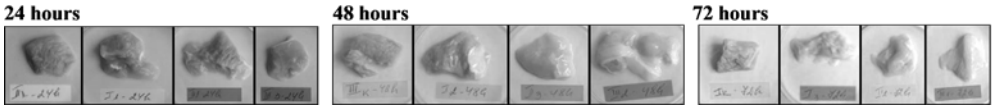

Figure 4. Control and experimental variants processed with papain

Turkey ligaments are mostly composed of collagen type I joined in firm fibers, which give the meat its toughness. With tenderization we aim for partial breakdown of this type of tissue and preservation of the muscle fibers. Treatment with proteolytic enzymes causes disorganization and disintegration of the collagen's structural elements by loosening its intermolecule bonds.

Electrophoresis in polyacrylamide gel SDS-PAGE is conducted with lyophilized samples of turkey ligaments after proteolytic enzyme treatment for 24 and 96 h. Figure 5 shows a photo of the conducted electrophoretic analysis on lyophilized samples of turkey ligaments post enzyme treatment with bromelain and papain.

The basic building unit of collagen – the tropocollagen molecule is composed of a triple coil with 3 polypeptide chains (two $\alpha 1$ and one $\alpha 2$). The control sample shows the typical visual representation of collagen. Two high-molecule mass fractions are observed. They most likely resemble the tropocollagen molecule in collagen type 1 (dimers and trimers).

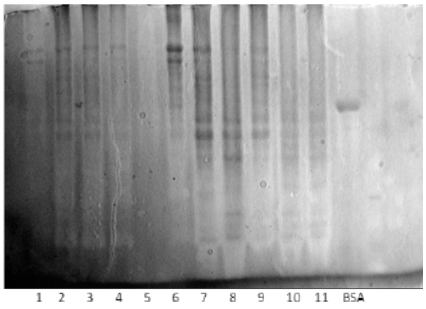

Figure 5. SDS-PAGE lyophilized turkey ligament samples post proteolytic enzyme treatment (bromelain or papain) for 24 or 96 h: 11 processing variants (1- *Control 24 h;* ***2*** *– variant I bromelain 24 h;* ***3*** *- variant II bromelain 24 h;* ***4*** *- variant III bromelain 24 h;* ***5*** *- Control 96 h;* ***6*** *- variant I bromelain 96 h;* ***7*** *- variant II bromelain 96 h;* ***8*** *- variant III bromelain 96 h;* ***9-*** *variant I papain 96 h;* ***10*** *- variant II papain 96 h;* ***11-*** *variant III papain 96 h* ***), BSA – standard – beef serum albumin.***

The rate of hydrolysis is contingent on the following factors – enzyme type, volume of enzyme concentration in the solution and treatment duration. Fractions with lower molecule mass are observed in the experimental variants. According to *Minh et al. (2012),* higher concentrations of the papain and bromelain preparations were able to hydrolyse the meat connective tissue proteins in a non-specific manner and generated a SDS–PAGE time course protein fragment profile. The samples treated with bromelain are noted to possess a smaller amount of fractions and a

lower breakdown rate, whereas the papain samples, particularly the high-concentrated ones, undergo complete breakdown of the connective tissue (multiple fractions with high Rf-value). Such intense hydrolysis leads to protein loss and deterioration of the organoleptic qualities of the meat.

Conclusion

Treatment with proteolytic enzymes results in disorganization and disintegration of the collagen structural elements, which in turn loosens and disrupts the intermolecule bonds. The higher the enzyme concentration and the higher the duration of processing are, the higher the rate of hydrolysis is.

The experimental variants processed with 50 and 100 U/ml bromelain and papain for the duration of 24hrs augment their water retention capacity by 20-25% with preserved colour and fresh look.

Enzyme concentration higher than 200 U/ml results in unwanted intense hydrolysis of the meat proteins and deteriorates the overall appearance and the gustatory qualities of the meat.

The optimal variant for hydrolysis with minimal protein loss and optimal preservation of the orgonoleptic qualities of the product is recorded in the processing of the raw turkey samples in bromelain and papain solutions with 50U/ml caseinolytic activity up to 24 hours.

Uticaj biljnih proteaza bromelaina i papaina na mekoću ćurećeg mesa

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Rezime

Glavni predmet proučavanja je efekat biljnih proteaza bromelaina i papaina na mekoću ćurećeg mesa. Eksperimenti su izvedeni sa uzorcima sirovog mesa u 3 različita nivoa koncentracije rastvora enzima (50U/ml 100U/ml i 200 U/ml) i u 3 različita vremenska perioda (trajanja) tretmana (24 h, 48 h, 72h).

Povećanje koncentracije enzima i trajanja tretmana dovodi do većeg stepena hidrolize proteina u ćurećem mesu. Uspostavljeni su optimalni uslovi za hidrolizu sa minimalnim gubitkom proteina i najvišim zadržavanjem organoleptičkih osobina uzoraka mesa.

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FAST AND SENSITIVE DETERMINATION OF CAMEL'S AND GOAT'S MEAT AND MILK USING SPECIES-SPECIFIC GENETIC MARKERS

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Original scientific paper

Abstract: For the fast and sensitive determination of camel's and goat's meat and milk, species-specific regions (SSR) of follicle stimulating hormone receptor (FSHR) gene in both camel and goat were amplified using PCR technique. DNA was extracted from small amount of muscles (0.05 gm) and very little of fresh milk (100 µl) to amplify specific DNA sequences of FSHR gene in both camel and goat using designed species-specific primer pairs. PCR amplified fragment size was 300 bp in camel's meat and milk, while the fragment size in goat's meat and milk was 855 bp. The use of species-specific regions of FSHR gene allowed direct and fast detection of adulteration and authentication of camel's and goat's meat and milk.

Key words: Meat, milk, species-specific, genetic markers, PCR

Introduction

In general, consumers are anxious about some of the issues such as food authenticity and adulteration, especially, when the identity of the ingredients in composite mixtures is not readily apparent (*Lockley and Bardsley 2000; Aida et al. 2005*). However, identification of species origin of meat and milk products has received considerable attention over the last ten years, particularly after the spread of commercial fraud. The common fraudulent practice found in the meat and dairy production line are the use of a less costly type of meat or milk in substitution of more expensive ones (*Calvo et al. 2002*). So, control tests of meat and milk products are very important to assure adulterant free for safe consumption. Previously, determination of species-specific meat and milk products could be achieved using numerous methods such as anatomical differences, enzyme-linked immunosorbent assays, histological differentiation of the hair that may possibly exist in the meat or milk, immune diffusion tests, level of glycogen in muscle tissue, properties of tissue fat, and sensory analysis (*Addeo et al. 1990; Chianese et al. 1990; Moio et al. 1990; Molina et al. 1999*). However, immunological,

electrophoretic and chromatographic methods are often not suitable and less sensitive for food products (meat and milk) identification, as well as complex, high cost and require data about the differences in protein compositions. Recently, food products like meat and milk products can be fast and accurate identified using molecular genetics methods such as PCR and PCR-RFLP (*Abdel-Rahman and Ahmed 2007; Ilhak and Arslan 2007; Abdel-Rahman et al. 2009*). Somatic cells from red meat and in milk (leukocytes and epithelial mammary cells) were used as a source of DNA which can be successfully applied for precise species differential using molecular genetics techniques (*Amills et al. 1997; Maudet and Taberlet 2001*). In this study, fast species-specific PCR technique was developed for determination and detection of camel's and goat's meat and milk.

Materials and methods

DNA extraction from meat. According to *Abdel-Rahman et al. (2009)*, genomic DNA was extracted from camel's and goat's muscle samples, where 50 mg of the tissue was homogenized and suspended in 500 μL STE (0.1 M NaCl, 0.05 M Tris-HCL and 0.01 M EDTA, pH 8). After adding 30 μL 10% SDS and 30 μL proteinase K (10 mg/mL), the mixture was vortexed and incubated at 50°C for 30 min. DNA was extracted by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume (50 μL) of autoclaved double distillated water (addH_2O).

DNA extraction from milk. Camel's and goat's genomic DNA was extracted from little fresh milk samples according to *Abdel-Rahman and Ahmed (2007)*, where 1400 μL of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0 and 0.5% SDS) and 30 μL of proteinase K (20 mg/mL) were added to 100 μL of camel's and goat's milk sample. The mixture was vortexed and incubated at 55°C for 20 min. DNA was extracted by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume (35 μL) of autoclaved double distillated water (addH_2O).

Species-specific primer pairs designing. Two PCR species-specific primer pairs were designed to amplify a specific segment of follicle stimulating hormone receptor (FSHR) gene coding ovulation in both camel and goat (Table 1). Using the online of the National Center for Biotechnology Information (NCBI), PCR primer pairs were designed (Primer-BLAST program) on the camel 531 bp linear DNA of FSHR gene sequence (GenBank accession number: GU990799.1) to

amplify a fragment of 300 bp (Figure 1). Also, PCR primer pairs were designed on the goat 860 bp linear DNA of FSHR gene sequence (GenBank accession number: AY765375.1) to amplify a fragment of 855 bp (Figure 2).

Table 1. Primer sequences of species-specific DNA regions and their annealing temperatures.

Species	Primer sequence 5' - 3' (Forward/Reverse)	Annealing temperature (°C)
Camel	ACTGGAATCTATCTGCTGCTC/GCTGCTGATGCCAAAGAGG	58
Goat	CGACAAGGCAAAACGGACAC/TCCTGGCAGAGGAAGACTCCA	51

Primers sequences alignment. Using the online BLAST program, the designed species-specific primers were confirmed and aligned to investigate whether there is a match or similarity with other species before proceeding to PCR. Table 2 shows the match of the forward primer which flanking the region of interest of FSHR gene in camel compared the other species using nucleotide sequence database in GenBank (NCBI). As expected, the primer matches to camel specie, but does not have matches to other target species (Animals producing meat and milk), that allow desired PCR amplification. Also in goat, the forward primer which flanking the region of interest of FSHR gene matches to goat specie, but does not have matches to other target species (Table 3).

```
>gi|329132912|gb|GU990799.1| Camelus bactrianus follicle stimulating hormone receptor
(FSHR) gene, partial cds (531 bp).

1   ACTGGAATCTATCTGCTGCTCATAGCATCCGTTGATATCCACACCAAAAGCCAGTACCACAACTATGCCATTGACTGGCAAACTGGA 87
  5'ACTGGAATCTATCTGCTGCTC3'.....
1   TGACCTTAGATAGACGACGAGTATCGTAGGCAACTATAGGTGTGGTTTTCGGTCATGGTGTTGATACGGTAACTGACCGTTTGACCT 87

88  GCAGGCTGTCATGCTGCTGGCTTTTTCACTGTCTTTGCCAGTGAGCTCTCAGTCTACACTCTGACAGCCATCACGCTGGAAAGATGG 174
88  CGTCCGACAGTACGACGACCGAAAAAGTGACAGAAACGGTCACTCGAGAGTCAGATGTGAGACTGTCGGTAGTGCGACCTTTCTACC 174

175 CATACCATCACCCACGCCATGCAGCTAGAATGCAAAGTGCAGCTCCGCCATGCTGCCAGTGTCATGCTGGTAGGCTGGGTCTTTGCT 261
175 GTATGGTAGTGGGTGCGGTACGTCGATCTTACGTTTCACGTCGAGGCGGTACGACGGTCACAGTACGACCATCCGACCCAGAAACGA 261

262 TTTGCAGTCGCCCTCTTTCCCCTCTTTGGCATCAGCAGCTACATGAAGGTGAGCATCTGCTTGCCCATGGATATTGACAGCCCCTTG 348
                  .....3'GGAGAAACCGTAGTCGTCG5'
262 AAACGTCAGCGGGAGAAAGGGGAGAAACCGTAGTCGTCGATGTACTTCCACTCGTAGACGAACGGGTACCTATAACTGTCGGGGAAC 348

349 TCACAGCTTTATGTGATGTCCCTCCTTGTGCTCAATGTCCTGGCCTTTATGGTCATCTGTGGCTGCTACACTCACATCTACCTCACA 435
349 AGTGTCGAAATACACTACAGGGAGGAACACGAGTTACAGGACCGGAAATACCAGTAGACACCGACGATGTGAGTGTAGATGGAGTGT 435

436 GTGAGGAACCCCAACATCATGTCCTCCTCTAGTGACACCAAGATTGCCAAGCGCATGGCCATGCTCATCTTCACGGACTTCCTCTGC 522
436 CACTCCTTGGGGTTGTAGTACAGGAGGAGATCACTGTGGTTCTAACGGTTCGCGTACCGGTACGAGTAGAAGTGCCTGAAGGAGACG 522

523 ATGGCACCC 531
523 TACCGTGGG 531
```

Figure 1. Primer pairs flanking a fragment (300 bp) of FSHR gene in camel. The forward primer begins from the nucleotide number one, while the reverse primer begins from the nucleotide number 300 in the opposite direction.

```
>gi|54399507|gb|AY765375.1| Capra hircus follicle-stimulating hormone receptor (FSHR) gene,
promoter region and partial cds (860 bp).

1 TTCCGACAAGGCAAAACGGACACGTGACCGAGGGTGGGATAGTTGGTGTCTACTAAGCCCAAAAACCCAGCCTGACCTGTTGGTCAC 87
   5'CGACAAGGCAAAACGGACAC3'→
1 AAGGCTGTTCCGTTTTGCCTGTGCACTGGCTCCCACCCTATCAACCACAGATGATTCGGGTTTTTGGGTCGGACTGGACAACCAGTG 87

88 TCAGCTGAGTCAGTTATCTCTGTAGACTACTCTTAAAAGTGCCTCCTACCATCTGTCCAGGGGCTCACTAACCCACTGCCTGTCTTC 174
88 AGTCGACTCAGTCAATAGAGACATCTGATGAGAATTTTCACGGAGGATGGTAGACAGGTCCCCGAGTGATTGGGTGACGGACAGAAG 174

175 TGCTACACCATATTTGGTTGTTAATTCCAGCAAGAAAGAGAATTAGTGGCTTTGACCCAGAAGTTCTGGTTTTGTATCAAGCAGCCT 261
175 ACGATGTGGTATAAACCAACAATTAAGGTCGTTCTTTCTCTTAATCACCGAAACTGGGTCTTCAAGACCAAAACATAGTTCGTCGGA 261

262 GGAGGAAGACATTGACACCAAGACTGGAAACAGGTCCCTGACCTTCACTGAGGAACTCTGTTAATGATGTTTCACACTGCAGATTGC 348
262 CCTCCTTCTGTAACTGTGGTTCTGACCTTTGTCCAGGGACTGGAAGTGACTCCTTGAGACAATTACTACAAAGTGTGACGTCTAACG 348

349 ATCTGTTTTGGAGAAAGTCAAGCGTGTCACTCTGTTTTGAGAAAAAAAAAAAAAAAGTGACCCACAGGGACAGTCTTACAGCGAATTT 435
349 TAGACAAAACCTCTTTCAGTTCGCACAGTGAGACAAAACTCTTTTTTTTTTTTTTCACTGGGTGTCCCTGTCAGAATGTCGCTTAAA 435

436 AATATAAGCTATTCTAGACATGCATCAAGTTTCAATTTGCAAACCCAACCAAAAAGGGTAAAGGGACAGCGTATCTTGCCACGCCCT 522
436 TTATATTCGATAAGATCTGTACGTAGTTCAAAGTTAAACGTTTGGGTTGGTTTTTCCCATTTCCCTGTCGCATAGAACGGTGCGGGA 522

523 CTACCTCTCCCTCCCCACCCCCACCAAAGTCACTGCTGTCACTCAGAAATTCTGCTATTTGTCTGGAAGTGACCGATAAAAAAGAAA 609
523 GATGGAGAGGGAGGGGTGGGGGTGGTTTCAGTGACGACAGTGAGTCTTTAAGACGATAAACAGACCTTCACTGGCTATTTTTTCTTT 609

610 AAAAGGAACACGGCCCTGGGCGGGTCACGTGACCCTACCAGCTCCCAACGCAGACCTCTTCTCAAAAGGGCTCAGTGTGGAGCCTCT 696
610 TTTTCCTTGTGCCGGGACCCGCCCAGTGCACTGGGATGGTCGAGGGTTGCGTCTGGAGAAGAGTTTTCCCGAGTCACACCTCGGAGA 696

697 GAAATCTGGGCAGGATTGTCTCTGCAGAGGCAGAAGCAAGCAGGTGGATGGATAAGTAAACATGGCCTTGTTCCTGGTGGCCTTGCT 783
697 CTTTAGACCCGTCCTAACAGAGACGTCTCCGTCTTCGTTCGTCCACCTACCTATTCATTTGTACCGGAACAAGGACCACCGGAACGA 783

784 CGCATTCCTGAGCTTGGGCTCAGGATGCCACCATCGACTCTGTCACTGCTCTAATGGAGTCTTCCTCTGCCAGGAGG 860
                                                    ←3'ACCTCAGAAGGAGACGGTCCT5'
784 GCGTAAGGACTCGAACCCGAGTCCTACGGTGGTAGCTGAGACAGTGACGAGATTACCTCAGAAGGAGACGGTCCTCC 860
```

Figure 2. Primer pairs flanking a region (855 bp) of FSHR gene in goat. The forward primer begins from the nucleotide number four, while the reverse primer begins from the nucleotide number 858 in the opposite direction.

Table 2. Alignments of the forward primer of the FSHR fragment in camel. Selected accession numbers are camel specie only, while the other accession number (not selected) is Pteropus specie (species of fox).

Descriptions

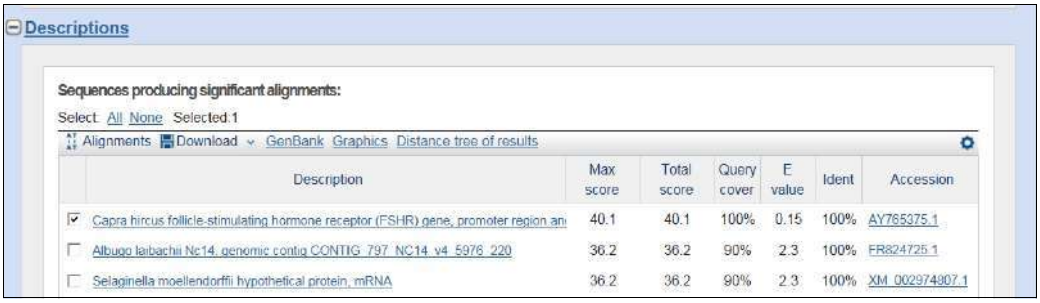
Sequences producing significant alignments:

Select: All None Selected:4

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185787.1
☑	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185786.1
☑	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185785.1
☑	Camelus bactrianus follicle stimulating hormone receptor (FSHR) gene, partial cds	42.1	42.1	100%	0.034	100%	GU990799.1
☐	PREDICTED: Pteropus alecto follicle stimulating hormone receptor (FSHR), transcri	38.2	38.2	90%	0.53	100%	XM_006909606.1

Table 3. Alignments of the forward primer of the FSHR fragment in goat. Selected accession number is goat specie only, while the next accession number (not selected) is Albugo laibachii (species of oomycete).



Descriptions

Sequences producing significant alignments:

Select: All None Selected:1

Alignments Download GenBank Graphics Distance tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	Capra hircus follicle-stimulating hormone receptor (FSHR) gene, promoter region an	40.1	40.1	100%	0.15	100%	AY765375.1
☐	Albugo laibachii Nc14, genomic contig CONTIG_797_NC14_v4_5976_220	36.2	36.2	90%	2.3	100%	FR824725.1
☐	Selaginella moellendorffii hypothetical protein, mRNA	36.2	36.2	90%	2.3	100%	XM_002974807.1

PCR amplification. PCR was performed in a reaction volume of 25 µL using 25 ng of genomic DNA of each specie, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/µL Taq DNA polymerase (Promega). Thermal cycling (MyGene Series Peltier Thermal Cycler) was carried out by initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 1 min, annealing temperature at 51 (goat) and 58 (camel) for 1 min, polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min, then the samples were held at 4°C. The amplified DNA fragments were separated on 2.5% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Results and discussion

In this study, genomic DNA from meat (red meat) and milk (leukocytes and epithelial mammary cells) was extracted to amplify specific segments or regions of FSHR gene encoding ovulation in two different species camel and goat. The amplification of species-specific DNA segments of the FSHR gene yielded PCR product with size of 300 bp in length in camel's meat and milk. While, in goat's meat and milk the PCR product was 855 bp (Figure 3A and B). To avoid the fraudulent and to assure consumers of accurate labeling, it was necessary to invent new precise and fast techniques for differentiation and detection of adulteration of meat and milk species-specific. Where, the common fraudulent practice found in the meat or milk products line is the use of a less costly type of meat or milk in substitution or mixing of more expensive or authenticated ones. In the current study, PCR technique was developed to identify and detect the adulteration of camel's and goat's meat and milk products. Where, two primer pairs (forward and

reverse) were specifically designed to generate two specific fragments in length of FSHR gene in both camel (300 bp) and goat (855 bp).

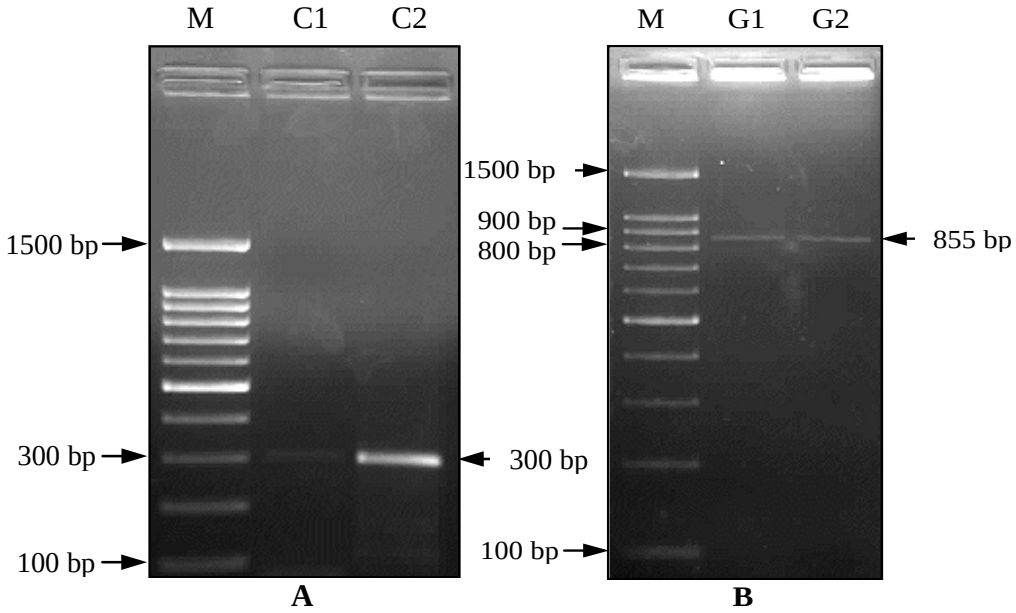


Figure 3. PCR products generated by species-specific designed primers in both camel (A) and goat (B). Lanes C1 and C2 are camel's milk and meat fragment size (300 bp), respectively. Lanes G1 and G2 are goat's milk and meat fragment size (855 bp), respectively. Lane M is a molecular weight marker.

Numerous studies have been previously carried for identification species-specific meat and milk, weather using immunological, electrophoretic and chromatographic methods (*Addeo et al. 1990; Chianese et al. 1990; Moio et al. 1990; Molina et al. 1999*) or molecular genetic methods such as PCR and PCR-RFLP techniques (*Baradakci and Skibinski 1994; Meyer et al. 1995; Meyer et al. 1996; Hopwood et al. 1999; Partis et al. 2000; Abdel-Rahman and Ahmed 2007; Ahmed et al. 2007; Abdel-Rahman et al. 2009*). In our previous studies, species-specific PCR and PCR–RFLP techniques were used to identify species meat and milk. Where, genomic DNA from buffalo's, cattle's and sheep's meat and milk and from cat's, dog's, donkey's, horse's and pig's meat was extracted to amplify the gene encoding species-specific repeat (SSR). The results of PCR amplification were 603 bp in length in buffalo and cattle, 374 bp in sheep, 672 bp in cat, 808 bp in dog, 221 bp in donkey and horse, and ≤100 bp in pig. To differentiate between buffalo's and cattle's meat and milk, as well donkey's and horse's meat, cytochrome-*b* gene was amplified (359 bp) and digested with restriction enzymes. By *Taq*I restriction enzyme, two different fragments (191 bp and 168 bp) were generated in buffalo, whereas no fragments were obtained in cattle. With *Alu*I

restriction enzyme, three different patterns were generated in horse (189 bp, 96 bp and 74 bp), while in donkey no digestion was obtained (*Abdel-Rahman and Ahmed 2007; Ahmed et al. 2007; Abdel-Rahman et al. 2009*). To avoid the similarity of the PCR products between the previous mentioned species and the two species under study, species-specific fragments in length 300 bp and 855 bp of FSHR gene were amplified in both camel and goat, respectively. However, the author's previous and present results of PCR product sizes for identification of species-specific meat and milk products are concluded in Table 4.

Table 4. PCR products of the species-specific meat and milk products descending ordered according to the fragment sizes.

Species	Primer sequence 5' - 3'	Annealing temperature (°C)	PCR product size (bp)
Goat	CGACAAGGCAAAACGGACAC TCCTGGCAGAGGAAGACTCCA	51	855
Dog	GGAGTATGCTTGATTCTACAG AGAAGTGGAATGAATGCC	52	808
Cat	CTCATTCATCGATCTACCCA GTGAGTGTTAAAACTAGTACTAGAAGA	52	672
Buffalo/Cattle	AAGCTTGTGACAGATAGAACGAT CAAGCTGTCTAGAATTCAGGGA	60	603
Sheep	GTTAGGTGTAATTAGCCTCGCGAGAA AAGCATGACATTGCTGCTAAGTTC	62	374
Camel	ACTGGAATCTATCTGCTGCTC GCTGCTGATGCCAAAGAGG	58	300
Donkey/Horse	TTCTGCTCTGGGTGTGCTACTT CTACTTCAGCCAGATCAGGC	55	221
Pig	GGAGCGTGGCCCAATGCA ATTGAATCCACTGCATTCAATC	57	≤100

Conclusion

PCR technique was developed for rapid and sensitive identification of camel's, and goat's meat and milk using designed species-specific primers. PCR amplified fragment size was 300 bp in camel's meat and milk, while the fragment size in goat's meat and milk was 855 bp. The proposed PCR assay represents a quick and sensitive method applicable to the detection and authentication of meat and milk species-specific.

Brzo i osetljivo određivanje kamiljeg i kozjeg mesa i mleka korišćenjem specifičnih genetičkih markera za vrstu

S.M. Abdel-Rahman, A.M. Elmaghraby, A.S. Haggag

Rezime

Za brzo i osetljivo određivanje kamiljeg i kozjeg mesa i mleka, regioni folikula specifični za vrstu (SSR) koji stimulišu hormon receptore (FSHR) gena i u kamile i koze su pojačani pomoću PCR tehnike. DNK je ekstrahovana iz male količine mišića (0.05 gm) i veoma malo svežeg mleka (100 µl) za amplifikaciju specifičnih DNK sekvenci FSHR gena i u kamile i koze korišćenjem dizajniranih prajmera parova specifičnih za vrstu. Veličina amplifikovanog PCR fragmenta je bila 300 bp mesa i mleka kamile, dok je veličina fragmenata u kozjem mesu mleku bio 855 bp. Upotreba regiona FSHR gena specifičnih za vrstu omogućava direktnu i brzu detekciju autentifikacije kamiljeg i kozjeg mesa i mleka.

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ECONOMIC EFFICIENCY OF BREEDING DAIRY SHEEP IN THE MOUNTAIN AND HILLY REGIONS OF BULGARIA

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Abstract: Sheep breeding is an important means of livelihood for the population living in the mountain and hilly regions of the country. The object of the present study is to analyze the economic efficiency of breeding dairy sheep in the mountain and hilly regions of Bulgaria. Object of the study are 2 models of farms with 100 ewes from the dairy type and the relevant categories lambs and rams. We compare and analyze two levels of milk productivity – 70 l per lactation in the first farm and 100 l per lactation in the second farm. The whole grain and roughage necessary for feeding ewes are calculated while hay is self-provided. The average fertility per ewe is 115%, the average wool yield is 2.2 kg and the repair of the flock is 20%. Incomes and costs are estimated at current prices for the 2013 – 2014. It was found that in the terms of the present study we may draw the conclusion that in case the farmer is not getting subsidy from the State Fund "Agriculture" will be efficiently to raise only ewes that have milk productivity 100 l per lactation; in support of the farm subsidies by the State Fund "Agriculture" and the two levels of milk production is appropriate breeding of dairy sheep in the mountainous and hilly regions of the country; in order to improve the economic efficiency farmers should pay attention to increasing the fertility of ewes and protection of the new-born lambs as well as increasing of milk productivity of ewes.

Key words: sheep-incomes-costs-profit-milk production

Introduction

Sheep breeding is an important means of livelihood for the population living in the mountain and hilly regions of the country. The favorable climate and forage conditions appear to be economic incentive for developing this industry because of

the rich pasture available, which helps reducing the costs for feeding animals. Unfortunately, in these regions there is a strongly declined demographic structure and, namely, ageing and depopulation of the villages. In consequence, the number of people breeding sheep strongly decreases. One of the factors for some overcoming this process is creating conditions for development of the small and medium-sized agribusiness. Because of the crisis in sheep-breeding is mostly economic, it can be overcome only by using economic mechanisms and particularly by creating better conditions for the sheep farms to work.

According to *Ozkan et al. (2009)* in terms of dynamic and competitive market only the most efficient agricultural producers will generate profit and survive. A farm is economically efficient when the total value of production overbalances production costs and there is an optimization of costs per production unit *(Tauer and Belbase, 1987).*

According to *Georgiev (1990),* economic efficiency in sheep breeding is a dynamic category where the results received and costs made as well as the factors generating them are variable measures.

A number of authors investigate economic efficiency of sheep breeding. *Stankov (2000)* analyse farms raising dairy sheep, merino sheep and meat-type sheep. According to them, to find the economic optimum where a unit of production growth is received by the lowest growth of costs is necessary to optimise the function forage-productivity.

In our previous study we analyzed economic efficiency of dairy and meat-type sheep farms in the intensive regions of the country *(Popova et al., 2007).* We established that in the dairy sheep farms there was a higher income generated and higher costs but lower efficiency of production.

Odzhakova et al. (2009) studied the economic efficiency of breeding Karakachanska breed and *Popova et al. (2011)* analyzed the economic efficiency of processing cow and sheep milk produced in the farm of OSZJ – Smolian. As a result the authors concluded that breeding sheep from the Karakachanska breed is not efficient without financial support from the state.

Mihailova-Toneva (2001) studied economic efficiency of breeding sheep from the Synthetic population Bulgarian Milk sheep in the flock of Institute for animal sciences in Kostinbrod. The biggest share in the structure of costs is that of costs made for providing forage – 75%. The incomes from market lambs and milk are approximately one and the same as relative part of the total income – about 25%. The subsidy received per year is 1/3 of the total income for the flock. The income per ewe is 110 BGN.

The object of the present study is to analyze the economic efficiency of breeding dairy sheep in the mountain and hilly regions of Bulgaria.

Materials and Methods

Object of the study are 2 models of farms with 100 ewes from the dairy type – Synthetic population Bulgarian Milk sheep and the relevant categories lambs and rams. We compare and analyze two levels of milk productivity – 70 l per lactation in the first farm and 100 l per lactation in the second farm. During the winter period (180 days) sheep are hand-fed as follows – 0.600 kg concentrate mixture, 2 kg roughage and 1 kg hay per ewe daily. During the summer period (180 days) animals are grazing. Before and during the mating period /about 45 days/ a ewe is given 0.300 kg hay daily. The whole grain and roughage necessary for feeding ewes are calculated while hay is self-provided. The average fertility per ewe is 115%, the average wool yield is 2.2 kg and the repair of the flock is 20%. There is only one person responsible for serving the sheep in the farm and another one to herd on the sheep on during the milking procedure. Milking is made by hand. Incomes and costs for 1 year are estimated at current prices for the 2013 – 2014.

Results and Discussion

The necessary quantities for feeding the animals are represented on table 1. The biggest share of the costs is for concentrate mixture – 64.51% /I option/ and 66.41% /II option/ and roughage – 27.12% and 25.67%. The quantity of concentrate mixture needed for reaching milk production rate of 100 l per lactation increased 8.75% compared to the quantity necessary for the rate of 70 l. Totally, the costs for forage increases 5.65% in case where the milk productivity is higher.

Table1. Necessary feeds for 1 year

Forage	Quantity, kg		Value, BGN	
	I option	II option	I option	II option
Silage	24000	24000	4080	4080
Meadow hay	21940	21940	1097	1097
Concentrate mixture	20224	21994	9707	10557
Starter mixture	180	180	162	162
Total	66344	68114	15046	15896

On table 2 production costs are represented. Costs made for fodder take the biggest portion – 62.83% /I option/ and 64.11% /II option/ followed by costs for labour – 28.06% /I option/ and 27.10% /II option /. Production costs in case where the milk production is expected to be 100 l per lactation are 3.55% higher which means 850 BGN additionally.

Table 2. Production costs, BGN

Indices	Value, BGN	
	I option	II option
Feeds	15046	15896
Labour costs	6720	6720
Water, Elec. energy, Fils	800	800
Medical costs	650	650
Amortization	400	400
Other costs	330	330
Total	23946	24796

The incomes from production are represented on table 3. The biggest part of income is coming from to the market lambs – 51.08% /I option/ and 47.44% /II option/. Incomes from the sale of milk take significant part – respectively 38.42% /I option/ and 42.82% /II option/. Incomes from sheep rejected and market wool is insignificant – respectively 10.49% and 9.74%. Therefore, the farmers should pay special attention to increasing fertility of ewes and protection of the new-born lambs and improving milk productivity.

Table 3. Incomes, BGN

Production	Quantity, kg		Value, BGN	
	I option	II option	I option	II option
Milk	7000	8400	9100	10920
Lambs	2200	2200	12100	12100
Waste Sheeps	1000	1000	2000	2000
Wool	220	220	484	484
Total	10420	11820	23684	25504

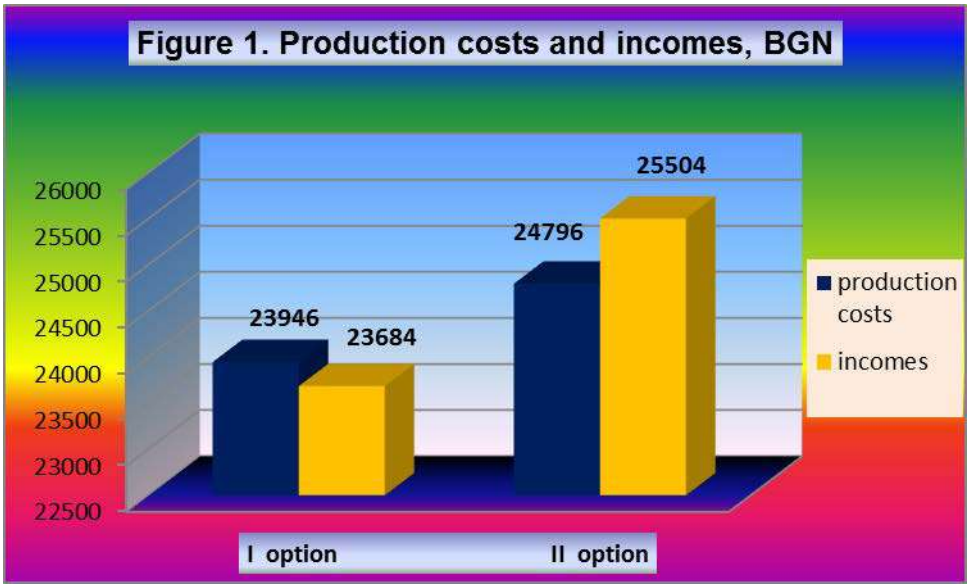

Figure 1. The production costs and the income from the farm.

On table 4 economic results are represented. The economic activities have been analyzed in both cases – with and without subsidies from the state, taking in account the subsidy is amounting to 41 BGN per ewe. In case the farmer is getting subsidy from the state the profit gained amounts to 3838 BGN /I option/ and 4808 BGN /II option/ as the profit per ewe is respectively 38.38 BGN and 48.08 BGN. In case the farmer does not receive subsidy from the State Fund "Agriculture" profit amounts to 708 BGN for the second option and for the first option there is a loss amounting to 262 BGN.

Table 4. Economic results

Indices	Value, BGN	
	I option	II option
Incomes, BGN	23684	25504
Costs, BGN	23946	24796
Subsidies, BGN	4100	4100
Profit with subsidies, BGN	3838	4808
Profit per sheep with subsidies, BGN	38,38	48,08
Rate of profitability with subsidies, %	16,03	19,39
Profit without subsidies, BGN	-262	708
Profit per sheep without subsidies, BGN	-2,62	7,08
Rate of profitability without subsidies, %	-1,09	2,86

The rate of profitability which is relative index of production efficiency and expresses the rate of return of production is the following – 16,03% /I option/ and 19,39%/II option/ in case the farmer is getting subsidy and 2,86% /II option / and -1,09% /I option / in case the farmer does not receive subsidy from the state.

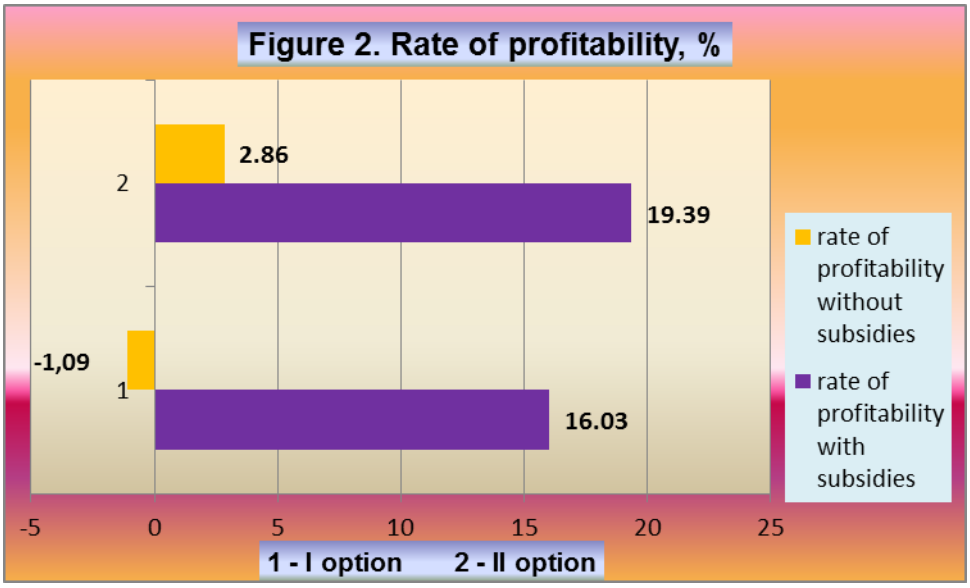

Figure 2. The rate of profitability with and without subsidies.

Profit in option II increases with 25.27% compared to option I in case the farmer is getting subsidy which means 970 BGN more. When analyzing the growth amounting to 970 BGN and the additional cost made for concentrate mixture in order to improve the milk productivity amounting to 850 BGN we ascertain the fact that for every 850 BGN additional cost that farmer makes to improve the feeding process and milk productivity he has return of 120 BGN additional profit but only if his activities are subsidized by the State Fund “Agriculture”.

Conclusion

It was found that:

In the terms of the present study we may draw the conclusion that in case the farmer is not getting subsidy from the State Fund “Agriculture” will be efficiently to raise only ewes that have milk productivity 100 l per lactation.

In support of the farm subsidies by the State Fund "Agriculture" and the two levels of milk production is appropriate breeding of dairy sheep in the mountainous and hilly regions of the country.

In order to improve the economic efficiency farmers should pay attention to increasing the fertility of ewes and protection of the new-born lambs as well as increasing of milk productivity of ewes.

Ekonomska efikasnost uzgoja muznih ovaca u planinsko-brdskom regionu Bugarske

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Rezime

Ovčarstvo je važan način da se obezbede sredstva za život za stanovništvo koje živi u planinskim i brdskim područjima zemlje. Cilj ove studije je da se analizira ekonomska efikasnost uzgoja muznih ovaca u planinskim i brdskim područjima Bugarske. Predmet istraživanja su 2 modela farme sa 100 ovaca i relevantne kategorije jagnjadi i ovnova. U radu se porede i analiziraju dva nivoa proizvodnje mleka - 70 l po laktaciji na prvoj farmi i 100 l po laktaciji na drugoj farmi. Žitarice i krmno bilje potrebno za ishranu ovaca se obračunava u kalkulaciji, a seno je obezbeđeno na samoj farmi. Prosečna plodnost po ovci je 115%, prosečan prinos vune je 2.2 kg i remont stada je 20%. Prihodi i troškovi se procenjuju u tekućim cenama za 2013 - 2014.

Utvrđeno je da, u slučaju da farmer ne dobija subvencije iz državnog fonda "Poljoprivreda", efikasnije je da se bavi odgojem samo ovaca koje imaju produktivnost mleka 100l po laktaciji; u znak podrške poljoprivrednim subvencijama od strane državnog fonda "Poljoprivreda," i dva nivoa proizvodnje mleka, prikladno je za uzgoj muznih grla u planinskim i brdskim područjima zemlje; u cilju poboljšanja ekonomske efikasnosti, farmeri treba da obrate pažnju na povećanje plodnosti ovaca i zaštitu novorođene jagnjadi, kao i povećanje proizvodnje mleka od ovaca.

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IMPACT OF NON-PROTEIN NITROGEN SUBSTANCES ON GRAPE POMACE SILAGE QUALITY

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Abstract: In this study grape pomace was ensilaged without and with the addition of NPN substances (Benural) at the dose of 0.5, 1.0 and 1.5% of the amount of husk and with the addition of inoculant based on homo and hetero fermentative lactic acid bacteria. The greatest effect on the nutritional value had application of Benural, especially in increasing the CP content from 126.9 to 178.3 g kg^{-1}DM, an increase of over 40%. Application of Benural increased the ammonia and soluble nitrogen several times, but even with the maximum addition of Benural percentage of ammonia nitrogen in the total nitrogen reached only slightly above 5% NH_3-N/Σ N (5.38%), while the percentage of soluble nitrogen in total nitrogen was 28.29%. The application of inoculants generally had no significant impact, both on the chemical composition and the fermentation process.

Key words: grape pomace, NPN substance, protein fractions, silage quality

Introduction

By-products are generally characterized by high water content, rapid fermentation and easy spoilage. Grape pomace is the residue after pressing grapes and juice extraction and consists mainly of epidermis, seeds, fruit and insoluble parts of the rest of the juice. Nutrient content of grape pomace is variable and depends on the grape variety, processing technology, the climate and soil conditions of production. For feeding of ruminants grape pomace can be used fresh, dried and ensiled. The use of fresh pomace for animal feeding in the long run is not possible due to rapid fermentation and spoilage. Dried grape pomace has been used in ruminant nutrition especially in fattening cattle *(Nikolić et al., 1980, Zeremski, 1982, Stojanović et al., 1989)*, but today it is not profitable since energy required for drying is too expensive. Ensilage of grape pomace is the best solution today.

Taking into account that the grape pomace is poor in protein and rich in

soluble carbohydrates, it is necessary to apply the procedure to increase the crude protein (CP), in order to optimize the nutrition of ruminants. One extremely simple and inexpensive method is to add a suitable source of non-protein nitrogen in the silage or meal. Symbiotic bacteria in the rumen will use the added nitrogen to synthesize their own proteins of high biological value, which will be digested and used in the small intestine of ruminants together with proteins from the meals *(Đorđević and Dinić, 2011)*. In accordance with this fact, the experiment is planned with an addition of commercially available non-protein nitrogen in the silage to increase the amount of CP.

The aim of this study was to investigate the possibility of ensiling grape pomace with the addition of different doses of NPN substances and inoculants based on lactic acid bacteria, and to determine detailed chemical composition and nutritive value of silage.

Material and methods

Grape pomace without stems from white grape "Rkaciteli" taken immediately after pressing from the "Rubin" Kruševac and ensiled by the method of two-factorial experiment (4 x 2) with three replications. Silage was conducted in the experimental containers holding 130 liters. The containers was loaded with sand layer thickness of 15 cm. The containers was open 65 days after ensilaging, and two examples was taken for analysis. The first research factor (A) is the addition of NPN commercial substance called "Benural" to pomace in four concentrations, and the second factor (B) is the addition of inoculants on two levels: A_1 B_1 – grape pomace without Benural and inoculant; A_1 B_2 – grape pomace with inoculant; A_2 B_1 – grape pomace with 0.5% Benural; A_2 B_2 – grape pomace with 0.5% Benural and inoculant; A_3 B_1 – grape pomace with 1.0% Benural; A_3 B_2 – grape pomace with 1.0% Benural and inoculant; A_4 B_1 – grape pomace with 1.5% Benural; A_4 B_2 – grape pomace with 1.5% Benural and inoculant (homofermentative-*Enterococcus faecium* and *Bacillus plantarum*, and heterofermentative-*Bacillus brevis*; concentration of 5×10^{10} cfu g^{-1})

As NPN substance was used Benural S which contains 42% urea, 56% bentonite and 2% sulfur. In the grape pomace and silage following parameters were determined: dry matter content (DM), crude protein (CP), crude fiber (CF), crude fat (EE), NDF, ADF, lignin. In the silage DM content, the degree of acidity (pH), ammonium and soluble nitrogen, content of acetic, butyric and lactic acids was determined. Chemical analisys were performed in the laboratory of the Institute for Forage Crops, Kruševac according to standard methods (AOAC, 2002). DM digestibility was determined by enzymatic method according *De Boevar et al. (1986)*. Data were processed by the analysis of variance (COSTAT) in a randomized block design. The significance of differences between arithmetic means was tested by LSD test.

Results and discussion

Quality forage is a decisive factor in the production of milk and meat of ruminants. Balance meals with regard to forage to concentrate ratio, then the content of nutrients: protein, minerals and vitamins, especially the relationship between energy and protein, is also a very significant impact on the quality of ruminant nutrition. The content of nutritive substances in the starting material and in the control silage (treatment A_1B_1) is similar and the differences are small (Table 1). CP content increases from the control silage (126.9 g kg^{-1}DM) to the silage with the highest level of Benural (178.3 g kg^{-1}DM). Nutritional value of grape pomace is significantly lower than other forages, resulting from high proportion of structural carbohydrates (CF, NDF and ADF). CF level is above 300 g kg^{-1}DM, and the concentration of NDF and ADF was also high at 650 g kg^{-1} DM and 590 g kg^{-1} DM, respectively (Table 1).

Table 1 Organic matter composition of starting material and silages, g kg^{-1} DM

Starting material		Crude Proteins	Ether extract	Crude Fibre	NFE	NDF	ADF
Grape pomace		118.4	91.3	339.6	417.0	651.4	490.8
Silages							
Level of Benural	Inoculalant	CP	EE	CF	NFE	NDF	ADF
A_1	B_1	125.7	109.1	346.2	365.8	628.6	594.7
	B_2	128.2	102.5	329.5	385.2	676.6	589.2
A_2	B_1	140.8	93.8	319.1	391.5	647.3	586.8
	B_2	139.7	98.5	325.1	401.1	657.5	609.1
A_3	B_1	153.4	94.5	327.4	359.6	673.4	587.2
	B_2	157.1	100.9	310.1	377.0	636.9	546.7
A_4	B_1	177.2	102.3	299.3	373.7	634.4	576.5
	B_2	179.3	94.1	323.3	346.7	640.3	607.5
$\overline{X}_{A_1}$		126.9^{d}	105.8^{a}	337.9	375.5^{ab}	652.6^{a}	591.9^{a}
$\overline{X}_{A_2}$		140.3^{c}	96.1^{a}	322.1	396.3^{a}	652.4^{a}	597.9^{a}
$\overline{X}_{A_3}$		155.3^{b}	97.7^{a}	318.7	368.3^{ab}	655.2^{a}	566.9^{a}
$\overline{X}_{A_4}$		178.3^{a}	98.2^{a}	311.3	360.2^{b}	637.3^{a}	592.0^{a}
$\overline{X}_{B_1}$		149.3^{a}	99.9^{a}	323.0	372.6^{a}	645.9^{a}	586.3^{a}
$\overline{X}_{B_2}$		151.1^{a}	99.0^{a}	322.0	377.5^{a}	652.8^{a}	588.1^{a}
Significance for A		*	ns	ns	*	ns	ns
Significance for B		ns	ns	ns	ns	ns	ns

ns – no significance; * ($p<0,05$); ** ($p<0,01$)

Nutritional value of raw material in this study is considerably lower compared to the research of many researchers, which is defined by much higher lignin content and a high proportion of lignin in the NDF (Table 2). The concentration of CP in this study in the starting material and in the control silage was within results of *Stojanovic et al. (1989), Zalikarenab et al. (2007), Alipour and Rouzbehan (2007)* and *Bahrami et all. (2010)*. The higher values are found by *Zheng et all. (2012)* and *Mirzaei-Aghsaghali et al. (2011),* who showed the highest value of 172.7 g kg^{-1}DM, which matches the results of these studies with the addition of 1.5% Benural. CF content in the starting material and in the silage in this study is within the above-cited results which values are above 300 g kg^{-1}DM. Significantly lower value (228 g kg^{-1}DM) was found by *Mirzaei-Aghsaghali et al. (2011)* and two times lower values were found by *Zalikarenab et al. (2007)*. The concentration of lignin in this study was almost two times higher than in most other research. These differences in the content of CF and lignin can be interpreted by varietal characteristics of grape, climatic conditions, the quality of the separation stems and quality of juice removal. For example DM content of grape pomace for the study was 400 g kg^{-1}DM, and this value in *Mirzaei-Aghsaghali et al. (2011)* was 225 g kg^{-1}DM. Results for NDF and ADF in this study is within the above-cited results. Significantly lower values for these parameters were determined by *Zalikarenab et al. (2007),* and in particular *Bahrami et al. (2010),* who showed 471 g kg^{-1}DM for NDF and 312 g kg^{-1}DM for ADF.

Table 2 Digestibility of silage from grape pomace, lignin content and share of lignin in NDF (%)

Levels of Benural	Inoculalant	Digestibility	Lignin	Lignin % in NDF
A_1	B_1	33.65	39.29	62.09
	B_2	32.75	39.15	58.73
A_2	B_1	33.18	37.98	59.07
	B_2	33.92	38.43	58.75
A_3	B_1	33.43	38.88	58.34
	B_2	34.61	37.10	57.84
A_4	B_1	35.03	37.29	58.80
	B_2	34.03	38.07	58.48
$\overline{X}_{A_1}$		33.20	39.22	60.41
$\overline{X}_{A_2}$		33.55	38.20	58.91
$\overline{X}_{A_3}$		34.02	37.99	5.,09
$\overline{X}_{A_4}$		34.53	37.68	58.64
$\overline{X}_{B_1}$		33.82	38.36	59.58
$\overline{X}_{B_2}$		33.83	38.19	58.45
Significance for A		ns	ns	
Significance for B		ns	ns	

ns – no significance; * ($p<0,05$); ** ($p<0,01$)

Sampling of silage was done 90 days after ensiling when chemical analyses of the process of lactic acid fermentation were performed. It was found that very good silage were obtained. The high DM content (400 g kg^{-1}DM) is established. It is known that levels of DM above 300 g kg^{-1} DM prevents the butyric acid and other undesirable bacteria that cause spoilage and decay of silage *(Ensilage, 1978, Djordjevic and Dinić, 2003)*, as shown by the results of this study because in any of the treatment has not been established presence of butyric acid (Table 3). It was found that increasing the dose of Benural caused statistically significant reduction in dry matter content, while inoculant differences were not statistically significant.

Table 3 Parameters of biochemical changes in silages, g kg^{-1} DM

Benural Level	Inoculalant	DM gkg^{-1}	pH	NH_3-N/ΣN, %	H_2O-/ΣN, %	Acetic acid	Butyric acid	Lactic acid
A_1	B_1	417	3.78	0.99	4.81	19.8	0.0	26.9
	B_2	417	3.76	0.97	4.87	20.4	0.0	24.3
A_2	B_1	403	3.85	2.82	12.02	20.6	0.0	27.5
	B_2	407	3.89	3.13	13.87	21.8	0.0	23.6
A_3	B_1	400	3.95	4.08	21.19	30.4	0.0	15.0
	B_2	392	4.02	4.26	23.67	22.7	0.0	18.7
A_4	B_1	392	4.08	5.52	27.98	20.2	0.0	25.0
	B_2	396	4.00	5.23	28.59	20.6	0.0	26.8
$\overline{X}_{A_1}$		417^{a}	3.77^{d}	0.98^{d}	4.84^{d}	20.1	0.0^{a}	25.6^{a}
$\overline{X}_{A_2}$		405^{ab}	3.87^{c}	2.98^{c}	12.94^{c}	21.2	0.0^{a}	25.5^{a}
$\overline{X}_{A_3}$		396^{b}	3.98^{b}	4.17^{b}	22.43^{b}	26.5	0.0^{a}	16.8^{b}
$\overline{X}_{A_4}$		394^{b}	4.04^{a}	5.38^{a}	28.29^{a}	20.4	0.0^{a}	25.9^{a}
$\overline{X}_{B_1}$		403^{a}	3.91^{a}	3.39^{a}	16.49^{a}	22.7	0.0^{a}	23.6^{a}
$\overline{X}_{B_2}$		403^{a}	3.92^{a}	3.35^{a}	17.75^{a}	21.4	0.0^{a}	23.3^{a}
Significance for A		*	*	*	*		ns	*
Significance for B		ns	ns	ns	ns	ns	ns	ns

ns - no significance, * ($p < 0.05$), ** ($p < 0.01$)

It is noted that the increase in the content of Benural helps to reduce the degree of acidity. Silage with pH values up to 4.20 are scored high marks, even for the lower DM content of silage *(Ensilage 1978)*. Different concentration of Benural caused statistically significant difference in the degree of acidity of the treatments, while the use of inoculants had no effect on the degree of acidity (Table 3). The presence of lower fatty acids in silages (lactic, acetic and butyric) is an

indicator of the successful lactic acid fermentation. Earlier it was pointed out the absence of butyric acid and relatively favorable ratio of lactic and acetic acids. In the present study, the application of inoculant did not have statistically significant impact on the content of the lower fatty acids (lactic, acetic and butyric), which can be explained by rapid decrease of pH to around 4.0.Application of Benural at 1% provided the greatest amounts of acetic and the lowest content of lactic acid, which was statistically significant.With increasing addition of NPN substances the proportion of ammonia and soluble nitrogen in the total nitrogen content of silage (% NH3-N / Σ N) increases from the control silage to silage with 1.5% Benural, but the values are small (max 5.38%). It was found that the increase in the content of Benural in the silage induced increase of % NH3-N / Σ N and% H_2O-N / N Σ, and the differences are statistically significant, while the use of inoculants had no effect on these values Table 3).

Conclusion

On the basis of the examination of ensiling grape pomace with addition of Benural and inoculants based homo and hetero fermentative lactic acid bacteria it can be concluded: Grape pomace can be successfully ensiled without additives. Add NPN substances contributed to a significant increase in crude protein, ammonia and soluble nitrogen, whereas there was no significant degradation of the protein in the silage. The application of inoculants generally had a significant impact on both the chemical composition and the fermentation process.

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Uticaj dodavanja neproteinskih azotnih supstanci na kvalitet silaže komine grožđa

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Rezime

U istraživaniima komina grožđa je silirana bez i uz dodatak NPN supstanci (Benural) u količini od 0,5; 1,0 i 1,5% od količine komina i sa dodatkom inokulantana na bazi homo i heterofementativnih mlečno-kiselinskih bakterija.. Najveći efekat na hranljivu vrednost imala je primena NPN, posebno u povećanju

sadržaja SP od 126,9 na 178,3 gkg^{-1}SM. Primena NPN supstanci je uticala na povećanje amonijačnog i rastvorljivog azota nekoliko puta, ali i pri najvećoj količini dodatog NPN supstanci udeo amonijačnog azota u ukupnom azotu je neznatno prešao vrednost od 5% (%NH3-N/ΣN 5,38) dok je udeo rastvorljivog azota u ukupnom azotu iznosio 28,29%. Primena inokulanata uglavnom nije imala značajnog uticaja, kako na hemijski sastav, tako i na proces fermentacije.

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THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

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