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Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164; e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

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THE MOST COMMON HEALTH DISORDERS AND WELFARE OF DAIRY COWS AND CALVES

B. Stanković[1], S. Hristov[1], D. Ostojić-Andrić[2], Z. Zlatanović[3], Lj. Samolovac[4], N. Maksimović[2]

[1]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[2]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[3]Higher Agricultural-food processing school, Ćirila i Metodija 1, Prokuplje, Republic of Serbia
[4]Institut PKB Agroekonomik, Industrijsko naselje b.b. 11213 Padinska Skela-Beograd, Republic of Serbia
Corresponding author: baxton@agrif.bg.ac.rs
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Abstract: Three farms of dairy cows (A, B and C) were observed for health disorders of dairy cows and suckling calves. Farm A is farm with tied system of rearing, with 1100 cows, while farms B and C have 400 and 600 cows kept loose with outdoor pens, respectively. Data regarding welfare criteria of dairy cows (health, feeding, housing and behaviour) were collected and analysed through Protocol of Welfare Quality (2009). Health disorders of dairy cows and suckling calves were collected and statistically analysed by chi-square test (χ^2 test). Welfare of all of three dairy farms were assessed as acceptable, meaning that provided welfare conditions meet the minimum requirements of animals. Principle of provision of good health was rated as acceptable (≥20 points) on farm A, while on farms with loose system (B and C) overall health rated as excellent (≥80 points). Occurrence rate of reproductive, locomotor, skin and claws disorders and digestive and systemic disorders of dairy cows and calves up to 4 months old were very different between three farms (A, B, C) with χ^2-values of 2901.71, 252.02, 204.08, 1152.31 and 184.23 respectively; $\alpha<0.01$). According presented data, it is obvious that the majority health problems were observed in tied system of rearing, on farm A, such as reproductive disorders and mastitis, as well as injuries and bad body score and lame cows. The most serious health problems of the calves were diarrhea and bronchopneumonia of different etiology.

Key words: calves, dairy cows, health disorders, welfare

Introduction

Among the different components of dairy cow welfare (health, feeding, housing and behaviour), the European Food Safety Authority (EFSA) reported that dairy cows are especially affected by poor health (*EFSA*, 2012). Welfare indicator includes many injuries that disturb health status of cattle causing pain through estimation of injuries like lameness and skin alterations; diseases through scrutinizing occurrence of coughing, nasal, ocular and vulvar discharge, hampered respiration, milk somatic cell count, diarrhoea, dystocia, downer cows and mortality, as well as pain induced by management procedures, such as dehorning and tail docking. In addition, many more diseases and disorders occur in farm conditions, reducing production and reproductive results and compromising welfare of different categories of cattle (*Broom and Fraser, 2007; Hristov et al., 2012; Stanković et al., 2012*). Health problems in dairy cows cause production losses (*Rajala-Schultz et al., 1999*), lead to treatment costs (*Kossaibati and Esslemont, 1997*), and are detrimental to animal welfare (*FAWC*, 1997). According to *Fleischer et al. (2001)* the incidence of health disorders has increased, possibly because they are associated with increased milk yield and production stress, as well as mortality rates (from 2 to 3.5% in 10 years), with locomotor disorders as one of the main causes (*Thomsen et al., 2004*).

Having in mind that many diseases often occur in dairy farms in Serbia, the objective of this paper was set to analyse welfare on three farms of different capacity with tied and loose system of rearing, particularly the principle of the good health in respect of the health disorders of these dairy cattle categories.

Material and Method

Three farms of dairy cows (A, B and C) were observed for welfare and health disorders of dairy cows and suckling calves.

Farm A is farm with tied system of rearing, with 1100 cows, while farms B and C have 400 and 600 cows kept loose with outdoor pens, respectively. Calves are kept individually during the first 15 days and then moved to groups on farms B and C, while on farm A they are being kept tied in stalls on the other side of the feeding corridor opposite to their mothers during the first 5-7 days of life and being transferred to group pens with 5-10 calves each.

Data regarding welfare criteria and principles of dairy cows (health, feeding, housing and behaviour) were collected and analysed through Protocol of Welfare Quality (*Anon., 2009*). Health disorders of dairy cows and suckling calves were collected and statistically analysed by chi-square test (χ^2 test). Data collected on these farms was processed by *Welfare Quality® scoring system*, using specific mathematical operation - *Choquet integral*, enabling adequate assessment/scoring

of each measure, criterion and principle adequately, according to its relevance and relative contribution to overall assessment of welfare on the farm. According to scores, criteria and principles, overall assessment classifies the welfare on farms into four qualitative categories: not classified, acceptable, enhanced and excellent.

Results and Discussion

Results of overall welfare assessment on dairy farms trough criteria and principles are presented in table 1.

Table 1. Welfare criteria assessment on three observed farms

Welfare criterion	*Farm A*	*Farm B*	*Farm C*
System of rearing	*Tied*	*Loose*	*Loose*
Absence of prolonged hunger	100,00	100.0	100.0
Absence of prolonged thirst	100,00	3.0	3.0
Comfort around resting	8,60	45.1	45.1
Ease of movement	15,00	100.0	100.0
Absence of injuries	16,40	99.3	98.7
Absence of diseases	36,70	74.3	86.0
Absence of pain induced by management procedures	28,00	100.0	100.0
Expression of social behaviours	100,00	100.0	100.0
Expression of other behaviours	0,00	0.0	0.0
Good human-animal relationship	70,50	40.7	76.1
Positive emotional state	16,90	58.4	42.0
Welfare principle			
Good feeding	100,00	14,60	14,60
Good housing	11,00	65,40	65,40
Good health	21,30	80,40	89,20
Appropriate behaviour	18,20	28,50	28,30
Overall welfare	*Acceptable*	*Acceptable*	*Acceptable*

Using welfare quality protocol (*Anon*, *2009*), welfare of dairy cattle on three farms were assessed as acceptable, meaning that provided welfare meet the minimum requirements of animals.

Principle of good feeding was assessed as excellent on farm A, while on farms B and C it was unsatisfying (≤ 20 points) for inadequate number of water bowls. Proportion of cows in poor condition on the farms studied corresponds to the interval (0 - 6%), which *Webster* (*2005*) found on farms of the highest level of welfare.

The principle of good housing conditions was rated as unacceptable (≤20 points) on farm A, while on the farms B and C as enhanced (≥55 points), where cows had freedom of movement and better comfort, which is in accordance with results of *Ostojić-Andrić et al*. (*2011*) and *Forkman and Keeling* (*2009*).

Principle of provision of good health was rated as acceptable (≥20 points) on farm A and farms B and C as excellent (≥80 points). It should be have in mind that skin alterations are consequence of various causes, housing conditions, spacing and calving parity (*Kielland et al*., *2009*) and unbalanced diet, creating predisposition (*Schulze et al*., *2009*). The presence of injuries in dairy cows on farms B and C does not represent a significant risk factor for their welfare, while on the farm A were determined a significant proportion of cows with skin lesions. Results within the principles of good health show that the average incidence of diseases such as nasal or vaginal discharge, cough, difficult respiration, tachypnoa, mastitis, diarrhoea and lying cow syndrome are not a risk to the welfare on farms B and C (2.25 - 5.00%), according *Forkman and Keeling* (*2009*). On farms B and C the absence of disease criterion was enhanced, and on the farm A acceptable. Established poor hygiene of cows might not increase the incidence of mastitis corresponding to the results of *Ellis et al*. (*2007*).

Criterion appropriate behaviour on farms B and C was acceptable, while on farm A unsatisfactory. The expression of social behaviour was assessed on all farms as excellent. The relationships of animals to humans and cows co-specific interactions have a major impact on the health, productivity and welfare, being important indicators of welfare (*Hemsworth and Coleman, 2011*). Good man - animal relationship was rated as enhanced (≥55 points) on farms A and C, while on farm B acceptable (≤55 points). According to the results of positive emotional state criterion of cows, farms B and C had enhanced quality of welfare, opposite to non-classified of the farm A. Welfare quality on observed farms could be described as acceptable to enhanced, but there are welfare problems as consequences of inadequate rearing conditions, compromising animals comfort, hygiene and freedom to move. Reproductive disorders, such as dystocia, and mortality rate menace dairy cattle welfare.

Generally, welfare level of dairy cattle is influenced by system of rearing and farm capacity, being better on smaller farms regarding lameness and skin lesions. Loose system of rearing has positive influence on cows' welfare, their health and emotional status, possibility of movement and higher comfort (*Ostojić-Andrić, 2013*).

Observed health problems were related to reproduction, udder health, locomotion, and respiratory, metabolic, and digestive disorders. The data regarding reproductive disorders rate of dairy cows on three dairy farms were presented in table 2.

Table 2. Reproductive disorders rate of dairy cows on three observed farms

Farm	A	B	C
size	1100	400	600
System of rearing	tied	loose, with outdoor pens	loose, with outdoor pens
Abortus	4.723	0	0
Ovarian cyst	8.45	17.5	7.5
Corpus luteum persistent	136.27	0	0.83
Endometritis	60.27	65.25	5.00
Pyometra	9.00	0	0
Febris puerperalis	24.09	0	0
Puerperal paresis	9.45	5.5	1.67
Mastitis	85.36	8.75	3.33
Partus gravis	8.09	0	1.83
Sectio Caesarea	2.27	0	0
Prolapsus uteri and vaginae	3.00	0	0
Retentio secundina	35.27	25.5	2.50
Rectovaginae	0.9	0	0
Tumor uteri	0.9	0	0
Urovaginae	0.73	0	0
Vaginitis	5.73	0	0
Thelitis	0.9	0	0
Udder quarters defects	2.36	0	0
Oedema uberis	15.36	0	0
Mumificatio and maceratio feti	0.73	4.25	1.00

Differences between occurrence rate of reproductive disorders of dairy cows on three dairy farms were very significant (χ^2=2901.71; α<0.01). Not only that listed reproductive disorders cause many problems in reproduction and consequently in dairy production, but they seriously jeopardize welfare of cows as well, causing pain, limited movement possibility and loss appetite.

The data regarding locomotor disorders rate of dairy cows on three farms were presented in table 3.

Table 3. Locomotor disorders rate of dairy cows on three observed farms

Farm	A	B	C
System of rearing	tied	loose, with outdoor pens	loose, with outdoor pens
Diagnosis	%	%	%
Arthritis and poliarthritis	13.00	0	0
Deformatio extremitates	5	0	0
Luxatio	0.181	0	0
Fracture	0.9	0	0

Differences between three farms in of respect locomotor disorders occurrence rate of cows were found to be very significant (χ^2=252.02; α<0.01), corresponding to the principle of provision of good health, as well as differences between three farms in respect of skin and claws disorders occurrence rate of cows were found to be very significant (χ^2=204.08; α<0.01), presented in table 4.

Table 4. Skin and claws disorders rate of dairy cows on three observed farms

Farm	A	B	C
System of rearing	tied	loose, with outdoor pens	loose, with outdoor pens
Diagnosis	%	%	%
Abcesus	0.36		
Phlegmona	3.55	0	0
Contusio	0.73	0	0
Vulnus	3.82	0	0
Panaritium	6.45	0	0

The data regarding digestive and systemic disorders rate of dairy cows on three dairy farms were presented in table 5.

Table 5. Digestive and systemic disorders rate of dairy cows on three observed farms

Farm	A	B	C
System of rearing	tied	loose, with outdoor pens	loose, with outdoor pens
Diagnosis	%	%	%
Cahexio	50.91	0	0
Diarrhea	2.45	0	0
Dislocatio abomasi	8.64	0	0
Indigestio	5.73	0	2.50
Intoxicatio	5.73	0	0
Enteritis	1.45	0	0
Hepatopatiae	3.64	0	0
Ketosis	29.91	0	0
Meteorismus	1.81	0	0
Mors per apoplexio	0.9	0	0
Sepsis	1.09	0	0
Conjuctivitis	0.181	0	0
Bronchopneumonia	5.27	0	0
Scabies	0.73	0	0

Differences between three farms in respect of digestive and systemic disorders occurrence rate of cows were found to be very significant (χ^2=1152.31; α<0.01). These disorders directly influence welfare of cows, due to the impact on principles of good health and good feeding.

The transition period is critically important to health and production of dairy cows. Milk fever, ketosis, retained foetal membranes, metritis, and displaced abomasum primarily impact cows during this period. Immunosuppression (*Mallard et al., 1998*) leads to increased susceptibility to environmental mastitis, with the greatest incidence around parturition (*Smith et al., 1985*).

According presented data, the majority health problems were observed in tied system of rearing, on farm A, such as reproductive disorders and mastitis, as well as injuries, poor body condition score and lame cows, which is in accordance with obtained welfare criterion good health. All of these findings are supported by previous investigations by *Stanković et al*. (*2014*).

Mastitis and lameness are the most important diseases of dairy cows (*Bareille et al., 2003*), which was confirmed through this study. The main locomotion disorders included laminitis, under-run heel or sole, sole ulcer, wall abscess, digital dermatitis, white line disease, interdigital granuloma, etc. (*Shearer et al., 2002*).

The occurrence of health disorders during the transition period, such as ketosis, retained foetal membranes and subsequent metritis, or displaced abomasum and secondary ketosis result in lost milk production (*Wallace et al.*, 1996; *Bareille et al., 2003*).

The most important problems of the welfare of cows in Serbia are related to housing with no outdoor outlets or pasture, occurrence of lameness, dystocia, downer cow syndrome and mortality, the manifestation of aggression between animals and bad stockmen attitude (*Ostojić-Andrić, 2013*). Early identification of injuries or disease is becoming difficult because surveillance is expected to decrease, due to use of automatic milking units and feeders that limits personnel requirements (*Frost et al., 1997*).

Significantly lower incidence of joint and udder injuries and less veterinary interventions were recorded if tied cows had possibility to move occasionally vs. cows constantly tied (*Ostojić-Andrić et al., 2011*), as well as clinical and subclinical mastitis rate (*Hristov et al., 2005*).

The data regarding health disorders rate of calves on three dairy farms were presented in table 6. Differences between three farms in respect of digestive and systemic disorders occurrence rate of health disorders of calves were very significant (χ^2=184.23; α<0.01). The most serious health problems of the calves were diarrhea and bronchopneumonia.

Table 6. Health disorders rate of calves (up to 4 months old) on three observed farms

Farm	A	B	C
Number	476	182	124
Diagnosis	%	%	%
Omphalophlebitis	1.05	0	0
Indigestion	0.84	0	0
Diarrhoea	30.25	54.95	16.13
Bronchopneumonia	98.74	28.57	9.68
Arthritis and poliarthritis	0.84	1.65	0
Sepsis	1.05	0	0

Calving can be a traumatic and risky event in the life of a calf. The most common cause of dystocia is excessively large calf birth weight and a resulting incompatibility of fetal-maternal size, especially at first calving (*Leslie, 2012*). The stress effects of a difficult calving greatly increase the risks of illness and death in young dairy calves. Difficult calving contributes almost 50% of all calf deaths (*Lombard et al., 2007*).

Calves, as all newborns are the most receptive category to pathogens, especially before colostrum intake. On farm A calves are kept tied in stalls on the other side of the feeding corridor opposite to their mothers during the first 5-7 days of life, even before they were offered colostrum, making them easy target for all pathogens in stalls environment through employees, birds, vehicles and open doors, which is common practice on many farms in Serbia.

Keeping good health is the most important condition of dairy cattle welfare. Health control measures include good hygienic, spatial and microclimate conditions of rearing, biosecurity on farms and during transport, programs for the control of major diseases, occurrence of lameness, claws care, control of mastitis and use of anaesthetics and analgesics as standard in operative procedure (SOP) of dehorning of the calves (*Hristov and Stanković, 2009; Anon., 2010; Ostojić-Andrić, 2013*).

Conclusions

According obtained data, dairy cattle welfare of all of three dairy farms were assessed as acceptable, meaning that provided welfare conditions meet the minimum requirements of animals. Principle of provision of good health was rated as acceptable (≥20 points) on farm A, while farms with loose system (B and C) overall health rated as excellent (≥80 points).

On the farm A was determined a significant proportion of cows with skin lesions, while presence of injuries on farms B and C does not represent a significant risk factor for their welfare. Results within the principles of good health show that the average incidence of diseases such as nasal or vaginal discharge, cough, difficult respiration, tachypnoa, mastitis, diarrhoea and down cow syndrome

are not a risk to the welfare on farms B and C (2.25 - 5.00%). On farms B and C the absence of disease criterion was enhanced, and on the farm A acceptable.

Occurrence rate of reproductive, locomotor and skin disorders and digestive and systemic disorders of dairy cows and calves up to 4 months old were very different between three farms of different size and system of rearing, with χ^2-values of 2901.71, 252.02, 204.08, 1152.31 and 184.23 respectively; $\alpha<0.01$). The most serious health problems of the calves were diarrhea and bronchopneumonia. According presented data, it is obvious that the majority health problems were observed in tied system of rearing, on farm A, such as reproductive disorders and mastitis, as well as injuries, poor body condition score and lame cows.

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Najčešći zdravstveni poremećaji i dobrobit muznih krava i teladi

B. Stanković, S. Hristov, D. Ostojić-Andrić, Zlatanović Z., Samolovac Lj., Maksimović N.

Rezime

Tri farme muznih krava (A, B i C) su analizirane u pogledu zdravstvenih poremećaja i dobrobiti krava i teladi. Na farmi A je zastupljen vezani sistem držanja sa 1100 krava, dok se na farmama B i C sa 400 odnosno 600 grla, krave drže slobodno.

Podaci o dobrobiti mlečnih krava (zdravlje, ishrana, smeštaj i ponašanje) su prikupljeni i analizirani primenom protokola za ocenu kvaliteta dobrobiti (*Anon, 2009*). Poremećaji zdravlja krava u laktaciji i teladi su prikupljeni i statistički analizirani hi-kvadrat testom (χ^2 test).

Dobrobit na sve tri mlečnih farmi je ocenjena kao prihvatljiva jer zadovoljava minimalne zahteve životinja. Princip obezbeđenja dobrog zdravlja je ocenjen kao prihvatljiv (≥20 bodova) na farmi A, dok je na farmama sa slobodnim sistemom (B i C) ocenjen kao odlično (≥80 poena). Pojava reproduktivnih, lokomotornih, digestivnih i sistemskih poremećaja mlečnih krava i teladi do 4 meseca starosti se veoma razlikovala između tri farme različite veličine i sistema uzgoja, sa χ2-vrednostima 2901,71, 252.02, 204.08 , 1152,31 i 184,23, redom (α <0,01). Prema iznetim podacima, češća pojava zdravstvenih problema je uočena u vezanom sistemu uzgoja, na farmi A, u pogledu reproduktivnih poremećaja, mastitisa, povreda, loše telesne kondicije i hromosti krava, kao i proliva i bronhopneumonija teladi različite etiologije.

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GENETIC POLYMORPHISM OF KAPPA CASEIN AND CASEIN MICELLE SIZE IN THE BULGARIAN RHODOPEAN CATTLE BREED

P. Hristov[1], B. Neov[1], H. Sbirkova[2], D. Teofanova[1], G. Radoslavov[1], B. Shivachev[2]

[1] Department of Animal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
[2] Department of Structural Crystallography and Materials Science, Laboratory of X-ray Diffraction analysis, Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.
Corresponding author: Denitsa Teofanova; E-mail: denyrt@yahoo.com
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Abstract: The present study aimed to compare the size of casein micelle in cow milk sample in function of kappa casein (*CSN3*) genetic polymorphism. Sixteen cows from Bulgarian Rhodopean cattle breed were genotyped by PCR-RFLP analysis. Milk samples from the three found *CSN3* genotypes (AB, AA and BB) were employed for the determination of casein micelles size by Dynamic Light Scattering (DLS). The results showed differences in the size and polydispersity of the casein micelles between the milks of cows with different genotypes. Hydrodynamic radii of micelles at a scattering angle of 90 °C varied from 80 to 120 nm and polydispersity varied from 0.15 to 0.37. In conclusion casein micelle size of *CSN3* AA cows (~ 120 nm) exceed with about 60% cows with AB (~ 80 nm) and BB genotype (~ 70 nm). These results could be useful for improving technological properties of the milk.

Keywords: casein micelle, Dynamic Light Scattering, kappa casein polymorphism

Introduction

The bovine casein locus contains four milk protein genes: αs1-casein (*CSN1S1*), β-casein (*CSN2*), αs2-casein (*CSN1S2*), and κ-casein (*CSN3*) (*Threadgill et al., 1990*). The genes are organized in a cluster of approximately 250 kB (*Rjinkels et al., 1997*). Among all known *CSN3* variants A and B are with highest frequency in *Bos taurus* (*Caroli et al., 2009*). In milk, the caseins exist as polydisperse, large, roughly spherical colloidal particles, 50–600 nm in diameter (mean ~ 150 nm), called ‘‘casein micelles’’ (*Fox et al., 2008*). The size, form and

structure of the casein micelle are of great importance for cheese-making properties of the milk (*Di Stasio et al., 2000*).

Since milk protein genes' polymorphism in genus *Bos* have been discovered and characterized its development is associated mainly to milk practice to clarify association between genetic variants and milk quantitative and qualitative traits (*Tsiaras et al., 2005*). This gives opportunity to usage some allelic variants as markers for milk composition and manufacturer properties of milk. Also researches of milk proteins polymorphism are focused to clarify origin, domestication and biogeography of modern cattle breeds (*Jann et al., 2004*)

Genetic variants of milk proteins can be detected by various identification methods. These techniques include, e.g., acrylamide electrophoresis in denaturing (SDS PAGE) or native conditions, isoelectric focusing (IEF), HPLC chromatography, Cryo-scanning electron microscopy etc. (*Hallen et al., 2009; Ren et al., 2013*). An inexpensive and fast method to determine the size distribution profiles of small particles in suspension or solution is Dynamic Light Scattering (DLS) (*Gebhardt et al., 2006; de Kruif et al., 2012*). DLS is an optical detection method that can directly measure some important structural parameters of biomacromolecules, such as hydrodynamic radius (R_h) and diffusion coefficient in solution. It exhibits many advantages in the analysis process, including sensing very small amounts of samples without destruction; real-time monitoring of the specimens under different conditions (temperature, pH etc.) and in addition is relatively simple and convenient for operating. Thus, DLS has been widely applied to the structural researches of proteins, polysaccharides and other bio-macromolecules (*Chu et al., 1995; O'Connell et al., 2001*).

In the present work, the correlation between *CSN3* different genotypes and the hydrodynamic radius (Rh) of casein micelles from individual milk samples are investigated.

Materials and Methods

Animals and sample collection

The experiments were performed on nasal swab and milk samples from pure breed cows of Bulgarian Rhodopean cattle (BRC). That breed originated from autochthonous Shorthorn cattle, upgraded mainly with Jersey cow.

From a herd of 80 Bulgarian Rhodopean cows a total of 16 unrelated cows were selected; number of lactations (2-5), age of the cows (3−8 years). All animals were genotyped for the *CSN3* gene by PCR-RFLP analysis as described previously (*Hristov et al., 2013*). *CSN3* genotyping showed three genotypes: AA (four cows), AB genotype (eight cows) and BB genotype (four cows). Milk production of each animal was recorded monthly for 305-d lactation period and protein and fat content were determined with MilkoScan 133-B (Foss Electric, Denmark). For DLS

analyses milk samples from each cow in mid lactation (100-130 days) were taken after morning milking and sodium azide (0.02%, m/m) was added to all tubes to prevent microbial growth.

Dynamic light scattering measurements

DLS measurements on milk solutions were carried out on a Brookhaven Instruments 90Plus (Brookhaven Instruments Corporation, NY, USA) apparatus at 22.0 °C and scattering angle of 90 °C (wavelength 657 nm and 35 mW). Time dependent fluctuations in the scattered intensity were measured using an avalanche photo detector (APD) and a digital correlator. To check for sedimentation or aggregation data collections were performed in triplicate as 2 minutes co-added runs (total time of 6 min). NIST traceable polystyrene solutions 3020 A, 22 nm ± 1.8 and 3090 A, 92 ± 2 nm (Thermo Scientific) and a blank, 0.02 μm filtered ultrapure water, were used as standards. The used buffer solution (50 mM TBS, pH 7.2) was filtered through 0.44 μm filter and also examined by DLS to account for eventual "dust" particles. Prior to DLS data collection and in order to remove major aggregates (fat fraction and unspecific precipitates) individual milk samples were centrifuged at 2 000 x g for 3 minutes at 4 °C. Then the "skim milk" fraction was diluted 100 times with 50 mM TBS (pH 7.2) and filtered through 0.44 μm syringe filters (*de Kruif and Huppertz, 2012*). DLS employs the Stokes−Einstein relationship between the diffusion coefficient (D) and the hydrodynamic radius (R_h):

$$D = \frac{kT}{6\pi\eta R_h}$$

where η is the viscosity. For obtaining size distributions the autocorrelation functions were deconvoluted using the non-negatively constrained least squares fit (multiple pass NNLS) algorithm. In addition, the intensity of scattered light is proportional to the particle size to the sixth power resulting in a higher scattered intensity for larger particles. Thus the intensity weight distributions measured by DLS were converted to number weighted distributions using the analysis software provided by Brookhaven (Brookhaven Instruments Corporation, NY, USA).

Statistical analysis

Descriptive statistics was used concerning the milk productivity and qualitative milk traits data. The calculated mean values (shown as mean value ± SEM) for milk productivity and qualitative traits were compared within different genotypes and evaluated by Student's t-test. These statistical assays were performed with GraphPad Prism version 5.04 (GraphPad software).

Results and Discussion

Effect of κ-CN genotypes on milk quantitative and qualitative traits

PCR-RFLP analysis showed three genotypes AB (eight cows), AA (four cows) and BB (four cows) for the 16 selected cows. To determine milk production, butter milk, fat and protein contents during the lactation period (305 d) a total of ten milk samples were collected on a 30 days basis from each animal. The average composition of milk (butter milk, fat and protein) for the different κ-CN genotypes is shown in Table 1. The results clearly demonstrate correlation between genotypes and milk production (AB > AA > BB). Milk production of heterozygous AB animals significantly exceeds that of homozygous BB cows, with 12% or about 500 L ($P < 0.01$) and with 5% (about 200 L, $P < 0.01$) that of animals homozygous by A allele of the gene. Regarding fat and protein contents there are only slight differences amongst the three genotypes (Table 1).

Table 1. Influence of the *CSN3* genetic polymorphism on the milk production and the milk quality traits in cows of the Bulgarian Rhodopean cattle

Genotype	Milk production L	Butter milk kg	Protein %	Milk fat %
AB	4099 ± 78.6^{a}	185.5 ± 0.5	3.54 ± 0.04	4.58 ± 0.09
AA	3896 ± 14.5^{b}	178 ± 2.5	3.76 ± 0.04	4.78 ± 0.07
BB	3598.5 ± 44.5^{c}	172.1 ± 0.9	3.56 ± 0.03	4.60 ± 0.2

Values express as means ± standard deviation; values within the same row not sharing a common letter differ significantly, $P < 0.01$.

Our results support the data by (*Bovenhuis et al., 1992*) suggesting a 15 % decrease of the milk production of the BB homozygous cows compared to the AB heterozygous cows. Some studies claim that the BB genotype is associated with higher (*Van Eenennaam et al., 1991*) or lower (*Bovenhuis et al., 1992*) milk yield whereas other studies indicated no effect (*Comin et al., 2008*). One should be very careful when crosschecking the results of different studies as in most cases they are not comparable due to differences in population size, breed of cows, frequency of occurrence of specific genetic variants under consideration, methods of expressing traits (whether test day or lactation averages) and the effect of other genetic variants.

DLS measurement of milk samples

Previous studies has linked the size distribution of casein micelle to the lactating stage (*de Kruif and Huppertz, 2012*), have investigatd the influence of the feeding regimes and investigated the micelle size of native and heated milk samples (*Devold et al., 2000*). This study focuses on the correlation between κ-CN genotypes and casein micelle size in individual milk samples. The resulting data from DLS measurements (hydrodynamic radius, polydispersity and multimodal distribution) is presented in Table 2.

Table 2. DLS measurement for individual milk samples

	Sample No / genotype	hydrodynamic radius (combined)	poly-dispersity	Multimodal distribution (size and relative intensity %)	
				Peak 1	Peak 2
		nm		nm*	nm
1	2682 AA	193.8 (1.5)	0.246	122	474 (<1)
2	2309 AA	182.5 (1.4)	0.193	117	324 (1.5)
3	2672 AA	234(6.8)	0.327	127	586(<1)
4	2688 AA	254(17)	0.339	111	643(<0.1)
5	2339 AB	174.7(2.4)	0.258	59	244 (<1)
6	2819 AB	201.6(6.8)	0.302	93	477(~10)
7	2152 AB	320.7(29.6)	0.342	67	-
8	2695 AB	188.7(2.0)	0.231	112	368 (1.5)
9	2663 AB	169.6 (0.4)	0.160	38	201 (<0.1)
10	2595 AB	284.1(20)	0.355	93	859 (7.6)
11	2296 AB	143.8 (5)	0.233	66	237 (<0.1)
12	2717 AB	167.6 (3.5)	0.215	99	292(<0.1)
13	2687 BB	162.9(0.9)	0.151	64	202(<1)
14	2726 BB	410.3(25.9)	0.377	70	1320 (<1)
15	2691 BB	161.0(3.5)	0.295	77	350 (<1)
16	2680 BB	191.2(2.9)	0.202	83	269 (<0.1)

* the relative intensity for peak 1 is 100%

Hydrodynamic radii (R_h) of micelles at a scattering angle of 90 °C varied from 40 to 120 nm. These values are in accordance with micelle size for Norwegian Red cattle (*Devold et al., 2000*) and Holstein-Friesian cows (*de Kruif and Huppertz, 2012*). DLS measurments were performed on 16 milk samples with distinct *CSN3* genotypes (AA/AB/BB) and the variation of the micelle size in function of genotype is shown on Figure 1. One can see that the micelle size for AA and BB genotypes is not fluctuating a lot. In contrast the micelle size of cows with AB genotype varies a lot. An over simplification of the data interpretation, links the highest observed values of casein micelle size (over 110 nm) to AA genotype. This finding is in agreement with data reported by *Bijl et al. (2014)* for skimmed milk of Holstein-Friesian cows. According to the data from Table 1 one can eventually suggest a correlation only to the highest protein and fat content in

milk for *CSN3* AA genotype (3.76 ± 0.04 %; 4.78 ± 0.07 %, respectively). However, there is no clear correlation between casein micelle size and observed milk quantitative and qualitative traits.

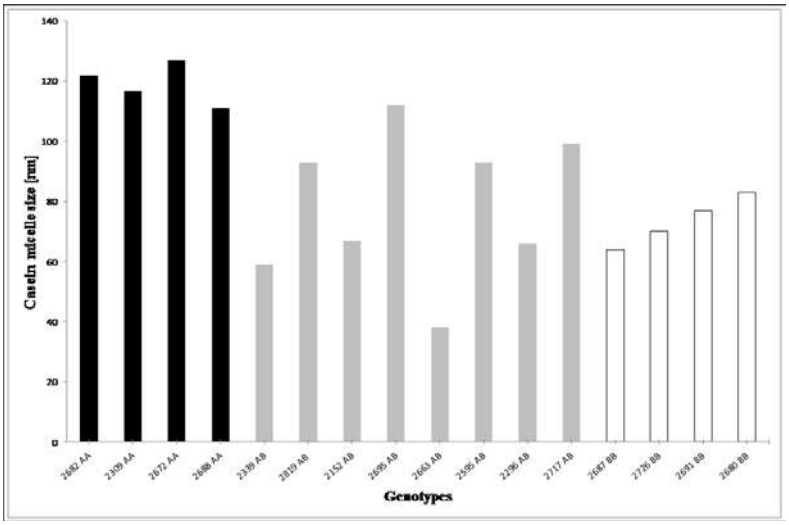

Figure 1. Genotypes vs. particle size (radius) distributions (normalized) of micelles obtained with dynamic light scattering

A major complication of light scattering studies is due to the presence of dust particles in the sample, therefore careful filtering procedures have to be applied. In the case of casein micelles an efficient filtering is not always possible, since dust particles and micelles are of similar size. It is thus essential to work with relatively concentrated solutions (resulting in higher polydispersity index (PDI)). Habitually polydispersity values below 0.1 are suited for DLS experiments while more elevated values of polydispersity are linked to either more concentrated samples or a multi modal distribution. As can be seen from Table 2, polydispersity of casein micelles varied from 0.15 to 0.37 related to a bimodal distribution. As one can see from the multimodal distribution "number vs. diameter" (Figure 2) the number average reveals that the dominant species has smaller size, consistent with the lower average intensity (Peak 1 in Table 1) while the contribution of aggregates with bigger size is minimal.

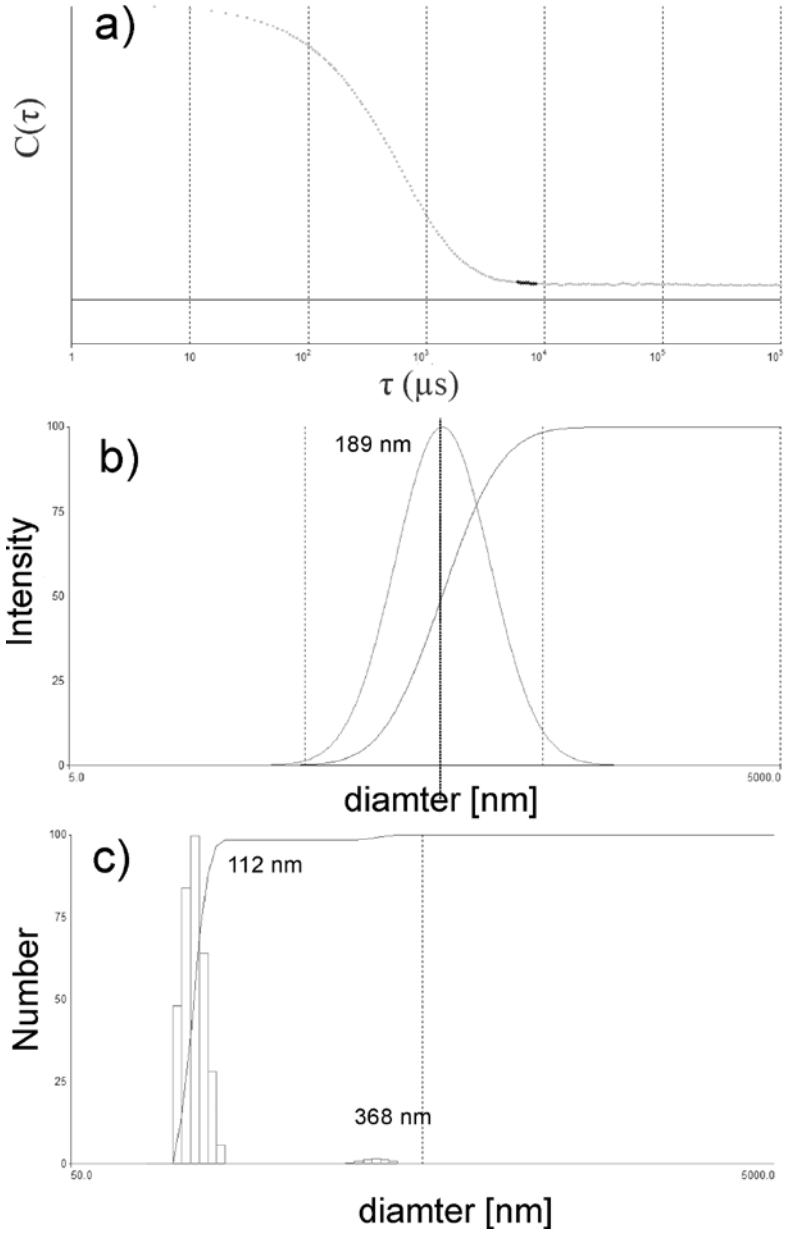

Figure. 2. Representative DLS distributions for sample 2695 a) correlation function b) intensity particle size distribution and c) multimodal number particle size distribution

Conclusion

This study reveals for the first time the correlation between κ-CN genotypes and casein micelle size in individual milk samples. *CSN3* AB genotype showing distinct variations of micelle size. DLS data suggest that there is a correspondence with *CSN3* genotype e.g. AA genotype shows bigger size of casein micelle. In contrast, protein and fat content in milk cannot be correlated to casein micelles size.

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Genetski polimorfizam kapa kazeina i veličina kazein micela u goveda bugarske rodopske rasa

P. Hristov, B. Neov, H. Sbirkova, D. Teofanova, G. Radoslavov, B. Shivachev

Rezime

Ova studija ima za cilj da uporedi veličinu kazein micela u uzorku kravljeg mleka u funkciji kapa kazein (CSN3) genetičkog polimorfizma. Šesnaest krava bugarske rodopske rasa goveda su genotipizirane korišćenjem PCR-RFLP analize. Uzorci mleka tri pronađena *CSN3* genotipa (AB, AA i BB) su upotrebljeni za određivanje veličine kazein micela metodom Dynamic Light Scattering (DLS). Rezultati su pokazali razlike u veličini i polidisperzitetu kazeina micela između mleka krava različitih genotipova. Hidrodinamički radijusi micela pod uglom rasejanja od 90°C varirali su od 80 do 120 nm a polidisperzitet od 0,15 do 0,37. U zaključku, veličina kazein micela *CSN3* AA krava (~ 120 nm) prelazi sa oko 60% krava sa AB (~ 80 nm) i BB genotipa (~ 70 nm). Ovi rezultati mogu biti korisni za poboljšanje tehnoloških svojstava mleka.

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CHANGES IN BLOOD VALUES OF GLUCOSE, INSULIN AND INORGANIC PHOSPHORUS IN HEALTHY AND KETOTIC COWS DURING AN INTRAVENOS GLUCOSE TOLERANCE TEST

R. Đoković[1], V. Kurčubić[1], Z. Ilić[2], M. Cincović[3], I. Davidov[3], M. D. Petrović[1], J. Trifković[4]

[1]Department of Animal Science, Faculty of Agronomy-Čačak, University of Kragujevac, Cara Dušana 34, Čačak
[2]Department of Animal Science, Faculty of Agronomy, University of Priština, Kopaonička bb. Lešak.
[3]Department of Veterinary medicine Faculty of Agriculture, University of Novi Sad, Trg D.Obradovica 8, Novi Sad
[4]Department of Animal Science, Faculty of Agroculture, University of East Sarajevo, Vuka Karadžića 30, East Sarajevo, RS, BiH.
Corresponding autor: radojicadjokovic@gmail.com
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Abstract: The aim of the present study was to determine the degree of blood glucose utilization by peripheral tissue on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy (n=8) and cows with ketosis (n=7) after intravenous infusion of glucose solution. After intravenous infusion of a total of 500 ml of 50 % of glucose solution, glucose and insulin blood values in both groups of cows increased significantly within 10 and 30 minutes of the experiment ($P < 0.05$). After intravenous infusion of glucose, it was established that values of inorganic phosphorus were decreased ($P < 0.05$) in blood in both groups of cows. Within testing period there was a significant decrease ($P < 0.01$) in the blood value of inorganic phosphorus in ketotic cows in comparison with healthy ones. This is linked with the active entry of glucose into the glucolytic pathway of peripheral tissues. It can thus be concluded that there is a higher degree of blood glucose utilization by peripheral tissues in ketotic cows.

Key words: ketosis, glucose utilization, glucolytic pathway, peripheral tissue

Introduction

The optimal supply of liver and mammary gland with glucose has an important role in preserving the health of dairy cows in the early stage of lactation.The first metabolic change in primary ketosis in dairy cows in early

lactation is hypoglycaemia. It causes serious metabolic changes in cow body, which are manifested through lipomobilization from body reserves and ketogenesis and lipogenesis in the liver (*Veenhuizen et al., 1991, Vazquez-Anon et al.,1994*). The glucose tolerance test is used for estimating the ability of the endocrine pancreas for synthesis and secretion of insulin in ruminants. The insulin concentration in blood is reduced in ketotic cows, compared to healthy animals in the early lactation, before and after infusion of a glucose solution (*Hove 1978, De Cupere et al., 1991, Sakai et al. 1996, Šamanc et al., 1996, Djoković et al., 2009*). Similar results have been reported by other authors (*Peters and Elliot 1984, Šamanc et. al., 1996, Djoković et al., 2007*), after an intravenous infusion of propionate solution, because propionic acid directly stimulates pancreatic secretion of insulin in ruminants.

During the glucose tolerance test, it is very difficult to estimate on the basis of glycaemia whether the metabolic disorder was induced by liver disease or hypofunction of the endocrine pancreas. At the same time, an estimation of the concentration of inorganic phosphorus in blood can be helpful. Namely, the decrease of its concentration in blood after intravenous infusion of glucose is linked with the active utilization of glucose into the glucolytic pathway of peripheral tissue, while a considerably smaller amount of glucose is used for glycogenesis in the liver (*Sakai et al., 1993, Gründberg et al., 2006, Djoković et al., 2007, Djoković et al., 2009)*.

The aim of the present investigation was to determine of blood glucose utilization by peripheral tissue on the basis of changes in blood concentrations of glucose, insulin and inorganic phosphorus in healthy and ketotic cows during an intravenous glucose tolerance test.

Materials and Methods

A total of eight healthy and seven cows with ketosis in the earliest stage of lactation (7-14 days post-partum) were chosen from a Holstein dairy herd. The diagnosis of ketosis was based on clinical symptoms (decreased appetite, rumen atony, behaviour changes), including high concentrations of β-hydroxybutyrate (2.60 ± 0.45 mmol/L) in the blood (>2.6 mmol/L; *Oetzel, 2004*) and ketone bodies in the urine. The Lestradet test was used to examine the presence of ketone bodies in the urine. Healthy cows did not show clinical symptoms of ketosis and urinary ketone bodies were not determined in those cows. The blood concentrations of BHB in healthy cows were 0.53 ± 0.18 mmol/L (> 1.2 mmol/L is frame for subclinical ketosis; *Oetzel, 2004*). Cows were of similar body mass (560-580 kg), 4-6 years old, an average of 3 lactations with a mean milk yield of 7750 L (calculated over 305 days) in the previous lactation. The meal was prepared in the way to meet the energy needs of animals in early lactation. Early lactation cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Chemistry characteristic

of meal were: 87,15 MJ NEL; crude protein 13,58% of DM; rumen undegradable protein 35,91% of crude protein; fat 3,09 %DM, fibre 23,26% DM.

The test was carried out in the morning at 09 h about 3 h after feeding. A solution of glucose (500 mL of 50 %) was injected intravenously during 5 minutes into a jugular vein of each animal. Blood samples were taken from the opposite jugular vein before (0) and10, 30, 60, and 90 minutes after injection. Blood samples were allowed to clot for 3 hours at 4°C and then, they were centrifuged (1500g, 10 minutes), following which the serum was carefully harvested and stored at -20°C until analysis. Blood samples collected into fluoride-containing tubes were immediately centrifuged in the same manner, and plasma glucose levels were determined. The concentrations of insulin in the blood serum were determined by ELISA methods (Cusabio) using Rayto reader. Glucose (glucose oxidase test, GOT) and inorganic phosphorus (modificate classical phosphomolybdate method) were measured using a Randox kit and Rayto spectrophotometer.

Statistical analysis: Influence of time during intravenous tolerance test to concentration of glucose, insulin and inorganic phosphorus was analysed by ANOVA-procedure with posthock LSD test. For all purposes we used statistic software Statgraphic Centurion (Statpoint Technologies Inc.Warrenton, Va, Virginia, USA).

Results and Discussion

Basically, the glucose tolerance test is used to estimate the functional ability of the beta cells for the synthesis and release of insulin (*Hove 1978, Sakai et al.,1993, Sakai et al.,1996, Gründberg et al. 2006, Djoković et al.,2009*). Changes in glucose, insulin and inorganic phosphorus values in the peripheral circulation of the cows given glucose intravenously are shown in Figs 1, 2 and 3.

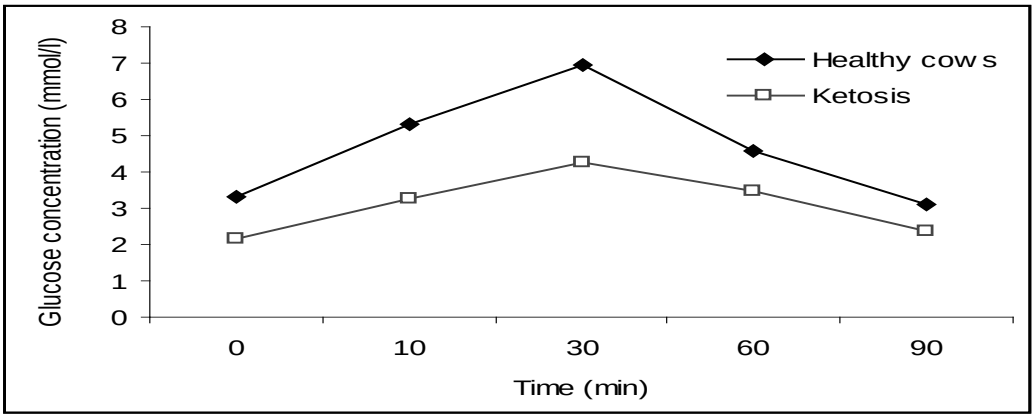

Figure 1. Changes in blood values of glucose of healthy and cows with ketosis after intravenous infusion of glucose solution

The initial blood glucose values in the healthy cows were within physiological range 2.5 - 4.2 mmol/L *(Radostits et al., 2000*), whereas in ketotic cows were determined hypoglycemia. The initial blood glucose values were significantly lower in ketotic cows (2.17 ± 0.16 mmol/L) than in healthy cows (3.30 ± 0.24 mmol/L; $P < 0.01$). Glucose injection led to a significant increase in glycaemia within 10, 30 and 60 min of the experiment in both groups of cows ($P < 0.05$), which peaked within 30 min and then slowly declined. In the group of cows with ketosis, the mean blood glucose value was significantly lower than in healthy group of cows during whole test period ($P < 0.01$). The fact that glucose values after intravenous administration showed similar increments and similar rates of decline in healthy and ketotic cows, indicated that the mean of glucose disposal was similar in both groups of animals. Thus, the pancreatic islets were subjected to almost identical glucose stimuli. These results are in accordance with previous data (*Hove 1978, Šamanc et al., 1996, Djoković et al., 2009*).

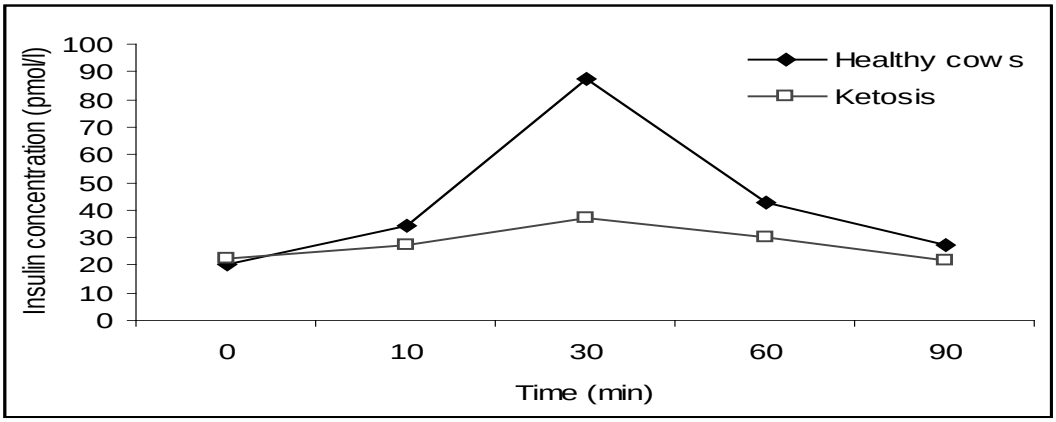

Figure 2. Changes in blood values of insulin of healthy and cows with ketosis after intravenous infusion of glucose solution

Mean initial blood insulin values were lower but without significant diferences ($P > 0.05$) in the healthy group (22.42 ± 2.67 pmol/L) than in the ketotic group of cows (20.37 ± 4.65 pmol/L). The glucose injection led to a significant increase ($P < 0.05$) in insulinemia within 10 and 30 minutes of the experiment in both groups of cows and confirm the possibility of glucose to influence the synthesis and release of insulin from beta cells of the endocrine pancreas. Namely, the value increased to 87.12 ± 24.47 pmol/L within 30 minutes in healthy cows compared with 37.00 ± 12.24 pmol/L in ketotic cows ($P < 0.01$). After 30 minutes blood insulin concentrations decreased in both group of cows and marked difference in insulinemia among groups was maintained within 60 and 90 minutes after glucose administration ($P < 0.05$). The low secretory responses of

insulin in ketotic cows, are therefore probably a result of a pancreas with a low secretory capacity for insulin, developed during the days or weeks of hypoglycemia which regularly accompanies high ketosis. The obtained results show the preserved function (relative insufficiency) of the beta cells of the endocrine pancreas of ketotic cows. Other authors have reported similar results (*Peters and Elliot 1984, De Cupere et al., 1991, Sakai et al., 1993, Sakai et al.,1996, Djoković et al., 2009*).

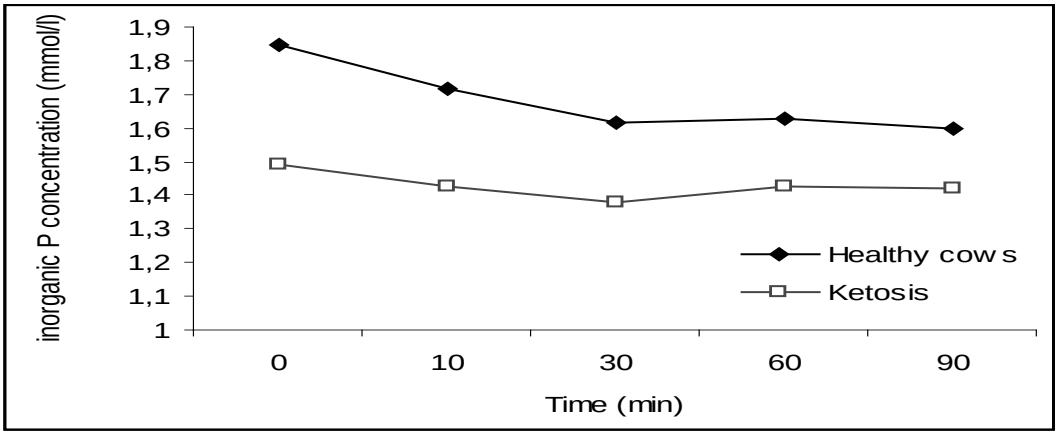

Figure 3. Changes in blood values of inorganic phosphorus of healthy and cows with ketosis after intravenous infusion of glucose solution

The inorganic phosphorus blood values in the healthy group of cows before the infusion of a glucose solution were 1.85 ± 0.12 mmol/L, whereas they were 1.48 ± 0.10 mmol/L in ketotic cows ($P < 0.01$). After intravenous infusion of a glucose solution, a decrease of the inorganic phosphorus values in the blood in both groups of cows were determined during the test period ($P < 0.05$). Among the tested groups of cows, statistically significant differences of the values of inorganic phosphorus in the blood were determined during the test period ($P < 0.01$). The significant decrease of the value of inorganic phosphorus in the blood of both groups of cows after glucose injection and a significant lower level in ketotic cows compared to the healthy ones, during whole testing period could be a sign of the increased usage of glucose in blood by the peripheral tissue in ketotic cows. Therefore, the view that blood glucose is used for energy purposes by peripheral tissue is confirmed. These results are in accordance with the previus observation (*Sakai et al. 1993, Gründberg et al. 2006, Djokovic et al. 2007,2009*).

Conclusion

- Significantly lower ($P < 0.01$) level blood inorganic phoshorus in ketotic cows compared to the healthy ones, during whole testing period could be a sign of the increased usage of glucose in blood by the peripheral tissue in ketotic cows.
- A solution of glucose (500 mL of 50 %) injected intravenously is an efficient therapeutic mean in treatment of ketosis in dairy cows

Acknowledgement

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Promene vrednosti glukoze, insulina i neorganskog fosfora u krvi kod zdravih i ketoznih krava za vreme intravenoznog opterećenja glukozom

R. Đoković, V. Kurćubić, Z. Ilić, M. Cincović I. Davidov, M. D. Petrović, J. Trifković

Rezime

Cilj ovog rada je bio da se proceni stepen iskorištavanja glukoze od strane perifernih tkiva na osnovu promena vrednosti glukoze, insulina i neorganskog fosfora kod zdravih (n=8) i ketoznih krava (n=7) nakon infuzije rastvora glukoze. Nakon intravenozne infuzije 500 ml 50% rastvora glukoze utvrđeno je da se vrednosti glukoze i insulina značajno povećavaju ($P < 0.05$) tokom 30 i 60 minuta eksperimenta. Nakon infuzije rastvora glukoze, vrednosti neorganskog fosfora su se značajno smanjivale ($P < 0.05$) u obe grupe krava tokom ispitivanog perioda.U okviru ispitivanog perioda utvrđene su značajno niže vrednosti ($P < 0.01$) inorganskog fosfora u krvi kod ketoznih krava u odnosu na zdrave krave. Smanjivanje vrednosti inorganskog fosfora u krvi dovodi se u vezu sa aktivnim ulaskom glukoze u periferna tkiva, pa se može zaključiti da je veći stepen iskorišćavanja glukoze od strane perifernih tkiva kod krava obolelih od ketoze u odnosu na zdrave krave tokom testa opterećivanja glukozom.

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INFLUENCE OF STARTER CULTURE, TEMPERATURE AND PROCESSING TECHNOLOGY ON THE QUALITY OF MACEDONIAN WHITE BRINED CHEESE

E. Sulejmani[1], Z. H. Musliu[2], S. Srbinovska[3]

[1] Faculty of Food Technology and Nutrition, Tetovo, Macedonia
[2]Faculty of Veterinary Medicine, Skopje, Macedonia
[3]Faculty of Agriculture Science and Food, Skopje, Macedonia
Corresponding author:erhan.sulejmani@unite.edu.mk
Original scientific paper

Abstract: The effect of the starter culture, temperature of curdling and processing technology on the composition, cheese yield and process optimization of Macedonian White cheese (MWC) was studied during 60 days of ripening in brine. Three treatments of cheese were made using current technological process and yogurt as starter–culture gained along processing of previous day (MWCK), freeze dried culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* 3:1; F–DVS YF–3331 Yo-Flex version: 2 PI-EU-EN (MWCB1), and (MWCB2) with same starter culture as previous but whith 5 minute earlier processing of curd and temperature of curdling at 39°C. As ripening progressed, titratable acidity (°SH), salt and protein contents of the (MWCB2) treatment continuously increased, whereas their fat-in-dry-matter and lactose contents decreased. In same production conditions depending on the used temperature. Way of processing and starter cultures the cheese from (MWCB2) treatment was with highest acidity of 66.63 $\pm$ 2.73^{o}SH until the end of ripening of the cheese. Moisture of cheeses remained stable during ripening. The pH of cheese at the 1 day of ripening, which decreased by increasing the temperature of curdling (5.03, 5.11 and 5.00 for MWCK, MWCB1 and MWCB2, respectively), significantly ($P < 0.05$) affected most of the chemical characteristics of cheese. The content of salt at the end of storage at (B1) and (B2) variant is 5.23 $\pm$ 0.31 and 5.52 $\pm$ 0.31 respectively. Higher temperature of curdling decreased moisture and pH, whereas cheese protein content increased. The consumption of milk for production of a 1 kilogram of cheese ranged from 7.8 to 8.3 liters of milk. It was concluded that starter cultures have possstively influenced and improved the quality of white cheese.

Key words: Lactose, starter – culture, white cheese

Introduction

The use of starter cultures containing lactic acid bacteria is an essential requirement in the manufacture of most cheeses (*Cogan and Hill, 1993*) including Macedonian White cheese (MWC). Their major function is to produce lactic acid and in some cases, flavour compounds (*Fox et al., 2000*). It is well known that reduction in milk pH due to acidification by starter cells at the appropriate rate and time is the key step in the manufacture of a good quality cheese (*Bintsis and Papademas, 2002*). For this reason, the changes of acidity of different treatments were studied separately during ripening and ripening. The basic composition and structure of cheeses are determined by the manufacturing operations like pH at renneting, but it is during ripening that the individual and unique characteristics of each cheese variety develop (*Fox et al., 1993*). The ripening cheeses do not have typical sensory properties immediately after hooping and salting. These are developed only during the cheese ripening. One of the most important biochemical processes determining the taste and texture of a cheese is proteolysis which includes microbiological enzymatic and physic–chemical processes *(Fox and Law, 1991*). The production of Macedonia White brined cheese depends on the hydrolysis of lactose by lactic acid bacteria to produce lactic acid (*Sulejmani, 2010*). The breakdown of the degradation of lactose in the curd has a major effect on the quality of the ripened cheese; for example, excessive lactic acid in cheese curd leads to a low pH, strong, acidic, harsh taste, and a brittle structure. The selection, maintenance and use of starter cultures are perhaps, the most important aspects of cheese making, particularly in the context of a modern mechanized process for which predictability and consistency are essential *(Bintsis and Papademas, 2002; Özer, 1999).*

Macedonian White cheese (Belo Sirenje) is brine-salted cheese variety with salty, acid taste and close texture resembling Beyaz Peynir (Turkish White cheese) and Feta but differs from Feta in the way it is made (*Sulejmani et al., 2011*). It is for example manufactured without dry salting of curd and slime formation on the curd surface before brining which are essential for the development of the characteristic Feta flavour during ripening. At the industrial level, the ripening period is 20 to 60 day.

The fact that classical White cheese is often produced traditionally without the addition of starter cultures frequently leads to indifferent quality of the product. This study was aimed in optimizing the manufacture process of Macedonian White brined cheese in which case was determined influence of the starter culture, optimal temperature of milk curdling and optimum time of curd cutting on the quality and yield of the cheese.

Materials and methods

Macedonian white−brined cheese was manufactured in triplicate from pasteurized cow's milk in a local dairy plant (Mlekara Tetovo, Tetovo, the Republic of Macedonia). Raw cow's milk supplied from the whole Tetova region in Macedonia was pasteurized (75°C for 15 second using a plate pasteurizer) and cooled at 5°C. The chemical composition of the milk used in the manufacture of white cheese was 12.68% total solids, 4.28% fat, 3.28% protein, 0.61% ash, and 4.41% lactose. The pH of the milk was 6.49. Cheese making procedures were described in a previous paper (*Sulejmani et al., 2011*). Briefly 3 treatment of the cheese were made where at the first control cheese (MWCK) yogurt as starter culture was added. The second and third treatment was inoculated with freeze dried culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* 3:1; F–DVS YF–3331 Yo–Flex version: 2 PI–EU–EN and processing of curd were applied 5 minute earlier from current. The temperature of curdling at MWCK and MWCB1 cheeses was 37°C while at MWCB2 cheese was 39°C. Cow milk was coagulated with chymosin renet (Chy Max^{TM} Liquid Plus derived by fermentation of *Aspergillus niger* var. *awamori* Christian Hansen Inc Danish, 200 IU) in 30 min. The curd was cut in cubes of 1 cm^3 and left of 5 minutes. Cheese of square shape 12 cm (length) × 10 cm (width) × 10 cm (height), were pressed for 3 hour than brine salted for 18 hour and ripened at 17°C for 20 days and hold afterword at 6°C.

Titratable acidity of cow milk was determined by the Soxhlet-Henkel method, and its total solids were determined Milkoscan 4000 (Foss Electric, Hillerød, Denmark) (*IDF, 2000*). The pH of milk and cheese samples was measured using a digital pH meter (digital pH meter, model MP120FK Mettler Toledo, Greifensee, Switzerland). Cheese was analyzed for lactose content by Gravimetric method (*AOAC, 2005*). Cheese samples were analysed for moisture by the oven-drying method at 102°C (*IDF, 1982*), fat and salt by the methods described by Ardö and Polychroniadou (1999), and total nitrogen by the micro-Kjeldahl method (*IDF, 1993*). All chemical measurements were done in triplicate or more. Cheese samples were chemically analyzed at d 1, 10, 20 and 40 of ripening. Apparent yield was calculated as the weight of cheese before brining (after 19 to 20 h ripening at 23 to 25°C) divided by the weight of the milk used.

The cheeses were evaluated at 60 d of ripening by 7 trained panelist's familiar whith the cheese according to the described procedure for Belo cheese (*Sulejmani, 2010*). The samples were evaluated by criteria appearance (scale 0-5), odour (scale 0-5), texture (0-10), and flavour (scale 0-15). Water and bread were provided to panelist to rinse their mouths between samples. Sensory analysis was conducted in 3 replicate trials and cheeses were evaluated in duplicate by each panelist.

The experiment was replicated 3 times in a randomized complete block design, which incorporated 3 treatments (MWCK, MWCB1 and MWCB2), 4 ripening periods (10, 20, 40, and 60 d) was used to analyze the response variables related to cheese composition and yield. The ANOVA was performed using a general linear model procedure (SAS Institute, 1995), where the effect of treatment and replicates were estimated for response variables. The Tukey multiple-comparison test was used as a guide for pair comparisons of treatment means. The level of significance of differences between treatments was determined at $P < 0.05$.

Results and Discussion

The composition of cow's milk cheeses and the differences in chemical composition between the cheese treatments are summarized in Table 1.

Table 1. Mean (± SE) of chemical composition of cheeses made with different starter[1] (g 100 g^{-1}) (*n*= 3)

Cheeses	Age (day)	Acidity, (°SH)	Moisture, %	Salt, %	Protein, %	Fat-in-dry matter, %	Lactose, %
MWCK	1	20.81 ± 2.72^{a}	53.28 ± 0.67^{a}	3.98 ± 0.25^{a}	14.43 ± 0.23^{a}	53.44 ±2.14^{a}	2.68 ± 0.44^{a}
	10	27.21 ± 2.93^{b}	55.12 ± 2.15^{b}	4.48 ± 0.16^{a}	14.25 ± 0.86^{a}	53.24 ± 2.43^{a}	1.96 ± 0.05^{b}
	20	43.73 ± 2.21^{c}	53.46 ± 0.48^{a}	4.36 ± 0.48^{a}	14.03 ± 0.41^{a}	55.83 ± 1.23^{b}	1.86 ± 0.05^{b}
	60	47.86 ± 3.83^{d}	53.32 ± 0.48^{a}	5.43 ± 0.23^{b}	13.41 ± 0.24^{a}	49.31 ± 3.25^{c}	1.30 ± 0.08^{b}
MWCB1	1	19.86 ± 1.44^{a}	53.06 ± 0.52^{a}	3.63 ± 0.18^{a}	13.52 ± 0.45^{b}	51.42 ± 3.33^{e}	2.56 ± 0.38^{a}
	10	27.73 ± 6.10^{b}	52.91 ± 0.40^{c}	4.36 ± 0.23^{a}	14.17 ± 0.54^{a}	54.21 ± 3.15^{b}	1.96 ± 0.03^{b}
	20	41.86 ± 2.39^{c}	48.05 ± 0.40^{c}	4.55 ± 0.44^{a}	15.45 ± 1.19^{b}	54.56 ± 1.69^{b}	1.91 ± 0.03^{b}
	60	54.35 ± 7.13^{d}	49.15 ± 0.35^{c}	5.23 ± 0.31^{b}	14.70 ± 0.24^{a}	46.21 ± 3.05^{f}	1.06 ± 0.26^{b}
MWCB2	1	24.13 ± 1.26^{a}	51.85 ± 0.39^{c}	3.82 ± 0.48^{a}	14.56 ± 1.02^{a}	53.32 ± 1.68^{a}	2.63 ± 0.40^{a}
	10	31.24 ± 5.73^{b}	53.94 ± 0.50^{a}	4.71 ± 0.35^{a}	14.13 ± 0.95^{a}	52.43 ± 3.21^{e}	2.03 ± 0.07^{a}
	20	54.82 ± 1.15^{c}	51.51 ± 1.13^{d}	4.31 ± 0.46^{a}	14.86 ± 1.08^{a}	57.04 ± 0.44^{d}	1.86 ± 0.05^{b}
	60	66.63 ± 2.73^{d}	51.21 ± 0.91^{d}	5.52 ± 0.31^{b}	14.83 ± 0.20^{a}	48.14 ± 2.70^{f}	1.01 + 0.33^{b}

$^{a–i}$Means within the same column with different superscripts differ significantly ($P < 0.05$). MWCK= Macedonian cheese made using yogurt as starter – culture, MWCB1= Macedonian cheese made using comercial freeze dried culture, curdling at 37°C, MWCB2= Macedonian cheese made using comercial freeze dried culture, curdling at 39°C.

The highest level of acidity (°SH), protein and fat at day 1 of ripening were (mean ± SE) observed at the MWCB2 cheeses containing 24.13, 14.56 and 25.68% respectively. MWCB2 treatment has higher titratable acidity value than others during the ripening (Table 1), ranging from 24.13 to 66.63°SH.

The influence of different starter culture and processing technology on the pH values of the cheeses during ripening is shown in Figure 1. The starter culture and temperature of curdling had no significant effect on the pH values of cheeses;

however the lowest and highest level of pH values durig ripening was observed in the MWCB2 or MWCB1 cheeses respectively.

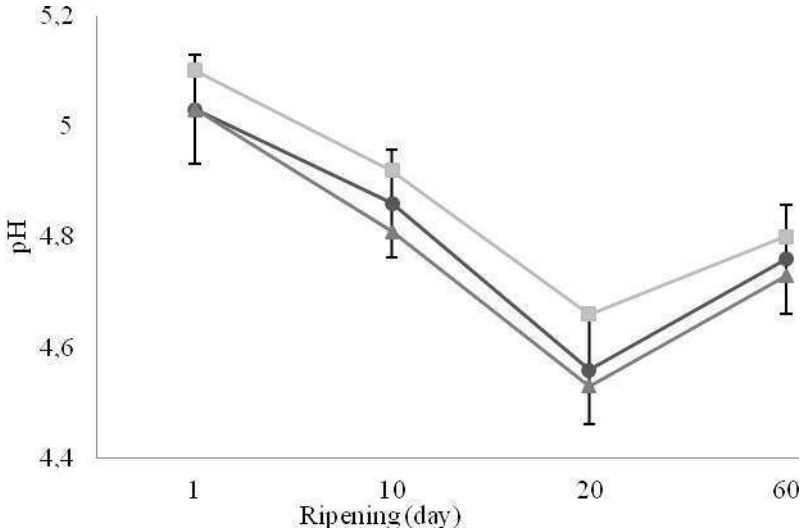

Figure 1. Mean values of pH in Macedonian white cheese made using yogurt as starter – culture MWCK (circles), cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCB1 (squares), cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCB2 (triangles).

The protein content except MWCB1 treatment decreased in the first 10 days of ripening. The results showed that protein of White brined cheese with different starter culture ranged from 14.43 to 13.41% for MWCK, 13.52 to 14.70% for MWCB1 and 14.56 to 14.83% for MWCB2 cheeses. From the statistical analysis it is observed that the lactose content of White brined cheese was not significantly ($P > 0.05$) decreased during ripening. An increased reduction of lactose in all treatments has been shown during the first 10 days of ripening. At the day 60 of ripening White cheese is containing traces of lactose with a level ranging from 1.01 to 1.30 in MWCB2 or MWCK cheese treatment, respectively.

The salt content, expressed as a percentage (Table 1), ranging from 3.98% to 5.43% for MWCK cheeses produced whith yogurt as starter culture, while results obtained from MWCB2 cheeses at the end of ripening were slightly higher.

The chemical composition of all cheese treatments was generally within the range typical for white brined cheese. Macedonian white brined cheese may be characterized as soft (50–60%) moisture, high fat cheese (25–30%), protein (12–21%) high salt (3–5%) content and the final pH range of 4.20 – 5.05 (*Mojsova et al., 2013*). Similarly to our results, it is reported a decrease in pH during ripening

of Iranian White cheese in brine (10%, pH = 7.4), mainly because of completion of lactose fermentation and the liberation of amino and free fatty acids (*Azarnia et al., 1997*). The results for protein content of Macedonian White cheese are in agrenment with the sutdies reported for Turkish White cheese (*Hayaloglu et al., 2005*). Differences in term of acidity (°SH) among the cheeses were significant ($P < 0.05$) during ripening. The moisture content decreased during ripening in all cheeses (Table 1) wich is in accordance with results of semihard ewe cheeses (*Juan et al., 2007*). The protein of cheese increased with use of commercial starter culture and with increase of clotting temperature of milk. Thermophilic starters induce a dynamic whith increasing of protein concetration in MWCB2 cheese. Also with increasing of clotting temperature of milk in concert with termophilic starter culture especially in MWCB2 treatment has cappability to ceep the protein concentration durable.

The differences in the protein content of cheeses during ripening are due to hydrolysis of proteins to water soluble nitrogenous compounds and to the diffussion of these products into the brine. The parameters for proteins, moisture content and pH are similar with the parameters determined in Feta cheese (*Abd El-Salam and Alichanidis, 2004).* Fermentation of lactose takes place from the very beginning the process of cheese manufacturing (*Shakeel-Ur-Rehman et al., 2004*). The results of lactose in cheese from the first day in all treatments were greater than the results reported for Feta - cheese (*Bintsis, 2001*). The same author, for the same cheese, on the 60 day of cheese, obtained smaller value of the concentration of lactose, ie from 0.2 to 0.6% compared with our results.

During production of cheese large amounts of lactose from the milk is lost in the whey, a small degree of lactose that remains in coagullum rapidly metabolized by the activity of starters or non starter cultures present in raw milk or environment (*McSweeney, 2004*). Lactate produced by the activity of starter−cultures is an important starting point for a series of roadsigns that contribute positively or negatively the development of aroma in cheeses. The concentration of salt in cheese depends on the initial condition of the cheese, the percentage of salt in brine, the type of salt, temperature and pH of the cheese (*Pavia et al., 2000*). The results of Macedonian white cheese yield (Figure 2) are in corespondance whith results for white brined cheese Minas Padra (*Anonymous, 2004*).

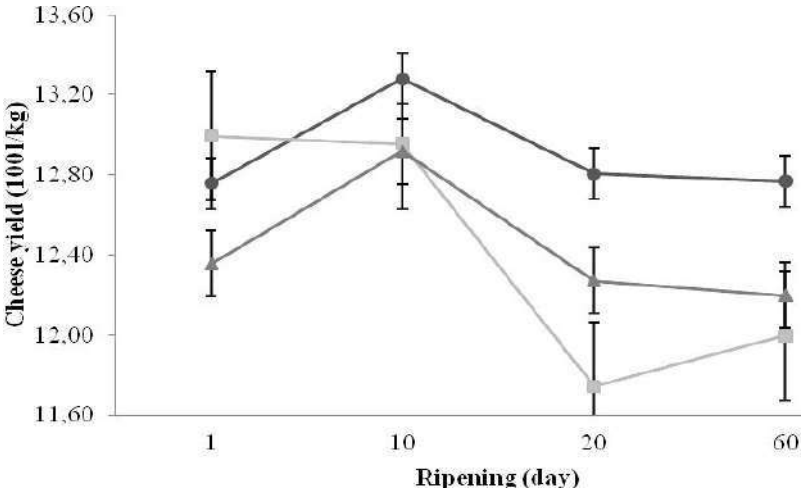

Figure 2. Mean values of yield of Macedonian white cheese made using yogurt as starter – culture MWCK (circles), cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCB1 (squares), cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCB2 (triangles).

The sensory evaluation results of the 60 days old cow cheeses are shown in Figure 3.

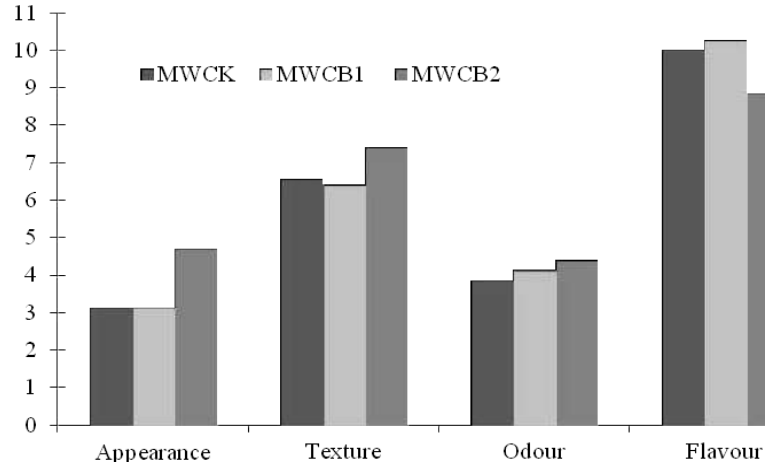

Figure 3. Mean values of sensory evaluation of Macedonian white cheese made using yogurt as starter – culture MWCK, cheese made using freeze dried culture F–DVS YF–3331 curdling at 37 °C MWCB1, cheese made using freeze dried culture F–DVS YF–3331 curdling at 39 °C MWCB2.

Overall cheeses were characterized by a salty and acidic taste and had a semi-hard texture except controll cheeses (MWCK). Flavour and total sensory scores were significantly influenced by the use of starter culture, processing technology and temperature of curdling. Cheeses made using freeze dried culture F–DVS YF–3331 curdling at 39°C MWCB2 had a more satisfactory appearance, texture and odour and quality than others Macedonian White cheese treatments (*Sulejmani et al., 2011*). Use of a thermophilic starter culture resulted in higher quality scores than did use of yogurt as starter culture.

Conclusion

The results obtained from this study indicate that a commercial sturter culture was suitable for Macedonian white cheese manufacturie. Differences in chemical and sensory attributes were correlated with differences in the starter culture and manufacture protocols used in the cheese treatments. The titratable acidity and dry matter content was significantly higher in the cheese manufacturd whith commercial starter culture. The ripening of cheeses produced whith traditional technology (without added commercial starter culture) is longer while the cheeses with commercial starter culture had the best organoleptic socres due to the fact that the aroma principles were fully expressed. Further studies with SPME-GC-MC and olfactometry techniques may be useful to establish key odorants and their role in Macedonian white cheese flavour characteristics.

Uticaj starter kulture, temperature i obrade na kvalitet makedonskog belog salamurenog sira

E. Sulejmani, Z. H. Musliu, S. Srbinovska

Rezime

Uticaj starter kultura, temperature koagulacije i tehnologije prerade na sastav, prinos sira i proces optimizacije makedonske belog sira (MWC) je ispitivan tokom 60 dana zrenja. U okviru tri tretmana, sir je napravljen korišćenjem tekućeg tehnološkog procesa i jogurt kao starter kulture dobijen u obradi prethodnog dana (MWCK), zamrznuto osušenih kultura *Lactobacillus delbrueckii subsp. bulgaricus* i *Streptococcus thermophilus* 3:1; F - DVS IF -3331 Jo - Flek Verzija: 2 PI - ES - RU MWCB1) i (MWCB2) sa istim starter kulturama kao i prethodni, ali je pet minuta ranije tretman i temperatura koagulacije na 39°C. Kao sazrevanja napreduje, titraciona kiselosti (°SH), soli i sadržaj proteina (MWCB2) raste, dok se

masti u suvoj materiji i sadržaj laktoze smanjuje, u istim proizvodnim uslovima zavisno od upotrebljene temperature. Način obrade i starter kultura sira (MWCB2) varijante je sa najvećom kiselosti 66.63 + 2.73°SH do kraja zrenja sira. Vlažnost sira je ostala stabilna tokom zrenja. pH sira na prvi dan zrenja, koji je smanjen povećanjem temperature koagulacije (5.03, 5,11 i 5.00 za MWCK , MWCB1 i MWCB2), značajno ($p < 0,05$), koje su uticale na hemijske karakteristike sira. Sadržaj soli na kraju skladištenja u (MWCB1) i (MWCB2) varijante je 5.23 $\pm$ 0.31 i 5.52 $\pm$ 0.31, respektivno. Viša temperatura zgrušavanja smanjuje vlagu i pH, dok se sadržaj proteina povećao. Potrošnja mleka za proizvodnju 1 kilograma sira kretala se od 7.8 do 8.3 litara. Zaključeno je da je korišćenje starter kultura uticalo na poboljšanje kvaliteta belog sira.

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EFFECTS OF ADDING DIFFERENT FORMS OF SELENIUM IN DIETS FOR FATTENING LAMBS

D. Ružić-Muslić[1], M.P. Petrović[1], V.Caro- Petrović[1], Milan M. Petrović[1], Z. Bijelić, Z. Ilic[2], J. Stojković[2]

[1]Institute for Animal Husbandry, Belgrade, Serbia
[2]Faculty of Agriculture, University of Pristina, Lesak, Serbia
Corresponding author: muslic.ruzic@gmail.com
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Abstract: The study included lambs of Mis population, divided into two groups (experimental and control) .All the animals fed with identical portions of meals consisted of alfalfa hay and fully concentrate mixtures. Meals are only different in the fact that the experimental group received organic selenium and contained 2000 mg of selenium / kg preparations, while the control group received inorganic selenium in the form of sodium selenite (Na2SeO3). Each animal consumed the same amount of selenium than 0.3 mg / kg of dry matter in the organic or inorganic form, which added in the mineral-vitamin premix. Diet of lambs has been ad libitum. The body weight of lambs in both (control and experimental groups), were balanced at 60 days (19.60 kg : 19.65 kg) and 100 days (31.06 kg : 32.88 kg). The result on average daily gain of the control and experimental groups were almost similar and there were no statistically significant differences (P> 0.05) in the measured values from 28 to 60 days (259.0 : 255.0 g), from the 60-100 days (286.0 : 330.0g), and on average from 28 to 100 days (274.0 : 297.0. g). Both treatment have no significant effect on lambs' performance (body weight and growth). The study results showed that the diet of lambs experimental group, based meal supplement organic selenium resulted in significantly higher concentration of Se in MLD, kidneys, liver and spleen, compared with the control group, which are consumed inorganic form of selenium. The differences between the Se content in MLD the experimental and control groups were on significance level P <0.05, while the differences in the content of Se in kidney, liver and spleen, the aforementioned treatments were statistically highly significant (P <0.01). So fattening lambs are better utilizing organic source of selenium, which is associated with better absorption of this element.

Key words: sheep, lambs, selenium, body growth, selenium content

Introduction

Selenium (Se) was discovered in 1817, and considered a toxic element for humans and animals, until *Rotruck et al. (1973*) have not yet established that it is incorporated in selenocysteine (SeCys) which is essential element for the normal life processes. Selenium (Se), in the form of selenocysteine, is the central structural component of a number of specific enzymes, and especially catalase, glutathione peroxidase, which allows the host defense against oxidative stress. An adequate intake of selenium is needed to reduce the risk of myopathy, immunodeficiency, cardiovascular disease, cancer *(Rock et al., 2001; Hartikainen, 2005).* For animals and especially lambs, selenium deficiency is associated with white muscle disease. Selenium from food mainly derived from plants, which are adopted from the soil selenium in inorganic form, and synthesize the most selenomethionine *(Mezes and Balogh, 2006*). Selenium is an essential micronutrient in sheep and the deficiency of it can limit lamb growth and survival *(Stewart et al., 2012).* Selenium has a variety of role and is an essential element in the diet of animals. Generally, there are two forms of selenium, inorganic (selenate and selenite) and organic (selnomethione and selenocysteine), *Sunde, (2006*) and that both forms can be a good source of selenium dietary (*Terry et al., 2012*). The soils contain inorganic selenites and selenates that plants accumulate and convert to organic forms, mostly selenocysteine and selenomethionine and their methylated derivatives. Selenium is a naturally occurring metalloid element that is essential to human and animal health in trace amounts but is harmful in excess. Any deficiency or excess in the diet affected animal health, the intake of selenium being dependent on the amount of selenium taken up by plants as bioavailable selenium *(Fordyce, 2005*). While light soils and lush legume-dominant pastures are most often associated with selenium responsive conditions in animals, there are many exceptions *(Karimi-Poor et al., 2011).* Selenium behaves antagonistically with copper and sulfur in humans and animals inhibiting the uptake and function of these elements (*Khanal & Knight, 2010*). It serves as an anti-oxidant that works in conjunction with vitamin E to prevent and repair cell damage in the body, also involved in immune function and is necessary for growth and fertility **(***Khanal and Knight, 2010; Karimi-Poor et al., 2011*,**)**. Moreover, Se is a component of selenoproteins and is involved in immune and neuropsychological function in the nutrition of animals (*Meschy, 2000).*Various selenium contains amino acids that occur in nature and play important physiological roles especially in grazing sheep. Selenium after absorb from plants roots transferred to tissues and milk accompanying with plasma protein. More than 80% of protein-bound Se is selenocysteine. *Ullrey, (1987),* pointed out that the forms of Se in animal tissues have not completely understood but some is bound to protein, perhaps by a selenium-sulfide linkage, and that some had integrated into proteins. Therefore, regulation and synthesis of those proteins

and its behavior in the different organs and tissues are dependent highly on selenium supply *(Karimi-Poor et al., 2011).*

However, the content of Se in the plants in our area (experimental farm of the institute) is low and in order to alleviate the consequences of nutritional deficits, it is necessary to supplement this element in diets for feeding of lambs. As source of selenium is mainly used selenite and selenate. However, organic sources of selenium in the form of selenomethionine, has certain advantages. *Weiss (2005)* suggests that the digestibility of sodium selenite in sheep is around 50%, while the adoption of organic forms around 66%. Also well known that Se organic sources, is incorporated more efficiently into tissue than of Se inorganic sources (*Ehlig et al., 1967; Van Ryssen et al., 1989*).

The aim of this study was to compare the effects of different Se sources on growth performance of lambs and to determine the selenium content in muscles and organs of fattening lambs.

Material and methods

The study included 30 lambs of Mis population, with an average age of 28 days. The lambs were divided into two groups (experimental and control) which were completely uniform in all relevant parameters (body weight, age, sex, type of birth). All the animals fed with identical portions of meals consisted of alfalfa hay and fully concentrate mixtures containing 18% of protein. Composition of concentrate mixtures were the following (%): whole grain corn-58.4; whole soybeans -23.6; wheat bran 10%; yeast-5; minerals-2; premix-1. The mixture contained: 88.83% dry matter; 18.73% of the total protein and 1.208 NU, kg / kg. With the achieved body weight of 15 kg onward, lambs continued to feed concentrate with 16% of the total protein and alfalfa hay. The mixture contained 87.77% of dry matter of 16.47% as the total of protein and 1.198 NU, kg / kg. Meals are only different in the fact that the experimental group received organic selenium, which is a product of the American company Alltech and contained 2000 mg of selenium / kg preparations, while the control group received inorganic selenium in the form of sodium selenite (Na2SeO3). Each animal consumed the same amount of selenium than 0.3 mg / kg of dry matter in the organic or inorganic form, which added in the mineral-vitamin premix. Diet of lambs has been ad libitum. In addition to the meals, lambs supplied with water through automatic drinkers. The measurement of lambs was performed on 28, 60 and 100 days when they were calculated the average daily weight gain of 28 to 60 days, from 60 to 100 days and an average of 28-100 days of fattening.. The average daily intake, feed conversion and nutrient material had accompanied by the same dynamics. At the end of the experiment, animals have weighed and slaughtered seven lambs per treatment in the experimental slaughterhouse of the Institute for Animal

Husbandry. After slaughtering and primary processing, which is performed by standard methodology, the samples were taken from muscle (Musculus longissimus dorsi), kidney, liver and spleen in order to determine the selenium content. Statistical analysis of the obtained data was performed using the program Statistica 10.

Results and Discussion

Table 1. Body weight and growth of lambs

Treatments	Body weight, kg			ADG, g		
	Initial body weight	60 days	100 days	28-60	60-100	28-100
Control (inorganic Se)	11.30	19.60	31.06	259	286	274
Experimental (organic Se)	11.49	19.65	32.88	255	330	297

a,b -P<0.05

The average body weight and average daily gain of lambs are shown above (Table 1). The body weight of lambs in both (control and experimental groups), were balanced at 60 days (19.60 kg: 19.65 kg) and 100 days (31.06 kg: 32.88 kg). The result on average daily gain of the control and experimental groups were almost similar and there was no statistically significant difference (P> 0.05) in the measured values from 28 to 60 days (259.0: 255.0 g), from the 60 -100.days (286.0: 330.0g), and on average from 28 to 100 days (274.0: 297.0. g). Both treatments had no significant effect on lambs' performance (body weight and growth). The result we obtained was comparable with the findings of (*Chladek et al., 1999; Antunović et al., 2009)*. Likewise, in the study of *Antunović et al., (2014),* found non-significant differences in body weights of fattening lambs depending on dietary treatments with selenium. *Vignola et al. (2009)* who tested the influence of different sources and levels of selenium in diets for feeding of lambs of the Apennine breed have stated that the treatment had no significant effect on the average daily gain. *Luthman and Lindh, (1990),* gave their explanation to the specified results that "unless there is an evident lacking of Se, selenium supplementation does not affect the growth performance of lambs". Different result found by *Kumar et al., (2009),* in their study, supplementation of organic as well as inorganic Se, has found to improve the growth rate, of the lambs and that between the two sources, organic Se was more effective than inorganic Se.

Table 2. Average daily intake of nutritive substances of meals and consumption of nutritive substances per kg of gain

Indicator	Average daily intake of nutritive substances of meals		Consumption of nutritive substances per kg of gain	
	Experimental	Control	Experimental	Control
	28-60			
Dry matter, kg	0.580	0.517	2.295	1.995
Total protein, g	119	109	472	420
NU, kg/kg	0.637	0.569	2.522	2.193
	60-100			
Dry matter, kg	0.790	0.814	2.390	2.855
Total protein, g	146	148	441	519
NU, kg/kg	1.014	1.024	3.070	3.593
	28-100			
Dry matter, kg	0.695	0.680	2.345	2.481
Total protein, g	134	130	452	476
NU, kg/kg	0.844	0.818	2.847	2.987

In table 2, the differences were minimal, in favor of the Experimental group in periods 28-60: 28-100 days on the Average daily intake of nutritive substances of meals on the following indicator and differences (experimental : control); Dry matter, kg-0.063:0.015; Total protein, g-10:4; NU, kg/kg-0.068 : 0.026. In favor of control group at 60 100 days with such difference on the following indicator: Dry matter, kg-0.024, Total protein, g-2, NU, kg/kg- 0.01. Concerning the consumption of nutritive substances per kilogram of gain was higher in the experimental group in period 28-60 with the following differences in the indicator: Dry matter, kg - 0.3, Total protein, g - 52, NU, kg/kg - 0,329. At 60 - 100 and 28 - 100 in favor of the control group with the following differences: Dry matter, kg - 0.465 : 0.136, Total protein, g – 78 :24, NU, kg/kg - 0.523 : 0.14.

Table 3. Selenium contents in muscle and organs

Tissue	Experimental group (organic Se)	Control group (inorganic Se)
M. longissimus dorsi, mg / kg	195.06^{a}	130.32^{b}
Kidney, µg/kg	1350.24^{A}	1131.62^{B}
Liver, mg / kg	710.22^{A}	591.13^{B}
The spleen, mg / kg	390.43^{A}	301.25^{B}

^{a,b} $P<0.05$ ^{A,B} $P<0.01$

The study results showed that the diet of lambs experimental group, based meal supplement organic selenium resulted in significantly higher concentrations of Se in MLD, kidneys, liver and spleen, compared with the control group, which are consumed inorganic form of selenium. The differences between the Se content in MLD The experimental and control groups at a significance level $P < 0.05$, while the differences in the content of Se in kidney, liver and spleen, the aforementioned treatments were statistically highly significant ($P < 0.01$). So fattening lambs are better utilizing organic source of selenium, which is associated with better absorption of this element. Previous studies in ruminants *(Aspila, 1988)* have pointed to the better absorption of organic selenium compared to inorganic selenium (65, 50). The weaker absorption of inorganic selenium is probably the result of reducing the availability of selenium from food from insoluble forms (Se element or selenides) in the rumen (*Varady et al., 2005).* As seen in table 3, can noticed that the accumulation of selenium in the internal organs significantly higher ($P<0.01$) in comparison with that of the MLD ($P<0.05$) at both investigated treatments and statistically significant differences ($P<0.05$) in favor of organic selenium and indicating a better bioavailability. Organic selenium supplementation gave 49.67% higher selenium contents in lamb meat than inorganic selenium supplementations. Our result was similar with the findings of *Steen et al., (2008),* of which they have noted that organic selenium supplementation gave 50% higher selenium concentration in lamb meat than inorganic selenium supplementation. The result obtained by *Antunović et al., (2009)* on Se content in the muscle of OS was higher than that found for the C lambs confirmed with ours. In our study, the organic selenium supplementation has higher percentages (than inorganic supplementation) of selenium acquired in internal organs: 19.32% in kidney, 20.15% in liver, 9.7% in the spleen. In both treatments has found the highest content of selenium in kidney of which is complementary with the findings of other authors. *Combs & Combs, 1986; Supczyñska et al., 2009,* reported the amount of Se in the tissues, ranked the highest in the kidney, followed by the liver and the least in skeletal muscle. The Se contents in liver in each treatment had 3 to 4 times higher than the muscle. As stated by *Lee et al. (2004),* which indicating that "a high Se concentration in the liver compared with muscle might result from the fact that liver acts as a major pool of Se in the body", uphold our findings. Moreover, in the studies of *Juniper et al.,2009*; *QIN et al., 2007; Van Ryssen et al., 1989,* in both groups, the highest Se concentration was also found in the kidneys, which is true in our results. *Pehrson, (2005),* terminated in his study that "the supplementation of farm animal diets with organic selenium instead of inorganic selenium will increase selenium status of lambs and slaughter lambs" in accordance with the results we obtain. A different result found by *Antunović et al., (2009),* where Se content of kidney was not significantly affected by treatment but they concluded that Se in organic form had a better bioavailability compared to the inorganic form. *Joksimović et al., (2012),* have noted in their papers that, "In difference to

inorganic selenium, organic selenium is deposited more effectively in tissues. Several authors who conducted similar studies have expressed their views regarding their findings. *Kim and Mahan, (2001*), informed that Selenium concentrations in tissues are affected by the dietary concentration and chemical form of Se. Whereas, *Ehlig et al., (1967); van Ryssen et al., (1989)* commented in their papers that Se from organic sources is also well known as more efficiently incorporated into tissue than inorganic sources of Se. The distribution of Se in tissues was dependent upon an organically bound source of Se fed to animals, and that this could be due to the molecular forms of Se present in organic Se sources (*Lawler et al., 2004;Wu et al., 1997).* Furthermore, various findings from feeding of different chemical forms of dietary Se to animals showed that organic Se was more bioavailable than inorganic Se of which resulted in an increase of Se contents in tissues *(Lawler et al., 2004; Lee et al., 2006).* In addition to selenium enriched yeast (organic form) most occupies part of selenomethionine which must undergo enzymatic transformation to selenocysteine lyase prior to the release of specific selenium without the production of reactive intermediates (*Foster et al., 1986). Hakkarainen (1993)* pointed out that the microbial population of the rumen can incorporate selenomethionine from selenized yeast in their proteins and thereby reduce selenium ingested food to insoluble forms, such as elemental selenium, and thus make it less available for absorption. The content of selenium in tissues is associated with a higher metabolic activity viscelarnih organs such as; kidneys, liver, spleen and pancreas. Absolutely the greatest concentration of Se was observed in the renal cortex. The explanation for this phenomenon is based on the fact that the proximal renal tubules main site of synthesis of three specific selenoproteins: a phospholipid hydroperoxide glutathione peroxidase, type I deiodinase iodotironin -5 and plasma glutathione peroxidase (*Mony and Larras Regards, 2000*).

Conclusion

Based on the conducted research and the results obtained, we can conclude:

• Source of selenium (inorganic and organic form) in meals of lambs, did not significantly affect the production performance of fattening lambs.

• Accumulation of selenium in the internal organs of lambs was significantly higher compared with the content in MLD at both trial treatments, a statistically significant difference in favor of organically bound selenium, indicate its better bioavailability.

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Efekti dodavanja različitih oblika selena u obroke za tovnu jagnjad

D. Ružić-Muslić, M.P. Petrović, V.Caro - Petrović, Milan M. Petrović, Z. Bijelić, Z. Ilic, J. Stojković

Rezime

Selen (Se) je esencijalni mikroelement za ljude i životinje i predstavlja centralnu strukturnu komponentu niza specifičnih enzima a pre svega glutation peroksidaze. Uglavnom potiče iz biljaka, koje iz zemljišta usvajaju selen u neorganskom obliku. Sadržaj Se u biljkama na našem području je nizak. Da bi se ublažile posledice nutritivnog deficita, neophodna je dopuna ovog elementa u obrocima za ishranu životinja. U tu svrhu se koriste organska i neorganska forma selena.
Cilj ovog istraživanja je upoređivanje efekata različitih formi selena na proizvodne performanse i retenciju selena u tkivima tovne jagnjadi. Istraživanjem je bilo obuhvaćeno 30 jagnjadi Mis populacije, prosečnog uzrasta oko 28 dana, podeljenih u dve grupe. Grla su hranjena identičnim obrocima koji su se sastojali od sena lucerke i potpune smeše koncentrata. Obroci su se razlikovali u tome što je ogledna grupa dobijala organski selen koji je bio proizvod američke firme Alltech i sadržao je 2000 mg selena/kg preparata, dok je kontrolna grupa dobijala neorganski selen u obliku natrijumselenita (Na_2SeO_3). Svako grlo je konzumiralo identičnu količinu selena od 0.3mg/kg suve materije u organskom odnosno neorganskom obliku koji su dodavani u mineralnovitaminsku predsmešu. Rezultati ogleda su pokazali da su telesne mase jagnjadi kontrolne i ogledne grupe bile ujednačene, kako 60. dana (19.60 kg; 19.65 kg), tako i 100. dana (31.06 kg; 32.88 kg). Dnevni prirasti jagnjadi kontrolne i ogledne grupe su takođe bili slični i nije bilo statistički značajnih razlika ($P>0.05$) u vrednostima izmerenim od 28. do 60. dana (259.0 : 255.0 g), od 60.-100. dana (286.0 : 330.0g), i prosečno od 28. do 100. dana (274.0 :297.0. g). Izvori selena nisu značajnije uticali na prosečno konzumiranje suve materije (0.695 : 0.680 kg), ukupnih proteina (134.0:130.0 g), OHJ (0.844:0.818), kao ni na konverziju hranljivih materija: suva materija (2.345: 2.481 kg); ukupan

protein (452 :476 g), OHJ (2.847: 2.987 kg), u periodu od 28-100. dana ogleda. Ishrana jagnjadi ogledne grupe, obrokom na bazi suplementa organskog selena je rezultirala znatno većim koncentracijama Se u MLD, bubrezima, jetri i slezini, u poređenju sa grlima kontrolne grupe, koja su konzumirala neorgansku formu selena.Ustanovljene razlike između sadržaja Se u MLD jagnjadi ogledne i kontrolne grupe su na nivou značajnosti $P<0.05$, dok su razlike u sadržaju Se u bubrezima, jetri i slezini, na navedenim tretmanima, bile statistički veoma značajne ($P<0.01$). Dakle, tovna janjad su bolje iskoristila organski izvor selena, što se dovodi u vezu sa boljom apsorpcijom ovog element.

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THE CONTENT OF CALCIUM, PHOSPHORUS AND MAGNESIUM IN THE BLOOD SERUM OF SHEEP DEPENDING ON THE SEASON AND PHYSIOLOGICAL STATE

J. Stojković[1], Z. Ilić[1], M. P. Petrović[2], V. Caro Petrović[2], D. Ružić Muslić[2], V. Kurčubić[3], R. Đoković[3]

[1]Faculty of Agiculture, Kosovska Mitrovica-Lešak, 28000, Serbia
[2]Institute for Animal Husbandry, Belgrade-Zemun, 11080 Zemun, Serbia
[3]Faculty of Agronomy, Čačak, 32000, Serbia
Corresponding author: Jovan Stojković e-mail: jovanips@gmail.com
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Abstract: The large number of sheep, especially the ones that spend the majority of time on pastures, is been fed with the meals, which do not contain all the necessary mineral substances. The level of calcium, phosphorus and magnesium on natural pastures is too low in relation to the needs of sheep. Therefore, the irregularity in the feeding of sheep occurs because of the absence of the other food sources. These irregularities are in the range from the acute mineral deficit or illness to the mild temporary forms, which could hardly diagnose, but they affect the level of production. The content of calcium, phosphorus and magnesium in the blood serum of sheep, as one of the indicators of the supplementation of sheep with these substances, has given in this paper. The average level of calcium in the blood serum of the control group of sheep was 2.61 mmol/l, phosphorus 1.04mmol/l and magnesium 1.31 mmol/l of serum. In the blood serum of the tested group, the average contents of calcium was 2.33 mmol/l, phosphorus 0.92 mmol/l and magnesium 1.37 mmol/l.

Key words*:* sheep, calcium, phosphorus, magnesium, serum

Introduction

The knowledge of the nutritive values of the grass mixture, with which sheep meet their needs during nearly one half of the year, is very important from the standpoint of the evaluation of the food quality (*Kargin et al., 2004; Khan et al., 2006*). Pasture is very important as the full-value source of food in the feeding of sheep, of which could not be easily substituted in the extensive breeding of ruminants. The quality of the pasture and its nutritional value depend on many factors but primarily on the quality of the soil and the climatic factors. In the

conditions of breeding sheep in the Šar Mountains, learning about the quality of pasture gives us the possibility of taking appropriately measures in its improving. It would be a starting point for resolving issues of proper nutrition of sheep during the summer, because that period represents an important phase of a changing in physiological states (lactation, and preparation for successful impregnation).

Certainly, these tests are not able to discover the genesis of their foundation, on which we can derive the conclusions for guidance of the direction of the further development by applying Hemo - Agro - Hido and other measures, which were dictated by the ecological factors. Because these researches do not have pretensions of such a capacity and profile, their goal is to show the picture of the chemical composition of the grass mass of tested pastures, as a very useful component of the main research, which related to the dynamics of calcium, phosphorus and magnesium in stages of vegetation, or the exploitation of the pastures. In many areas of the Šar Mountains, the content of stodgy cattle food is not enough tested. From the results obtained based on our previous researches of mineral substances in stodgy food, a low content of phosphorus is established in them, and appeared much more frequently than it is the case with calcium. This phenomenon is especially present during dry years and certain periods, which often occur at the Šar Mountains where the testing had performed. It should be added that in the Šar Mountains, there are soils in which the content of physiologically active phosphorus (P_2O_5) is extremely low, as was found by *Mirić (2000)*, and a low content of this element in the soil adversely affects its content in plants. To this, we should add the statement of *Stojković (2006)* that a substantial number of plant species from these areas has a low content of phosphorus.

Materials and Methods

The testing of the influence of the physiological conditions and the seasons on the dynamics of the content of calcium, magnesium and phosphorus in the blood serum of the crosses F_1 (Sharplaninska sheep x Wurttemberg sheep) was conducted during two years in farm of Štrpce. There were 60 sheep in the experiment, of which 30 were in the control group – the futile sheep (treatment 1, 2, 3, 4 and 5) and 30 in the experimental group - the fertile sheep (treatment 1, 2, 3, 4 and 5). At the end of each treatment, the blood has taken from the jugular vein of the sheep for the preparation of the serum. The testing of these mineral elements was carried out in the blood serum of the sheep and the food used in their meals. The sheep were staying in a cottage at the Šar Mountains started from the late May until the early October (2007). The main meal was their pasture with the addition of 200 g of corn grits. In addition, from October until May (2008), the sheep were in their home environment near Štrpce. The main meal was their meadow hay from 1.5 to 2.0 kg and corn grits from 200 to 300 g per day. The cattle salt in the form of the mineral plates was also available to the sheep.

Statistical analysis was performed using Statistica, version 6, SatSoft. Inc. (2003).

Table 1. The contents of nutrients in the feed for feeding sheep

Type of food	Chemical composition, (%)						
	Water	Protein	Cellulose	Ash	Ca/g	P/g	Mg/g
Meadow hay	11.15	8.37	30.67	7.80	0.76	0.26	0.20
Lucerne hay	11.90	9.80	28.90	8.58	0.98	0.15	0.34
Corn grits	11.51	8.51	1.80	1.56	0.10	0.35	0.19
Spring grass	20.71	9.67	24.90	6.79	0.76	0.15	0.05
Summer grass	18.68	6.80	30.22	5.56	0.77	0.16	0.06
Autumn grass	18.12	5.65	28.40	8.23	0.78	0.30	0.08

Results and Discussion

The data about the dynamic of the content of calcium in the blood serum of the sheep are shown in table 2.

Table 2. The contents of calcium in the blood serum of sheep (mmol/l)

	X±SD	SG
Control group – futile sheep		
Treatment 1 - August	2.47±0.18	0.47
Tretmenrt 2 - November	2.59±0.18	0.47
Treatment 3 - January	2.58±0.13	0.35
Treatment 4 - July	2.57±0.24	0.64
Treatment 5 - September	2.60±0.17	0.44
Average	***2.61±0.19***	***0.47***
Tested group – the fertile sheep		
Treatment 1 – first half of the pregnancy	2.54±0.25	1.05
Treatment 2 – second half of he pregnancy	2.34±0.11	0.45
Tretmen 3 - the beginning of lactation	2.02±0.13	0.54
Treatment 4 – the end of lactation	2.29±0.15	0.60
Treatment 5 – the period of becoming infertile	2.50±0.11	0.47
Average	***2.33±0.15***	***0.62***

After the fertilization of the sheep, the content of calcium in the blood serum was 2.54 mmol/l, while in the futile sheep at that time was 2.74 mmol/l. In the second half of the pregnancy, the content of calcium decreased to 2.34 mmol/l, while in the futile sheep this decrease was much lesser (2.59 mmol/l). In the phase of the most opulent secretion of the milk in the lactating sheep, the decrease in the

content of calcium is most pronounced, and it was slightly below the physiological limits (2.02 mmol/l) then. At that time, the content of calcium in the blood serum of the futile sheep was at the same level (2.58 mmol/l). In the phase of reduced secretion of the milk in the lactating sheep, the increase in the content of calcium of 4.00% was noted, and later by becoming infertile, the level of calcium in the blood serum climbed to 2.50 mmol/l. At that time, in the futile sheep, the content of calcium held at the same level (2.57 and 2.60 mmol/l). By the dynamics of calcium content in the blood serum of the fertile and futile sheep, general conclusion can be made, that, normocalcemia existed in the conception of the sheep and that the changes, that occurred in the fertile sheep, the result of normal moving, were caused by the different needs of the organism in accordance with the physiological conditions. Considering the content of calcium in the blood serum of the sheep it can be concluded that calcemia was normal and that there were no significant differences ($P>0.05$) in physiological conditions, or season.

The dynamics of the content of calcium in the blood serum shows a regular rhythm when we correlate it with changes in the physiological status of the sheep, making a concave parabola with the lowest point in the time of the most opulent secretion of milk. At the same time, the contents of calcium in the serum in the futile sheep ranged slightly above average the normal values (between 2.57 and 2.74 mmol/ l) and despite the season it was not subjected to changes. The percentage of variation in the content of calcium was significantly lower in the futile sheep, which is understandable.

According to the data from *Underwood (1976)*, this value was lower for 5.15% of the average content of calcium in our previous experiments, *Stojković (2006, 2009)*. Although, in literature there are the data according to which the content of calcium in the blood serum of sheep can be much higher, *Pavličević et al., (1998)*, as was the case when the minerals with high calcium content were added to the meals. But there are also data on cases of the expressive hypocalcemia such are indicated by *Mirić et al., (2000)* in whose experiments the level of calcium in the blood reach the level of only 1.98 mmol /l.

The data about the content of phosphorus in the blood serum of the sheep are shown in the table 3.

Table 3. The content of phosphorus in the blood serum of the sheep (mmol/l)

	X±SD	SG
Control group – futile sheep		
Treatment 1 - August	0.98±0.16	0.43
Tretmenrt 2 - November	1.01±0.15	0.39
Treatment 3 - January	1.02±0.08	0.22
Treatment 4 - July	1.07±0.06	0.15
Treatment 5 - September	1.13±0.04	0.11
Average	***1.04±0.09***	***0.26***
Tested group – the fertile sheep		
Treatment 1 – first half of the pregnancy	1.13±0.15	0.62
Treatment 2 – second half of he pregnancy	0.86±0.09	0.37
Tretmen 3 - the beginning of lactation	0.74±0.08	0.33
Treatment 4 – the end of lactation	0.92±0.08	0.35
Treatment 5 – the period of becoming infertile	0.97±0.19	0.40
Average	***0.92±0.10***	***0.41***

In the first half of the pregnancy, the content of phosphorus in the blood serum of the sheep was 1.13 mmol/l. in the second trimester the content decreased to 0.86 mmol/l. During the period of full lactation, the maximum decrease was 0.74 mmol/l. From this, along with the decrease in the secretion of the milk, the level of phosphorus increased to 0.92 mmol/l, only to reach the level of 0,97 mmol/l in the infertile sheep in September. In the futile sheep, the content of phosphorus in the blood serum in August was 0.98 mmol/l and it was constantly increasing during the year (1.01 mmol/l, 1.02 mmol/l, 1.07 mmol/l and 1.133 mmol/l) to reach in the beginning of September 1.13 mmol/l. In the fertile sheep, the smallest deviation from the average values between the certain sheep was notice after the fertilization and they were constantly growing from the period of becoming infertile. In terms of percentage of the variations of the content phosphorus in the blood of the futile sheep, the flow was reverse. Varying is highest at the time when should be impregnated the sheep and it constantly declines until September.

From the data presented, it can be noticeable that the content of phosphorus in the blood serum of the sheep during the year is in all physiological states, under the physiological limits. Hypophosphatemia reached its peak in the fertile sheep in the phase of the most opulent secretion of the milk. Concerning that, hypophosphatemia was very pronounced in the fertile sheep, and the average varying of the inorganic serum of phosphorus during the year was higher than in the futile sheep. Differences in the content of phosphorus per season and physiological condition were in the level of the statistical significance ($P < 0.01$).

The content of phosphorus for all groups in the experiment was in the average 0.98 mmol/l of the serum. With these values it was located below the

physiological limits of the normal range of the content according to the data from the literature (1,45-2,00 mmol/l).

The lowest values recorded in the most opulent stage of lactation (0.74 mmol/l) and the highest in the first half of the pregnancy (1.13 mmol/l). With lactation passing off and the arrival of the non-productive lactation period (in autumn), the content increased to 0.97 mmol/l. In terms of variation, it is characteristic that, from the beginning to the end of pregnancy of the monitored period, the variety grew more and more (from 5.03 to 15.50%). Although, in the futile sheep, the average content of phosphorus in the serum was higher than in the fertile sheep by 11%, the general level was very low, and the changes were subordinate more to the factor of diet, which was not the same case and in the fertile sheep. The content of phosphorus in the futile sheep was constantly increasing during the year, starting from 0.98 mmol/l in August and to 1.13 mmol/l at the end of the observed period. Concerning the content of phosphorus in the serum of the fertile and the futile sheep, it can be terminated that hypophosphatemia is expressed during the whole year.

During the general hypophosphatemia, of both fertile and futile sheep, it is important to notice the appearance of a very different character of variation in the content of phosphorus per year, namely caused by the physiological state. In the futile sheep it can be seen a constant, although small increase in the content of phosphorus during the "vacation" until next season of fertilization, while at the same time narrowing of the variation span. Whereas, the pregnant sheep have a decrease in the phosphorus content, this goes along with the duration of stress of the organism (increased distress) in pregnancy in the first two months of lactation. In addition, it has increased with the arrival of the period of the physiological relief, with a permanent increase in the percentage of the variations in the content of phosphorus in the blood serum. These findings supports the assumption that in the states of hypophosphatemia in the temporary futile sheep, the balance of phosphorus is gradually improving to such an extent that they can, after a one-year break, manifest the sexual passion and be fertilized. In the "fertile" sheep, although in the second half of the lactation the balance of phosphorus in average improves, it cannot be improved in all animals, which is attested by the increased percentage of variation so that, slightly more than a half of the sheep came to a successful fertilization, while a smaller part remained unfertilized.

The displayed value of the content of phosphorus, it can be seen that there was virtually a low level of this element in the blood serum of the sheep with a minimal increase in the time of their pregnancy, and with the lowest value in the stage of lactation. A low content of phosphorus in the blood of the sheep has interpreted by its low content in food, especially hay, and reason for the low content in hay, by the interpretation of *Mirić (2000)*, lies in the fact that, in this region exist large areas of land on which the content of physiologically active phosphorus (P_2O_5) is extremely low. This is confirmed by the findings of *Stojković*

(1997, 2009) in which a large number of plant species from these areas have a low content of phosphorus. Its enhanced secretion in milk can interpret a particularly low content of phosphorus in the blood serum of the sheep in lactation.

The data about the dynamics of the content of magnesium in the blood serum of the sheep are shown in the table 4.

The content of magnesium in the blood serum of the sheep was within the normal range and it showed no significant differences with changes in the physiological state of the sheep, which is a characteristic of its behaviour. Similar or the same range of the levels of magnesium in the blood in the same intervals had established in the futile sheep, too. Differences in the content of magnesium in the blood serum of the sheep, both in season and in the physiological conditions were not significant (P> 0.05).

The content of magnesium in the blood serum of the sheep was within the normal physiological range of 1.34 mmol/l and showed no significant differences during the changes in the physiological state, which is the characteristic of its behaviour. Yet, although to a lesser extent, there was a reduction of its content in the blood of the sheep at the end of lactation (1.27 mmol/l). There were not any notable individual changes in the content of magnesium, or changes in groups of the sheep with different lactation. However, averages per groups indicate the existence of a reverse trend of a change in the content of this element in the blood serum in relation to the trend of a change in the level of calcium and phosphorus. This means that the content of magnesium was the highest in the blood serum of the sheep with the highest lactation, and the lowest in the infertile sheep.

Table 4. The content of magnesium in the blood serum of the sheep (mmol/l)

	X±SD	SG
Control group – futile sheep		
Treatment 1 - August	1.28±0.12	0.27
Tretmenrt 2 - November	1.32±0.17	0.19
Treatment 3 - January	1.29±0.19	0.16
Treatment 4 - July	1.35±0.11	0.21
Treatment 5 - September	1.35±0.23	0.10
Average	***1.31±0.16***	***0.18***
Tested group – the fetile sheep		
Treatment 1 – first half of the pregnancy	1.33±0.15	0.62
Treatment 2 – second half of he pregnancy	1.31±0.21	0.37
Tretmen 3 - the beginning of lactation	1.43±0.23	0.40
Treatment 4 – the end of lactation	1.27±0.10	0.59
Treatment 5 – the period of becoming infertile	1.55±0.18	0.61
Average	***1.37±0.17***	***0.51***

The data on the content of magnesium, which had obtained in these researches, correspond with the results of *Adamović and Pavličević (1990)* and *Pavličević and co. (1999)*. The explanation for the relatively small changes in the content of magnesium in the blood of the sheep, according to *Underwood (1976)* and *Stojković (2009)*, is that the magnesium in soft tissues is not reduced even when the sheep loose up to 30% of magnesium from the skeleton.

Conclusion

The content of calcium, phosphorus and magnesium in the blood serum of sheep, as one of the indicators of supplementation of animals in these materials, has investigated. The average content of calcium in the examined blood serum of the sheep was 2.47 mmol/l, phosphorus 0.98 mmol/l and magnesium 1.34 mmol/l of the serum. The values of calcium were at the upper limit of normal content referred to in the literature. The changes in the content of calcium were not significantly manifest in relation to the season and the physiological state of the sheep. The values of phosphorus were below the deficit, and these values decreased the most in the beginning of the lactation period of the sheep. The content of magnesium was within the normal physiological range. The changes in the content of magnesium were not significantly manifest in relation to the season and the physiological state of the sheep, but had a reverse trend compared to the trend of the change in calcium and phosphorus.

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Sadržaj kalcijuma, fosfora i magnezijuma u krvnom serumu ovaca po godišnjem dobu i fiziološkom stanju

J. Stojković, Z. Ilić, M. P. Petrović, V. Caro Petrović, D. Ružić Muslić, V. Kurčubić, R. Đoković

Rezime

Veliki broj ovaca, naročito onih koji dobar deo vremena provode na paši, hrani se obrocima koji ne sadrže sve potrebne mineralne materije. Nivo kalcijuma,

fosfora i magnezijuma na prirodnim pašnjacima je suviše nizak u odnosu na potrebe ovaca. Tako se nepravilnost u ishrani javlja kod ovaca u odsutnosti drugih izvora hrane. Ove nepravilnosti kreću se od akutnog mineralnog deficita ili bolesti, pa do blagih prelaznih formi koje se teško dijagnosticiraju, ali se odražavaju na nivo proizvodnje. U radu je dat sadržaj kalcijuma, fosfora i magnezijuma u krvnom serumu ovaca, kao jednog od indikatora obezbeđenosti ovaca ovim materijama. Prosečan sadržaj kalcijuma u krvnom serumu kontrolne grupe ovaca iznosio je 2,61 mmol/l, fosfora 1,04 mmol/l i magnezijuma 1,31 mmol/l seruma. Kod ogledne gupe prosečan sadržaj kalcijuma bio je 2,33 mmol/l, fosfora 0,92 mmol/ i magnezijuma 1,37 mmol/l seruma.

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MILK YIELD, COMPOSITION, NUTRITIVE AND TECHNOLOGICAL VALUES FROM EWES FED DRIED DISTILLERS' WHEAT GRAINS WITH SOLUBLES (DDGSw)

M. R. Yossifov

Dept. «Animal nutrition and feed technologies»
Institute of Animal Science – Kostinbrod, P.C. 2232, Bulgaria
Corresponding author: e mail: m_vet@abv.bg
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Abstract: The aims of this study were to evaluate the effects of lactating dairy ewes diets supplementing with different vegetable protein sources (sunflower meal (*SFM*) vs. dried distillers' wheat grains with solubles (*DDGSw*)) at the pick of lactation (27 – 72 lactating day). The experiment was carried out with sixteen lactating dairy ewes, Bulgarian Dairy Synthetic Population (BDSP) in 60-d feeding trial (7-d preparatory + 45-d experimental + 8-d closing periods). Animals were allotted randomly (by age, lactation, milk yield, % milk fat, % milk protein) into two dietary treatments: 1./ a SFM- based control diet (*CD*), and 2./ DDGSw- based experimental diet (*ED*). Diets were iso- nitrogenous, iso- fibrogenous, iso- caloric and equal in protein truly digestible in the small intestines (*PDI*), calcium (*Ca*) and phosphorus (*P*). Compared with the *CD*, supplementation with *DDGSw* decreased ($p<0.001$) the average daily milk yield (5.8 %) and 6.5 % fat-corrected milk (6.8 %). There're no significant differences in milk composition between treatments (*CD* and *ED*): solids non-fat (*SNF*) + 0.8; dry matter (*DM*) + 0.1 %; fat content of milk (*MF*) - 1.1 and milk protein (*MP*) content + 0.4 % per sheep for *ED*, compared with *CD*. *DDGSw*- based diet did not affect nutritive and technological parameters of raw milk: *MP*/*MF* (+ 1.3 %), *MP*/*DM* (- 0.1 %) and *MF*/*DM* (- 1.0 %) ratios. In conclusion, observed data indicates that *DDGSw* at level of 17 % *DM* basis affected ewe milk yielding negatively, without affecting milk composition, nutritive and technological parameters.

Key words*:* Lactating dairy ewes, Dried distillers' wheat grains with solubles (DDGSw), Feed conversion and efficiency, Milk yield, composition, nutritive and technological parameters.

Abbreviations: *DDGSw*-Dried distillers' wheat grains with solubles; *SFM*-sunflower meal; *ED*-experimental diet; *CD*-control diet; *SNF*-solids non fat;

Ca-calcium; *CP*-crude protein; *DM*-dry matter; *MF*-fat content of milk; *MP*-milk protein.

Introduction

The „Green thinking" and the „boom" of biofuel production caused an increase of by-products from this industry available as livestock feeds (*Kozelov and Yossifov, 2013; Lyons, 2007*). Bioethanol in EU, e.g. Bulgaria, is produced mainly from grains – wheat and corn (*Paul et al., 2012; Piron et al., 2009***)**. So, the main byproduct is dried distillers' wheat grains with solubles (*DDGSw*). It contains high concentrations of digestible fibre, so it's an ideal feed for ruminants. Simultaneously, the *DDGSw* is high in protein (40 %) and with optimal levels of fats (3 – 7 %) and fiber (8 – 9 %) with high digestibility (*Aldai et al., 2009; Gibb et al., 2008; Paul et al., 2012; Yossifov, 2012*).

Commonly, *DDGS* have been recognized at low/moderate levels in feedlot rations as substitute for both traditional protein and energy sources (*Yossifov and Kovelov, 2012*). But recent studies indicate that adding *DDGS* to dairy rations improve cow' dry matter intake (*DMI*) (*Zhang et al., 2010*), milk yield (*Anderson at al., 2006; Chibisa et al., 2010*), milk protein and fat (*Schingoethe et al., 1999; Sasikala-Appukuttan et al., 2008*). Nevertheless, there have been relatively few studies reported in which *DDGS* has been fed to sheep. Some suggested that it can be successfully fed to lactating dairy sheep without any effect on animal performance, milk composition and rennet abilities (*Dimova et al., 2009; Yossifov, 2014a*).

So, our experiment was intended to explore the concept of supplementing lactating dairy ewe' diets with *DDGSw*. The objectives of this were to verify if higher (than recommended) *DDGSw* levels affect milk yield at the pick of lactation curve, and if the *ED* affect milk composition and milk nutritive and technological parameters. Such differences, if existing, may be used from animal nutritionists to balance dairy sheep total mixed ration.

Material and methods

Experimental animals and diets. The experiment was conducted at the Experimental Farm of the Institute of Animal Science, Kostinbrod, BG using 60-d feeding trail (7-d preparatory + 45-d experimental + 8-d closing periods). Sixteen lactating dairy ewes of Bulgarian Dairy Synthetic Population (*BDSP*) breed were randomly (by age, lactation, milk production, % milk fats, % milk protein) divided into two diet treatments (n=8): control (*CD*) and experimental diet (*ED*) in order to

evaluate the effects of *DDGSw* supplementation during the pick of lactation (27 – 72 lactating day).

The experimental design is shown in table 1. Daily ration (as *DM* basis) contained 75 % forage (meadow hay + corn silage) and 25 % concentrate mixture. *CD* concentrate mixture consisted of corn (7.72 %), wheat (8.79 %), sunflower meal (*SFM*= 8.26 %) and supplement (0.65 %). Part of the wheat and whole *SFM* of the *CD* were replaced by 16.75 % *DDGSw* in *ED*. Diets were formulated to be iso-caloric, iso-fibrogenous and equal in *PDI*, *Ca* and *P* to meet and exceed all nutrient requirements of lactating dairy ewes (*NRC, 2007*). The supplement provided *Ca* (limestone), ammonium sulphate and vitamin-mineral premix (per kg of diet: *Mg* - 60.0 mg, *Fe* - 1.3 mg, *copper* - 1.0 mg, *I* - 1.6 mg, *Zn* - 60.0 mg, *Co* - 1.0 mg, *Vit. A* – 5000 IU, *Vit. D* - 2000 IU, *Vit. E* -10.0 mg). The diets were fed twice daily – 7.00 AM and 6.00 PM throughout the experimental period. Feed intake was being adjusted daily. Animals were provided free access to fresh water and salt blocks.

Feed sampling and analytical procedures. Feeds were sampled and analysed bimonthly. The feed refusals were collected and weighed daily and analysed twice a month. Samples were analysed for *DM* by drying in a forced-air drying oven at 65 ^{o}C for 48 h. Samples were ground to pass through a 1 -mm screen for further chemical analyses: crude

Table 1. Diet formulation

Item	**SFM- based diet**	**DDGSw- based diet**
		Forage:
Meadow hay	13.32	13.30
Corn silage	61.26	61.17
		Concentrate mixture:
DDGSw	–	16.75
SFM	8.26	–
Wheat	8.79	0.26
Corn	7.72	7.71
		Supplement:
Limestone	0.20	0.40
$(NH_4)_2SO_4$	0.45	0.41
SFM - Sunflower meal; DDGSw - Dried distillers' wheat grains with solubles.		

protein (*CP*) (Kjeldahl N x 6.25), ether extract (*EE*), crude fibres (*CF*), ash, calcium (*Ca*) and phosphorus (*P*) according to *AOAC (2002)*.

Milk sampling and Analysis. Milk yield was recorded twice a day – individually per ewe, during the morning and evening milking. Milk samples were taken and analysed weekly in accordance to the regulations for milk sampling (*country AC method*). Physicochemical characteristics of the raw milk samples were analysed with apparatus EcoMilk (Milkana KAM 98-2A – Bultech Company). The following milk composition parameters were investigated: solids non fats (*SNF*), dry matter (*DM*), milk fats (*MF*) and milk protein (*MP*).

Biostatistical Analyses. The amount of feed offered and refused was recorded daily for each treatment (*CD* and *ED*) of ewe and feed intake and dry matter intake (*DMI*) was calculated (average per sheep). Feed efficiency (*FE*) was calculated as ratio of average daily milk yield to the average *DMI* (M: F). The following indices and ratios for milk samples were calculated: *MP*/*MF*, *MP*/*DM* and *MF*/*DM* to evaluate the nutritive and technological qualities of raw sheep milk. All parameters were analysed using MS Office 2007 and Student t-test. Statistical significance was accepted at $p<0.05$ and $p\geq0.05$ but ≤0.1 was interpreted as indicating a trend towards significance.

Results and discussion

Diet composition. Chemical composition of feedstuffs is presented in **table 2**. *DDGSw* was higher in *DM* (9.6 %), *CP* (0.8 %) and *EE* (315 %) but lower in *CF* (235.3 %), Ash (31.5 %), *Ca* (241.7 %) and *P* (6.7 %) compared with SFM.

Table 2. Chemical composition of diet' ingredients (as % of DM):

	MH	Corn silage	SFM	DDGSw	Wheat	Corn
Dry matter	80.68	39.58	84.62	92.72	86.88	86.29
Crude protein	6.63	6.86	36.55	36.85	11.70	9.28
Ether extract	1.65	2.66	1.37	5.70	2.35	3.42
Crude fibre	30.66	16.74	21.96	6.55	2.62	3.84
Ash	6.34	5.21	7.14	4.89	1.96	1.47
Ca	0.38	0.41	0.41	0.12	0.07	0.06
P	0.09	0.11	1.04	0.97	0.33	0.24
MH- Meadow hay, SFM- Sunflower meal; DDGSw - Dried distillers' wheat grains with solubles.						

DDGSw' content of crude protein (*CP*) in our trial corresponded to that reported by other authors (*Kluth, 2010; Thacker, 2007*), but was lower than that

found by *Vilarino et al. (2007)* and *Dimova et al. (2009)*. Higher values of *CP* were found by *Oryschaк (2010); Slominski et al. (2010)*. Content of fats (*EE*) was twice as much in *DDGSw* as in *SFM*. Similar values were reported by *Kluth et al. (2010)* and *Thacker et al. (2007)*. On the contrary, significantly lower values were found by *Cozannet et al. (2009)*. Other reported higher values (*Cozannet et al., 2009*). The crude fiber (*CF*) values in *DDGSw* was twice lower in *SFM*, which corresponded with *Vilarino et al. (2007)*, but were lower than those found by *Kluth (2010)* and *Oryschaк (2010)*.

The chemical composition of total mixed ratios (*TMRs*) was similar in *DM* (2.1 kg) and ensured iso-caloric (on the average 2.3 *FUM* as net energy), iso-fibrogenous (on the average 0.6 kg *CF*) and equal in *PDI* (on the average 0.19 kg), *Ca* (0.017 kg) and *P* (0.009 kg). Balance of Protein in Rumen (*BPR*) was between - 0.001 and + 0.002 kg for *CD* and *ED*, respectively (table 3).

Intake. Average daily intake (*ADI*), dry matter intake (*DMI*) and consumption of nutrients from *TMRs* are summarized in figure 1. Sheep fed *ED* consumed higher levels of *TMRs* as fed basis (2.2 %), *DM* from forage (3.0 %), *DM* from concentrate mixture (5.4 %) and *DM* from *TMRs* (3.9 %). Thus, our results are in agreement with the reported DDGS-induced increase in feed consumption (as *DMI*). The higher values of average daily intake (*ADI*) of *CP* (33.2 %), Ash (6.4 %), Ca (22.1 %) and P (36.5 %) were found in *ED* compared to *CD*.

Table 3. Chemical composition and nutritive value of DDGSw- and SFM-based diets (g):

	SFM- based diet	**DDGSw- based diet**
		Chemical composition[1]
Dry matter	2134.69	2183.99
Crude protein	391.77	471.24
Ether extract	91.35	115.49
Crude fiber	626.18	590.98
Ash	184.40	186.80
Ca, %	15.71	17.60
P, %	7.87	9.70
		Nutritive value[2,3]
FUM[4]	2.37	2.29
PDI	179.37	195.54
BPR	- 2.19	+ 3.61
BPR/FUM	- 0.92	+ 1.58

[1] As DM basis (except DM); [2] Our own data (our unpublished data); [3] As fed basis; [4] According to Bulgarian feed evaluation system. PDI- Protein truly digestible in small intestines, BPR- Balance of protein in rumen, FUM- Feed units for milk as net energy.

The higher *EE* intake from *ED* (48.7 %) seem to be compensated by the lower consumption of fibres (5.5 %) and did not increase the net energy intake expressed as feed units for milk (2.2 %). Consumption of *PDI* was higher at *ED* (10.0 %) and balance of protein in rumen varied between *CD* (- 1.39) and *ED* (+ 3.23 %).

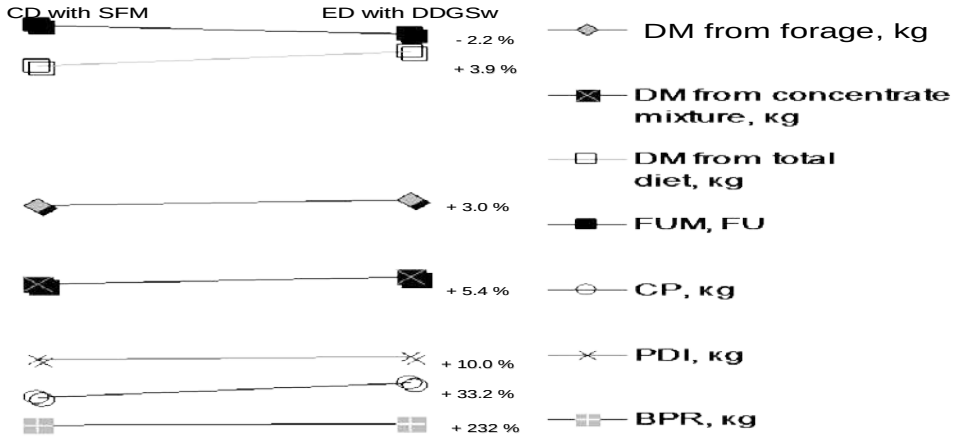

Figure 1. Average daily intake of forage, DM and nutrients

Animal performance. Ewe performance is shown in table 4. The *DDGSw* supplementation to the ewe diets decreased (5.8 %) significantly ($p<0.001$) the average daily milk yield for the studied segment of lactation curve (1.32 L (*CD*) to 1.24 L (*ED*)). The observed distance was higher (6.8 %) when milk yield was corrected to 6.5 % milk fat (*FCM*) as a difference between *ED* and *CD* ($p<0.001$).

Table 4. Yield, composition and technological parameters of sheep milk:

ITEMS / GROUP		SFM- based diet	DDGSw- based diet
			Productivity:
Average daily	Actual	1315.75 ± 196.90[a]	1239.90 ± 164.45[a]
milk yield, ml	6.5 % fat corrected	1437.44 ± 215.11[a]	1339.91 ± 177.71[a]
			Chemical parameters:
Solids non fats		10.93 ± 0.32	11.02 ± 0.24
Dry matter		18.03 ± 0.93	18.05 ± 0.49
Protein		5.65 ± 0.279	5.67 ± 0.199
Fat		7.10 ± 0.801	7.024 ± 0.540
			Ratios:
Protein /Fat		0.80 ± 0.05	0.81 ± 0.08
Protein /Dry matter		0.314 ± 0.005	0.314 ± 0.014
Fat /Dry matter		0.393 ± 0.024	0.389 ± 0.021

[aa] p<0.001.

Milk analyses. Milk composition is presented in figure 2. The differences between investigated milk parameters were not significant among the treatments. So, our data on the sheep milk content correspond to *Boikovski et al. (2006)* and *Djorbineva et al. (2002)*. Also, they're within the limits of dairy sheep and standards for *SBDP* breed (*Hinkovski et al., 1984; Nedelchev et al., 2003*).

Besides, we evaluated milk properties by physicochemical parameters, nutritive and technological ratios and indices in order to get more profound insight on its quality. Percentage of *SNF* and *DM* (fig. 2) among the groups was within the norms (10.93 – 11.02 and 18.03 – 18.05 %) and the differences were not significant (*CD << ED*). Similar values were reported by *Boikovski et al. (2005)*.

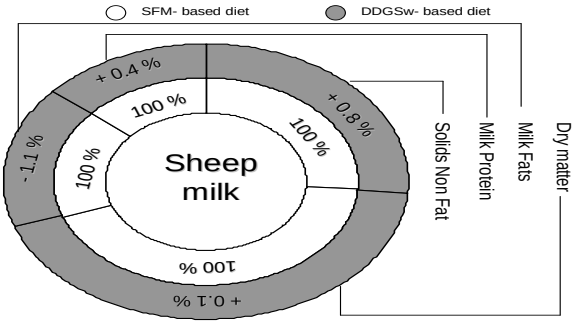

Figure 2. Chemical composition of sheep milk

However, some authors found lower values (*Stancheva, 2003*), while other reported higher (*Djorbineva et al., 2002*). The content of milk fat (fig. 2) showed downward tendency – ED << CD (1.1 %), but results were within the normal range and corresponded with the results found by *Stancheva (2003)*. Some found lower values (*Djorbineva et al., 2002*), and other – higher (*Stancheva et al., 2011*). Milk proteins were actually the same among the groups (5.66 %) and exceeded the values found by other authors (*Boikovski et al., 2005; Stancheva et al., 2011*).

The nutritive and technological parameters of raw sheep milk were characterized by the use of the following ratios (figure 3): *MP/MF, MP/DM and MF/DM*. All values were within the recommended standards (0.80, 0.31 and 0.39). The *MF/DM* and *MP/DM* values were lower at *ED* (1.0 and 0.1 %) than *CD* and corresponded to the values published by *Stancheva (2003)*, but were lower than values found by *Djorbineva et al. (2002)* and *Stancheva et al. (2011)*. The *MP/MF* ratio was higher at *ED* (1.3 %) than *CD* but the values were lower than those found by *Djorbineva et al. (2002)* and *Stancheva et al. (2011)*.

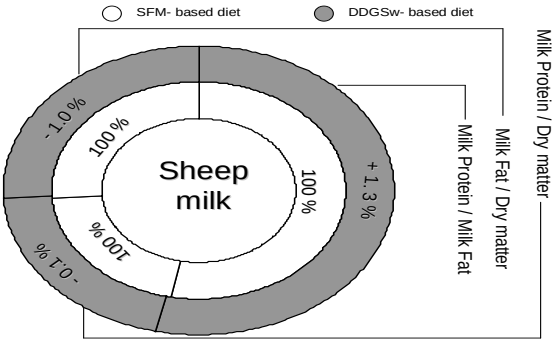

Figure 3. Nutritive and technological quality of sheep milk

Feed efficiency. The nutrients utilization and their biotransformation into milk production is summarized in fig. 4. The conversion of nutrients into 1 L milk production was less effective in animals consuming *DDGSw*- based diet, as compared with *CD*: *TMR* (9.7 %), *DM* (11.5 %), *CP* (14.1 %), *FUM* (4.4 %) and *PDI* (18.0 %). Feed efficiency, as presented by milk/ feed (*M/ F*) ratio, also was decreased (19.3 %) in *DDGSw*- based diet (fig. 4).

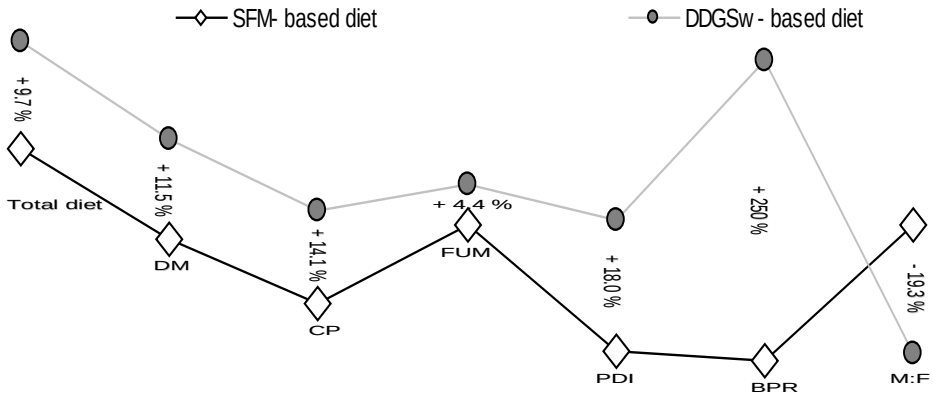

Figure 4. Feed conversion (g.L^{1}milk) and feed efficiency (as M/ F)

The nitrogen, as a limiting factor in high productive dairy animals was used to be established the effect of dietary protein source (*SFM* vs. *DDGSw*) on milk production (figure 5). So, animals fed with *CD* consumed (253 g) lower levels of dietary *CP* (as *N*), compared with *ED* (24.9 %). The percentage of *N* retained in milk rose in order *ED* << *CD* (- 5.4 %). Thus, the percentage of N utilization was higher in *SFM*- based diet (40.8 %) compared with *ED*.

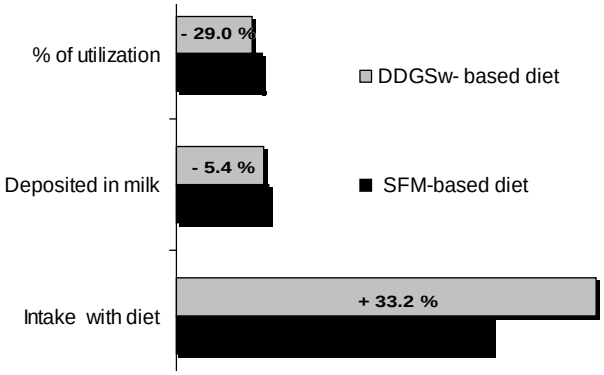

Figure 5. Nitrogen balance

The observed results of the present study indicate that DDGSw supplementation of lactating dairy sheep in early to peak of lactation, as protein source, decreases milk yield but has no effect on its composition, nutritive and technological qualities. Lower to moderate levels of supplementation should be tested (*Dimova et al., 2009*).

Conclusion

The results in our experimental conditions shows that:

- The data on the chemical composition of *DDGSw* were as follows: *DM* – 92.72 %; *CP* – 368.50 g/kg *DM*; *EE* – 57.00 g/kg *DM*; *CF* –65.50 g/kg *DM*; Ash – 48.90 g/kg; *Ca* – 0.12 g/kg *DM* and *P* – 0.97 g/kg *DM*;
- Sheep fed *ED* consumed higher levels of total diet as fed basis (2.2 %), *DM* from forage (3.0 %), *DM* from concentrate mixture (5.4 %) and *DM* from total diet (3.9 %), average daily intake (*ADI*) of *CP* (33.2 %), Ash (6.4 %), *Ca* (22.1 %) and *P* (36.5 %) as compared with *CD*. The higher *EE* intake from *ED* (48.7 %) compensated for the lower consumption of fibres (5.5 %) and didn't increase intake of feed units for milk (2.2 %). Consumption of *PDI* was higher in *ED* (10.0 %) and balance of protein in rumen varied between *CD* (1.39) and *ED* (3.23 %);
- Average daily milk yield for the studied segment of lactation curve was significantly ($p<0.001$) lower in *ED* < *CD* (5.8 %). The differences between treatments were significant and statistically proved as 6.5 % fat-corrected milk – *CD* >> *ED* ($p<0.001$);
- Differences between controlled physicochemical milk composition parameters (solids non fats (*SNF*), dry matter (*DM*), milk fat (*MF*) and

milk protein (*MP*)) and nutritive and technological parameters (*MP*/*MF*, *MP*/*DM*, *MF*/*DM*) were within the recommended range and were not affected by treatments;

- The conversion of nutrient ingredients into 1 L milk production was less effective in animals consuming *DDGSw*- based diet, relative to *CD*: Total diet (9.7 %), *DM* (11.5 %), *CP* (14.1 %), net energy as *FUM* (4.4 %), *PDI* (18.0 %).;
- The feed efficiency, presented as milk/feed (*M/F*) ratio was advantaged by the *SFM*- based diet (19.3 %).

So, we can conclude that higher (than recommended) DDGSw levels (17 and 44 % as fed total ration or concentrate mixture, respectively) in our experimental units affected animal response negatively. Thus, the ED decreased significantly milk yield, but did not affected milk composition, nutritive and technological parameters.

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Prinos, sastav, nutritivne i tehnološke vrednosti mleka ovaca hranjenih sušenom džibrom rastvorljivim materijama (DDGSv)

M. R. Yossifov

Rezime

Cilj ove studije je bio procena efekata korišćenja obroka dopunjenog različitim izvorima biljnih proteina (suncokretova sačma (SFM) vs. sušena džibra pšenice sa rastvorljivim materijama (DDGSw)) u ishrani mlečnih ovaca u vrhuncu laktaciji (27 - 72 dan). Eksperiment je izveden sa šesnaest mlečnih ovaca u laktaciji, bugarske mlečne sintetička populacija (BDSP) u 60-dnevnom hranidbena tretmanu (7-dnevni pripremni period + 45-dnevni ogledni period + 8-dnevni završni period). Životinje su nasumično dodeljene (po starosti, laktaciji, prinosu mleka, % mlečne masti, % proteina u mleku) u dva hranidbena tretmana: 1. Kontrolni tretman na

bazi SFM (CD), i 2. / DDGSw- zasnovan eksperimentalni obrok (ED). Obroci su bili izo-azotni, izo-fibrogenous, izo-kalorijski i jednaki u proteinima svarljivim u tankom crevu (PIO), kalcijumu (Ca) i fosforu (P). U poređenju sa CD-om, suplementacija DDGSw utiče na smanjenje ($p<0.001$) prosečnog dnevnog prinosa mleka (5,8%) i 6,5% mast-korigovanog mleka (6,8%). Tu su i značajne razlike u sastavu mleka između tretmana (CD i ED): nemasne čvrste materije (SNF) + 0,8; suva materijae (DM) + 0,1%; sadržaj mlečne masti (MF) - 1.1 i mlečnog proteina (MP) Sadržaj + 0,4% po ovaci za ED, u poređenju sa CD-om. Ishrana obrokom baziranim na DDGSw nije uticala na nutritivne i tehnološke parametre sirovog mleka: MP/MF (+ 1,3%), MP/DM (- 0,1%) i MF/DM (- 1,0%) odnosi. U zaključku, primetio podaci ukazuju da je DDGSw na nivou 17% suve materije uticao negativno na prinos mleka ovaca, bez uticaja na sastav mleka, nutritivne i tehnološke parametre.

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AVAILABLE CONTROL MEASURES FOR Q FEVER IN SHEEP

B.Vidić[1], S. Savić[1], S. Boboš[2], R.Radinović[2], N. Prica[1]

[1]Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republic of Serbia
[2]Agricultural faculty, University of Novi Sad, Department for veterinary medicine, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
Corresponding author: branka@niv.ns.ac.rs
Review papaer

Abstract: Q fever is a worldwide zoonosis caused by Gram-negative bacteria, *Coxiella burnetii*.This antropozoonosis is characterized by a wide spectrum of hosts and vectors. Primary role of domestic animals as reservoirs of human infections emphasizes the accurate prompt detection of Q fever in domestic animals.This microbe can survive for months and even years in dust or soil. Sanitation of endemic foci of Q fever is practically pretty close to impossible because of the high resistance of the causative agent, small infectious dose and various epidemiologies. Within the group of zooantroponoses, Q fever takes a leading position in the region of Vojvodina. This is why Q-fever is considered a specific problem of this province. Control programs against Q fever in sheep and cattle, most frequently recommend serological examination and vaccination of animals. In animals, the most effective vaccines are those composed of inactivated whole phase I bacteria. Bacterial shedding in placental tissue and milk was reduced in experimental infection or in natural C.burnetii infection of sheep and cows vaccinated by phase I vaccines. One of the recommended measures is excluding positive reactors from the flock followed by continuous monitoring and separation of seropositive animals. Milk from seropositive cows must be pasteurized. The aim of the vaccination after lambing season, is to prevent new infections until next lambing of young animals, which were not pregnant. Well-timed sequential vaccination before pregnancy reduces the risk of *C.burnetii* infection, highly prevents the abortions decreases the shedding rate of *C.burnetii* after the abortion.

Key words: Q-fever, sheep, epidemiology, control

Introduction

Q fever is a worldwide zoonosis caused by Gram-negative bacteria, *Coxiella burnetii.* This antropozoonosis is characterized by a wide spectrum of hosts and vectors (*Savić et al., 2013).* Animals such as cattle, sheep, and goats can

carry the Q fever microbe in tissues involved in birth--the uterus, placenta, and birth fluids. Infected animals also release the microbe in milk and manure. These particles are infected with a major route of infection for humans and animals. This microbe can survive for months and even years in dust or soil. *C. burnetii* is one of the most resistant of all non-sporulating bacteria. Sanitation of endemic foci of Q fever is practically pretty close to impossible because of the high resistance of the causative agent, small infectious dose and various epidemiologies. As regards disease transmission, agent inhalation in the dust is of much more importance than a vector-born disease spread by ticks.

Primary role of domestic animals as reservoirs of human infections emphasizes the accurate and prompt detection of Q fever in domestic animals (*Schliesser and Schmid, 1970; Biberstin et al., 1974; Rašeta and Mihajlović, 1983; Beaudeau, 2010*). Positive reactors are found in sheep, cattle, goats, swine, horses, poultry and cats (*Macellaro et al., 1993; Vidić et al., 1990; EFSA J., 2010*). From clinical point of view, Q-fever is not a negligible issue in veterinary medicine. The miscarriages have been registered primarily in sheep, cattle and goats, as well as some other reproductive disorders such as mastitis, poorly viable offspring, etc. (*Biberstin et al., 1974; Vidić et al., 1990b; Vidić et al., 1999; Boboš et.al., 201;*) which might lead to non-negligible economic losses infected herds (*Vidić et al., 2013b*).

Successful suppression and prevention of Q fever infections cannot be accomplished using common general preventive measures, and adequate specific prevention is not yet available worldwide. Control programs against Q fever in sheep and cattle, most frequently recommend serological examination and vaccination of animals (*Vidić et al., 1990*; *EFSA J., 2010*; *Hogerwerf et al., 2011*).

The main problem in Q fever prevention is the lack of adequate and specific protection measures as well as poor efficiency of general preventive measures. High rate of positive reactors among sheep, nomadic pastoralism and grazing system in sheep farming are major factors that negatively influence the epidemiological situation of Q-fever in Vojvodina (*Šeguljev et. al., 1988; Vidić et al., 1996).* Seroepizootiological investigation in Vojvodina revealed higher prevalence of Q fever in sheep than in cattle (*Šeguljev et. al., 1988; Vidić et al., 1996).* In Vojvodina Q fever persists in an endemic-epidemic form (*Šeguljev et al., 1997*).

Within the group of zooantroponoses, Q fever takes a leading position in the region of Vojvodina *(Šeguljev et al., 1993;Vidić et al., 1996).* This is why Q-fever is considered a specific problem of this province. Up to the beggining of 90s, Q-fever was a leading zoonosis in Vojvodina. Large epidemics of Q fever followed the line of nomadic sheep flocks movement. Since sheep are the main reservoir of the disease, Q fever demonstrated pronounced seasonal incidence with about 90% of affected patiens at the end of winter and beggining of the spring, during the lambing season (*Šeguljev et al., 1993*). Since1991, the number of

patients with Q fever has significantely decreased, therefore, during the last ten years, the average incidence is 0.82/100000 *(Vidić et al., 2012).* Q fever can now be seen as small family epidemics among owners of domestic animals and without pronounced seasonal character (*Vidić et al., 2013).*

Control measures

Successful suppression and prevention of Q fever infections cannot be accomplished using common general preventive measures, and adequate specific prevention is not yet available worldwide. High resistance of *C.burnetii* in the environment and various epidemiologies makes the sanitation of endemic foci almost impossible. In animals, the most effective vaccines are those composed of inactivated whole phase I bacteria. Bacterial shedding in placental tissue and milk was reduced in experimental infection or in natural *C.burnetii* infection of sheep and cows vaccinated by phase I vaccines (*Behumer et al., 1975; Brooks et al., 1986).* The phase I vaccine prevented abortion and reduced the shedding of C.burnetii, thereby reducing both environmental contamination and the risk of transmission to human (*Behumer et al., 1975; Vidić et al., 1990a; Arricau-Bouvery et al.,2005).*

Control programs against Q fever in sheep and cattle, most frequently recommend serological examination and vaccination of animals (*Guatteo et. al., 2008; Gidding et al., 2010; van der Hoek et al., 2010; Vidić et al., 2013a).* One of the recommended measures is excluding positive reactors from the flock followed by continuous monitoring and separation of seropositive animals. Milk from seropositive cows must be pasteurized.

Vaccination

In everyday practice, vaccination is recommended in infected herds and flocks; however, efficiency of different vaccination protocols has not yet been fully investigated, particularly with regard to the duration of vaccination program, animal categories to be vaccinated and time of vaccination. The latest research showed good results at the level of individual animal and at herd / flock level. Vaccination resulted in an apparent significant decrease in infection rate during the first years upon application of vaccination program, strongly suggesting the prolongation of this period. Reduction of clinical symptoms (abortions, infertility) is noticeable in the first year after vaccination; however, vaccination period of 3-4 years is required to stop and prevent shedding of bacteria. Efficiency of the application of all control measures including vaccination should be monitored using serology tests and PCR methods by systematic sampling of blood, milk, vaginal mucus and faeces. Vaccination offers a new conception of suppression and

eradication of this zoonozis, not only in a view of public health safety but also in creating Q fever free regions in endemic areas (*Behumer et al., 1975; EFSA J., 2010*).

At present, several vaccines for cattle and sheep are available in the market, such as bivalent vaccine *C.burnetti* and *Ch.psittacci* for sheep. Vaccine phase I *C.burnetii*, virulent one (encoding a complete LPS) demonstrated much higher efficiency than the phase II vaccine made of non-virulent strains (*Behumer et al., 1975; Arricau-Bouvery et.al., 2005*). Universal animal vaccination program is not feasible; vaccination practices are adjusted to the epidemiological situations in particular regions. Vaccination of dairy goats against Q fever with Coxevac was analysed in a study and it was shown that the percentage of animals in which bacteria were detected in uterine fluid, vaginal swabs, and milk was reduced. The biggest change was observed in prevalence in uterine fluid and in young animals. Vaccination may reduce environmental contamination, because shedding of bacteria is highest during parturition, abortion, and subsequent periods. This of course, contributes to reduction of risk for human exposure to Q fever (*Arricau-Bouvery et al., 2005.)*

There are other studies with similar results. *Guatteo et al., (2009*), performed a clinical trial and demonstrated that vaccine was effective in reduction of a chance for the appearance of bacterial shedding in animal, if it is given to uninfected animals before pregnancy. *Arricau-Bouvery et al. (2005)* showed that vaccination of 17 goats in a clinical trial decreased excretion of *C. burnetii*. On the other hand, *Rousset et al., (2008),* conducted a field study of a goat herd infected with *C. burnetii* where vaccination did not prevent shedding, but there was a reduction of bacterial load in vaginal swabs of primiparous animals. A definite association between vaccination and bacterial shedding is still to be looked into. It depends if the vaccination is done before first or subsequent pregnancy, or before, or after natural exposure.

With the aim of reducing the number of human cases of Q fever in some of the countries goats and sheep were vaccinated, and in other countries humans at risk are vaccinated against Q fever (*Hogerwerf et al., 2011)*. In France, cattle are vaccinated to prevent economic losses caused by abortions (*Rousset et al., 2009*). In any of these countries, not one human case of Q fever has been reported (*EFSA J., 2010*). After vaccination of the animals, it has been found that the total number of human cases of Q fever has dropped within one year, what can be related to the intervention measures. For sure there is a relationship between the shedding of causative agent, environmental contamination, and number of human cases, but further analysis is needed. It can be assumed that vaccination in dairy sheep and goats can lead to the lower shedding of *C. burnetii*. This could mean a lower risk for the human population.

In Australia, vaccination is applied as a preventive measure in sheep and humans potentially exposed to risk (*Gidding et al., 2010*). In Russian Federation,

vaccination is conducted in certain regions where Q fever occurs endemically or where infection foci are widespread in the nature. In France and Slovakia, depending on the epidemiological situation, vaccination is applied in cows and sheep (*EFSA J.,2010).*

Vaccination is a measure that prevents shedding of *Coxiella* and significantly reduces the risk of spreading Rickettsia, thus significantly reducing the risk of human infections (*Hogerwerf et al., 2011).* This does not necessarily mean that the infection will not occur in humans during the next year - this disease can be eliminated but not completely eradicated (*EFSA J., 2010*).The aim of the vaccination after lambing season, is to prevent new infections until next lambing of young animals, which were not pregnant. Well-timed sequential vaccination before pregnancy reduces the risk of *C.burnetii* infection, highly prevents the abortions decreases the shedding rate of *C.burnetii* after the abortion.

Antibiotic treatment

Antibiotic treatment is used effectively in humans to reduce clinical symptoms associated with Q fever. Antibiotic treatment was not demonstrated to be effective in preventing the shedding of bacteria in sheep and also it is not effective in influencing the epidemiology of infection in domestic ruminant populations (*Astobiza et al., 2010*). According to data from the literature, application of chemotherapy in infected animals was investigated. Administration of tetracycline to sheep, at a dose 8mg/1kg in drinking water, several weeks before lambing, was recommended as a possible prevention protocol (*Berri et al, 2005; Blain, 2007*).

Preventive measures

Q fever is an occupational concern for workers who have contact with animals, animal products, or animal waste. Immuno-compromised persons, pregnant women, and persons with cardiovascular problems (with evident valve failure) should avoid close contact with animals, particularly during the labouring season. Such individuals should seriously consider avoiding contact with sheep and goats, particularly during lambing or kidding time. Consult with your health care provider to determine if you are at high risk for contracting Q fever. Consuming pasteurized milk and use of pasteurized milk for dairy products (cheese) is highly recommended.

Furthermore, access to facilities or space at high risk of Q fever should be restricted, i.e. allowed only to vaccinated persons. Immunization of humans exposed to high rate of professional risk is a primary preventive step against Q fever (*EFSA J., 2010*). As an adverse effect of the vaccines, severe local reactions may occur in humans who were previously exposed to the infection or were

vaccinated – immune compromised persons. Having in mind the importance of this zoonosis, good communication on local and regional level and cooperation between human and veterinary medicine is indispensable.

Suppression of Q fever in domestic animals and prevention of environment contamination with *C.burnetii* are the most important measures in protection of humans from infection (*EFSA J., 2010*). Other measures that can significantly reduce the risk from infection include education of residents in rural regions about infection route, possible risks and precautions, education of farmers and other professionally exposed categories with an aim of establishing good agricultural practice. These actions can influence the reduction of risk of Q fever.

Good farm practice is always in use of reduction of human and animal health risks. Measures of self- protection and zoo hygienic measures are many that are incorporated into good farm practice. Personal hygiene during and after working with animals in addition with regular cleaning an disinfection during lambing or calving is one of the most important measures. Then, wearing protective clothing and shoes, using protective gloves when removing postpartum products and also with safe disposure of placenta, aborted and stillbirth animals are essential. Investigation of farm abortion and stillbirth outbreaks with determination of the causes of abortion and still birth and isolation of aborted animals until discharges cease is also important. Usage of additional equipment for self-protection (mask, goggles) when in contact with hazardous materials (after abortions, during Q fever epidemics, when cleaning the facilities for keeping sheep, goat and cattle) is essential when working on farms. One of the measures is certainly control of ticks, rodents and other parasites of livestock. The dust has to be reduced and quarantine used when purchasing new animals. Entrance has to be restricted for people and other animals (including dogs and cats) whenever possible.

Prevention of Q fever in animals is difficult, since infected animals may show no signs of infection with the organism. Isolation of any newly purchased animals from pregnant ewes or does is advised until all pregnant animals have birthed. Isolate any animals that abort from the remainder of the herd, and consult a veterinarian to discuss diagnostic testing. Dispose of bedding and equipment contaminated with tissues and fluids from an abortion in a sealed trash bag bury or burn. Individuals handling these materials should take protective precautions. Clean contaminated equipment and facility surfaces with soap and water and disinfect with a phenol disinfectant. More precautions for people are described below.

Experience from the Netherlands

The public was informed about the massive Q fever epidemic in humans Holland (*Karagiannis et al, 2009; EFSA J., 2010*). The outbreak begun in 2005,

and in the period 2007-2009, the number of patients in one region reached even 3523 persons. Investigation of epizootiological background of the epidemics revealed numerous abortions in goats in the region, which were identified as the source of infection. The following procedures were applied: all pregnant animals were culled from all infected farms; all animals were examined three times using serological ELISA; bulk samples of milk were examined two times applying PCR method; infected animals were eliminated; animals were vaccinated according to the age and herd size; access to farm was prohibited for visitors; use of milk was prohibited and appropriate biosafety measures were applied (*Karagiannis et al, 2009*). During this period, 35000 pregnant animals were killed, and costs were estimated to 6 million Euros. In the same period, Q-fever was identified in humans and animals in neighbouring countries - Germany and Belgium (*EFSA J., 2010*).

After the epidemic in Holland, data collected in Europe showed great variability with respect to laboratory diagnostic methods used and criteria for interpretation of the results depending on diagnostic goals (herd screening, identification of Rickettsia-shedding animal, epidemiological research or routine diagnostics). One of the conclusions of this Symposium was that professional knowledge is still insufficient, especially concerning epidemiology, identification of infection routes and potential reservoirs. Regular veterinary surveillance of the herd is indispensable for monitoring the infection and setting an accurate and timely diagnosis. Based on the current knowledge, a cornerstone of Q fever control is the vaccination of the animals with phase I *C.burnetii* vaccine.

The importance of particular domestic animals in the epidemiology of Q fever is differs among regions depending on their number, infection level, herd size, breeding system and zoo-hygiene. The epidemic course of Q fever Q fever disease is influenced by the range of factors such as airflow, rainfall, density of the population and geological characteristics of the terrain (*Šeguljev et al, 1997; Vidić et al. 2003*).

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Mere kontrole Q groznice kod ovaca

B.Vidić, S. Savić, Ž. Grgić, S. Boboš, R.Radinović, N. Prica

Rezime

Q groznica je oboljenje poznato širom sveta, a izaziva ga gram-negativna bakterija *Coxiella burnetii*. Ova antropozoonoza je poznata po širokoj paleti domaćina i vektora. Domaće životinje imaju primarnu ulogu kao rezervoari uzročnika i oboljenja za ljude i zbog toga je vrlo važna tačna i pravovremena dijagnostika Q groznice kod domacih životinja. Uzročnik može dapreživi više meseci, čak i godina u prašini ili u zemlji. Sanitacija endamskih žarišta Q groznice je praktično skoro nemoguća, zbog visoke rezistencije uzročnika, male infektivne doze i raznovrsnosti epidemiologije. U grupi antropozoonoza, Q groznca zauzima vodeću poziciju na regionu Vojvodine. Zbog toga se Q groznica smatra specifičnim problemom ove pokraine.

U programima kontrole Q groznice kod ovaca i goveda, uglavnom se preporučuju serološka ispitivanja životinja i vakcinacija. Najefikasnije vaccine kod životinja su one sa inaktivisanom fazom i bakterije. Izlučivanje bakterija u tkivo placente i mlekom je smanjeno prilikom eksperimentalne ili prirodne infekcije sa *C.burnetii* kod ovca i goveda vakcinisanih sa vakcinama koje sadrže fazu I. Jedna od preporučenih mera kontrole je isključivanje pozitivnih grla iz stada, kontinuirani monitoring i izdvajanje seropozitivnih životinja. Mleko seropozitivnih krava se mora pasterizovati. Cilj vakcinacije životinja nakon sezone jagnjenja je prevencija novih infekcija do sledećeg jagnjenja mladih životinja koje još nisu gravidne. Dobro planirananom vakcinacijom pre graviditeta se smanjuje rizik od infekcije sa *C.burnetii*, preveniraju se pobačaji i smanjuje izlučivanje *C.burnetii* nakon pobačaja.

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INFLUENCE OF DIFFERENT GROWING CONDITIONS ON PRODUCTION, MILK COMPOSITION AND BODY CONDITION SCORE FOR ALPINA GOAT BREED

N. Memiši[1*], N. Stanišić [2]

[1] AD Mlekara, Tolminska 10, 24000, Subotica, Republic of Serbia
[2] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
*Corresponding author: memisin@mlekara.rs
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Abstract: This paper presents the results of testing the impact of different farming systems on milk production, milk composition (milk fat, protein and dry matter without fat) and body condition score of Alpine breed goats in different growing systems during one production year. Control of the amount and chemical composition of milk included a total of 59 French Alpine goats at the age of 2-3 years (2-3 lactations), which are divided into two groups with approximate similar body weight. In the first group of goats a stable diet was applied. Goats had a sufficient amount of alfalfa hay available (ad libitum) and the addition of about 0.5 kg of concentrate that is administered twice a day. Goats in the second group in addition to 0.5 kg of alfalfa hay, received 0.25 kg of concentrate and in the period from April to October during the day stayed at the outlet and the surrounding pasture. Control of body weight of goats and body condition were performed once every two months from March to October, while the rate of body condition (BCS) was given score of 1-5. Somatic cell count and chemical quality of milk were controlled on a daily basis in the laboratory for raw milk AD Mlekara - Subotica on the device CombiFoss 6200 FC. Both groups of goats had a statistically significant increase in production of milk (about 45 l) and the average daily milk yield (of about 0.15 l) in the second compared with the third lactation ($p < 0.01$). It was also determined statistically significant effect of lactation on content of protein, dry matter without fat (DMwF) and the number of somatic cells in milk in both groups of goats. Body condition score of the analyzed groups of goats varied over time, and statistically significant differences were found in July ($p = 0.021$) and September ($p = 0.013$), where goats from the second group that remained at the pasture in the examined period had higher scores for BCS compared with the first group.

Key words: Alpina goat**,** nutrition, body condition score, somatic cell count, chemical composition of milk

Introduction

Milk from goats is of particular nutritional and economic importance in many parts of the world. Goat milk is primarily used for the production of traditional cheeses, yoghurt and ice-cream. The composition of milk is one of the major factors determining its value in the market. The nutritive value and technological properties of milk are largely influenced by its composition *(Morand-Fehr et al., 2007).* A number of animal (species, breed) or environmental (feeding regime, lactation stage, animal health and management) factors affect milk composition *(Chillard et al., 2003, Gorecki et al., 2004, Ataşoğlu et al., 2009).* Body condition is a very important factor in determining potential milk production. It is also a useful tool to help monitor adequate feeding and management.

Body condition score (BCS) is thus an estimation of muscle and fat development of an animal and is correlated with the direct measurement of backfat depth or the proportion of fat in the animal body providing a better estimate than body weight alone *(Sanson et al., 1993).* BCS is also a subjective way to evaluate the nutritional status of a flock and acts as a potential indicator for goat owners to increase the production efficiency in their flock *(Özder et al., 1995; Sejian et al., 2010).*

Goats with the score of body condition that is beyond the respective target ranges (from 0-2 and from 3.5-5) will produce less milk and meat with lower costs. Body condition score was successfully developed for dairy cattle, but applies equally to dairy goats (goats are graded on the scale from 1 to 5), and in fact are based on an estimate of the quantity of deposited fat. Today, there are many directions for feeding goats, which are based on NRC or similar official foreign tables for nutritional requirements and the composition of the meal (*NRC, 2007; AFRC, 1997; Sahli et al., 2004, Luo et al., 2004*). In combination with regular evaluation of body condition of goats in the growth and lactation, these tables for nutritional requirements should be adjusted upward or downward, in order to provide adequate nutrients under the given circumstances, with sufficient incentives to improve production and growth, or with sufficient tightness to prevent obesity and health risks (*Memiši and Žujović, 2010*). Such diet will correct the loss of production due to lack of nutrition and prevent the occurrence of the syndrome of fat goats (*Santucci et al., 1991*).

For this reason, the aim of this work is to show the influence of different farming systems (stable and stable-pasture) and nutrition on milk production, milk composition and body condition score of goats during lactation.

Materials and Methods

Description of the study location

Research was done on goat farm located in the vicinity of municipality of Subotica.

Experimental animals and treatments

Control of the yield, chemical composition of milk and somatic cells were covered by a total of 59 heads of French Alpine breed at age 2-3 years (2-3 lactations), which were divided into two groups, with an approximate similar body weight. In the first group of goats the stable diet was applied. Goats had free access to a sufficient amount of alfalfa hay (ad libitum) and the addition of about 0.5 kg of concentrate was administered twice during the day. Goats in the second group in addition to 0.5 kg of lucerne hay, received 0.25 kg of concentrate per day in the period from April to October while during the day they stayed at the outlet and the surrounding pasture. The botanical composition of the pasture was about two thirds of legumes (usually *Trifolium alestre, Trifolium montanum, Trifolium repens, Lotus corniculatus*, etc.). The blade of grass was very little represented (around one third - usually *Agrostis vulgaria, Festuca rubra, Alopecurus partenisis, Dactylis glomerata, Bromus moulgaria, Bromus mollis* etc.).The chemical composition of concentrate is shown in Table. 1. Calculation of nutritive value of concentrate mixture is made on the basis of recommendations by *Obračević (1990).*

Table 1. Chemical composition and nutritive parameters of concentrate mixture used in goat nutrition

F e e d	% in mixture
Corn	64,50
Wheat	12,00
Soybean meal (44%)	5,00
Sunflower meal (33%)	16,00
Di-calcium phosphate (16%P)	1,00
Premix	0,5
Salt, g	1,0
Nutritive Parameters	
Dry matter, % NEL MJ/kg CP/g	85.7 6,54 14,5

Animal feeding and management

Animals were given a two weeks adaptation period within which they were treated for internal parasites. External parasites were controlled through weekly spraying throughout the trial period using deltamethrin (decatix). Animals of second group were released to graze at 10:00 hours and returned in to pens by 17:00 hours. Concentrates were offered twice in a day; early in the morning before being released for grazing and in the evening at 17:00 hours after return from grazing. Free access was allowed to water and rock salt.

Milk production

The quantity of produced milk in all goats was determined on the 10th day after partus at the latest, all through to the end of lactation (dry off). Control of' milk production was performed on two occasions at equal time intervals (morning around 7 am and in the evening, about 7 pm), and in intervals of 28-32 days. The animals were in A control. Measuring the amount of milked milk was carried out in a graduated cylinder; with the lowest digit of 10 ml.

Milk samples (0.5 l/goat) for chemical composition were taken from each goat after mixing the yield from the evening and morning milking. Milk samples were collected in plastic containers and transferred to the laboratory immediately. They were kept in a refrigerator at 4 °C. The basic composition of milk samples was determined approximately 3-4 h after milking. Composition of milk (quantity of milk fat, proteins and fat free dry matter FFDM) was determined by the method of infra-red spectrophotometry using apparatus Milkoscan FT 6200, whereas the total somatic cell count (SCC) in milk was determined by fluoro-opto-electronic method on apparatus Fossomatic FC.

Body weight measurements

Initial body weights of goats were determined by two consecutive days of weighing and subsequent weights were taken every 60 days. All weights were taken before a day's morning offer of feeds. Average daily body weight gain was determined as a proportion of total weight change to the feeding period of 210 days. Body weight of goats and body condition scores were controlled once every two months starting from March until October, while the rate of body condition (BCS) was given grade of 1-5 (*Santucci et al., 1991*). Body condition score (BCS) was taken by an expert using a BCS scale of 1 (thin) to 5 (fat).

Statistical analysis

Statistical analysis of the data obtained was performed using Statistics 7 software (Statsoft, USA) on two factorial studies, where the first factor was group and the second lactation. Data are presented by groups (first and second groups of goats) and lactation (2 and 3 and the Total = Mean value of both lactation). From the statistical parameters shown is the mean value (X) and standard deviation (Sd).

Results and Discussion

Table 2 shows the mean value and variability of milk production, the contents of certain components in milk and the number of somatic cells in the experimental groups of goats.

Duration of lactation, milk production, average daily milk yield and milk composition did not differ significantly between the analysed groups of goats (Table 2.). Somatic cell count was slightly higher in the first than the second group, however, due to the high value of the standard deviation this is not a statistically significant difference between groups of goats in this parameter ($p = 0.063$).

Both groups of goats had significantly higher production of milk (about 45 liters) and average daily milk yield (about 0.15 l) in the second compared to the third lactation ($p < 0.01$). A statistically significant effect of lactation on protein content, dry matter without fat (DMwF) and somatic cell count of milk in both groups of goats. Milk fat content (MFC) was not significantly different ($p = 0.325$), although it was slightly higher in the third compared to the second lactation, in both groups of goats. For the parameters listed in Table 2., there were no significant interactions between groups of goats and lactation number ($p > 0.05$).

Table 2. Average values and variability of milk yield, chemical composition and somatic cell count

Variable/ Lactation in order		First group of goats		Second group of goats		group	lactation	group*lactation (interaction)
		X	Sd	X	Sd			
Duration of lactation	2.	241.40	20.27	254.50	14.45	0.241	0.074	0.225
	3.	258.00	19.40	257.77	12.86			
	Total	249.26	21.10	256.52	13.23			
Milk production	2.	337.44	25.23	354.10	18.37	0.650	0.000*	0.365
	3.	398.19	53.85	392.62	40.68			
	Total	366.22	50.78	377.94	38.45			
The average daily milk yield	2.	1.40	0.12	1.39	0.08	0.709	0.001*	0.912
	3.	1.54	0.12	1.52	0.11			
	Total	1.47	0.13	1.47	0.11			
Milk fat	2.	3.47	0.14	3.34	0.13	0.310	0.325	0.395
	3.	3.34	0.22	3.33	0.28			
	Total	3.41	0.19	3.33	0.23			
Protein	2.	3.02	0.10	2.98	0.12	0.463	0.001*	0.791
	3.	2.86	0.12	2.84	0.16			
	Total	2.95	0.13	2.89	0.15			
FFDM	2.	8.08	0.19	8.01	0.18	0.460	0.002*	0.641
	3.	7.86	0.12	7.85	0.18			
	Total	7.98	0.19	7.91	0.19			
SCC (000)	2.	1151.1	237.35	1017.2	169.52	0.063	0.013*	0.838
	3.	1370.7	262.80	1204.6	270.09			
	Total	1255.1	267.51	1133.2	250.04			

* ($p<0.01$); FFDM – fat free dry matter; SCC – somatic cell count

Compared to results obtained by other authors, duration of lactation in our research is similar to the level reported by *Gall (1980)* for duration of lactation in French Alpine breed from 200 to 300 days. Lower values were stated by *Pavliček et al., (2006)* in alpine goats reared in the private sector and whose lactation duration was from 201 to 203 days. Average daily milk yield determined in Alpine goats is lower than the values recorded in 30 goats (2.7 kg) of color improved breed (*Bernacka, 2006*), and Saanen goats (2.26 kg) in trials of Ataşoğlu et al. (2009). The total quantity of milk in both groups of goats is higher than the values measured in the two populations goats ((Montefalcone and Valfortorina goat) found by *Casamassima et al. (2007)* which amounted to 275 kg and 258 kg, throughout 180 days of lactation.

The average content of fat for the whole lactation was 3.38% and this value corresponded with data published by *Margetin and Milerski (2000)* but it was higher than that mentioned by *Zeng et al., (1997).* On the other hand, *Agnihotri et al., (2002)* found higher values of average fat content.

Milk protein and fat contents were lower to those found in the milk of Garganica, Maltese and Saanen goat *(AIA, 2005);* of color improved breed *(Bernacka, 2006);* Saanen and Alpine goats *(Marenjak et al., 2009).* The breed, level of milk production and stage of lactation, as well as reproductive cycle may influence the SCC in dairy goats *(Haenlein, 2002).*

Similar values in regard to somatic cell count in goat milk, depending on the order of lactation, were reported by *Raynal-Ljutovac et al., (2007). Kozačinski et al., (2002)* established in goat milk average SCC of $1.30x10^3$/ml and concluded that the limit for SCC in goat milk can be over $1.0x10^3$/ml, which is in accordance with results obtained in this study. Increased SCC in milk from dairy goat breeds reared in the USA is often, and above $1.0x10^3$/ml as stated by *Haenlein, (2002).* Similar values, even slightly higher for SCC, depending on the order of lactation are stated by *Pavliček et al., (2006)* in Alpine breed goats. The average number of SC ranged going from first to third lactation from 1.36 to $1.48x10^3$. Higher SCC in goat milk ($1.59x10^3$/ml) was also established by *Ying et al., (2002). Antunac et al., (1997)* stated that herds of dairy goats rarely have in the average milk sample SCC below one million.

Body condition score of the analyzed groups of goats varied over time, and statistically significant differences were found during July ($p = 0.021$) and September ($p = 0.013$), where goats from the second group had higher scores for BCS compared to the first (Table 3).

Table 3. Average values and variability of body weight and body condition of goats

Traits / Lactation in order		First group of goats		Second group of goats		group	lactation	group*lactation (interaction)
		X	Sd	X	Sd			
March BCS	2.	2.06	0.18	2.03	0.09	0.600	0.042*	0.199
	3.	1.90	0.15	1.99	0.16			
	Total	1.98	0.18	2.00	0.13			
March Body weight	2.	46.47	2.66	47.40	2.19	0.688	0.003*	0.601
	3.	50.12	3.23	50.00	3.71			
	Total	48.20	3.42	49.01	3.41			
May BCS	2.	2.16	0.17	2.14	0.05	0.181	0.023*	0.061
	3.	1.99	0.13	2.12	0.11			
	Total	2.08	0.17	2.13	0.09			
May Body weight	2.	48.69	2.97	49.49	2.53	0.221	0.113*	0.599
	3.	49.91	3.73	51.89	4.08			
	Total	49.27	3.31	50.98	3.69			
July BCS	2.	2.22	0.11	2.22	0.09	0.021*	0.181	0.023*
	3.	2.09	0.12	2.25	0.10			
	Total	2.16	0.13	2.24	0.10			
July Body weight	2.	49.69	2.80	50.58	3.76	0.247	0.058	0.737
	3.	51.40	3.42	53.00	3.23			
	Total	50.50	3.14	52.08	3.56			
September. BCS	2.	2.29	0.10	2.27	0.09	0.013*	0.131	0.003*
	3.	2.15	0.10	2.32	0.09			
	Total	2.22	0.12	2.30	0.09			
September. Body weight	2.	50.13	3.52	50.03	3.53	0.566	0.007*	0.509
	3.	52.77	3.60	54.25	4.02			
	Total	51.38	3.71	52.64	4.30			

* ($p<0.01$); (BCS) Body condition score

Body weight was not significantly different between groups of goats during the test period. Lactation had a significant effect on the difference in body condition in examined goats. Statistically significant differences were observed between the second and third lactation during months of March and May, while during the period September, lactation significantly affected only the body weight of the animals ($p = 0.007$).

The interaction of two factors analyzed (groups and lactation) is not significantly different in body weight of goats (Table 3.). For the BCS there was a statistically significant interaction of the first order for the month of July ($p = 0.023$) and September ($p = 0.003$), whereas during months of March and May, this trend has not been determined.

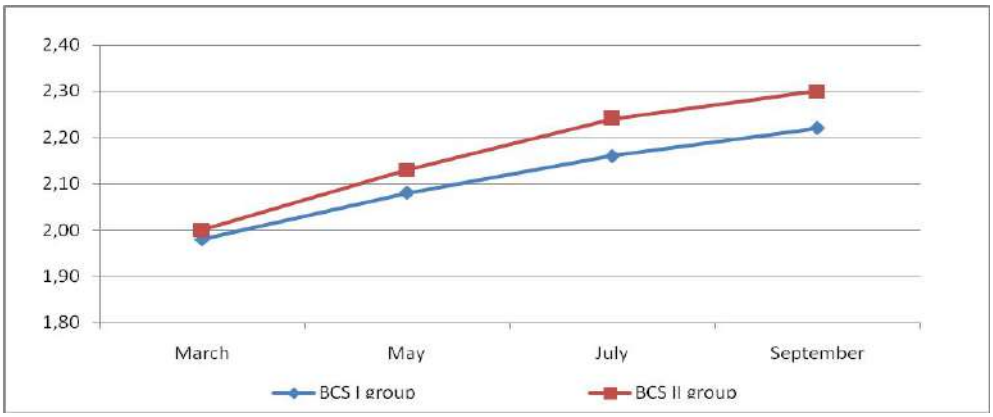

Figure 1. Changes in body condition during the lactating period in goat experimental groups

Ataşoğlu et al., (2009) have found in twenty one (2-4 years old) lactating Saanen goats grown in a semi-intensive system in which the nutrition of goats was based mainly on grazing on a woody and herbaceous pasture with the supplementation of mixed concentrate and vetch hay, body condition score differed (*P*=0.0021) among the sampling periods from April (2.64) to October (2.91). The authors note that body weight of goats was not affected (P = 0.1599) by sampling period. *Antunović et al. (2009)* found in the 30 French Alpine goats grown in the organic production during early lactation period (in the first 30 days) the BCS value to be 2.7 (from 2.2 to 3.5).

Conclusion

Based on test results for different cultivation systems on milk production, milk composition (milk fat content, protein and dry matter without fat) and body condition score of Alpine breed goats during one production year, it could be drawn the following conclusions:

- Length of lactation, milk production, average daily milk yield and milk composition did not differ significantly between the analyzed groups of goats ($p > 0.05$). The number of somatic cells in milk was slightly higher in the first group than the second, but without statistical significance. It was found a statistically significant effect of lactation on the content of protein, fat solids and somatic cell count of milk goats in both groups ($p < 0.01$).
- Body weight of the animals was not significantly different between the experimental groups of goats during the year. Lactation had a significant effect ($p < 0.01$) on difference in body condition of examined goats.

The interaction of two analyzed factors (groups and lactation) is not significantly different in body weight of goats. Body condition score of the analyzed group of goats varied over time, and statistically significant differences ($p < 0.01$) were found during July ($p = 0.021$) and September months ($p = 0.013$), where goats of the second group remained at the pasture, had higher BCS scores compared with the first group.

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Uticaj različitih sistema uzgoja na proizvodnju, sastav mleka i ocenu telesne kondicije koza alpina rase

N. Memiši, N. Stanišić

Rezime

U ovom radu prikazani su rezultati ispitivanja uticaja različitih sistema uzgoja na proizvodnju, sastav mleka (sadržaj mlečne masti, proteina i suve materije bez masti) i ocenu telesne kondicije koza rase Alpina u različitim sistemima uzgoja u toku jedne proizvodne godine. Kontrolom količine i hemijskog sastava mleka bilo je obuhvaćeno ukupno 59 koza francuske alpine u starosti 2-3 godine (2-3 laktacija), koje su podeljene u dve grupe sa približnom telesnom masom. Kod prve grupe koza primenjivan je stajski način ishrane. Koze su na raspolaganju imale dovoljnu količinu lucerkinog sena (ad libidum) kao i dodatak oko 0.5 kg koncentrata koji je davan u dva navrata u toku dana. Koze druge grupe su pored 0.5 kg lucerkinog sena, dobijale 0.25 kg koncentrata i u periodu od Aprila do Oktobra meseca su u toku dana boravile na ispustu i okolnom pašnjaku.

Kontrola telesne mase koza i ocena telesne kondicije kontrolisane su jednom u dva meseca počev od marta do oktobra meseca, pri čemu je pri oceni TK davana ocena od 1-5. Broj somatskih ćelija kao i hemijski kvalitet mleka, kontrolisan je svakodnevno u laboratoriji za sirovo mleko AD “Mlekare“ – Subotica na aparatu CombiFoss 6200 FC.

Obe grupe koza imale su statistički značajno veću proizvodnju mleka (za oko 45 l) i prosečnu dnevnu mlečnost (za oko 0.15 l) u drugoj u poređenju sa trećom laktacijom ($p<0.01$). Utvrđen je i statistički značajan uticaj laktacije na sadržaj proteina, suve materije bez masti (SMbM) i broja somatskih ćelija u mleku kod obe grupe koza. Ocena telesne kondicije analiziranih grupa koza varirala je tokom vremena, a statistički značajne razlike utvrđene su tokom jula ($p=0.021$) i septembra meseca ($p=0.013$), gde su koze druge grupe koje su u ispitivanom periodu boravile na pašnjaku, imale više ocene BCS u poređenju sa prvom.

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CLINICAL, HAEMATOBIOCHEMICAL AND RUMINAL CHANGES DURING THE ONSET AND RECOVERY OF INDUCED LACTIC ACIDOSIS IN SHEEP

M.M. Zein-Eldin, M.M. Ghanem, Y.M. Abd El-Raof, H.M. El-Attar, H.M.El-khaiat

Animal Medicine Department, Faculty of Veterinary Medicine, Benha University.
Corresponding author: E-mail: dr.mohamedmeselhy@yahoo.com.
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Abstract: A total number of five sheep were used in cross over design with an interval of three weeks for induction of lactic acidosis with sucrose, and treated with sodium bicarbonate as antacid, yeast as probiotics and gentian root powder as medicinal herbs. The acidoteic sheep showed significant ($P<0.05$) decrease in body temp, significant increase in respiratory rate, pulse rate and reduction of ruminal movement with depression, weakness, semisolid feces and stand with their head held lowered. There were significant changes in heamatobiochemical, ruminal parameters, these changes were more obvious at 24 hours after induction of acidosis. The clinical, heamatobiochemical, ruminal parameters of induced lactic acidosis were improved rapidly post-treatment with sodium bicarbonate and yeast, whereas these parameters showed slow improvement post treatment by gentian root powder. It was concluded that treatment of induced lactic acidosis in sheep by sodium bicarbonate and yeast give a good result and improve general health condition of the animal but it's preferable for treatment of lactic acidosis using a combination of both sodium bicarbonate and live yeast as sodium bicarbonate raise the ruminal pH rapidly and yeast stabilizes it. Treatment of lactic acidosis by oral administration of freshly grated gentian root showed slow improvement, so further investigation must be done before using gentian root alone in treating lactic acidosis.

Key words: gentian root, haemato-biochemical, lactic acidosis, sheep, sodium bicarbonate, yeast

Introduction

Acute ruminal acidosis is the most dramatic forms of ruminal microbial fermentative disorders and in some cases is lethal in less than 24 hours. The problem is more common when animals are grouped than when they are separate; probably because the psychology of competition induces them to over - consume

(Radostits et al., 2007). The severity of ruminal acidosis and disease signs vary considerably, depending on the amount and type of carbohydrate-rich feed consumed and the degree of prior ruminal microbial adaption to the carbohydrate substrate *(Gentile et al., 2004).* There are two major phases involved in the etiology of acidosis. The first phase - abrupt increase in the ingestion of readily fermentable carbohydtates accompanied by altered ruminal microbial population profile and subsequent accelerated ruminal fermentation to acids. The second phase - absorption of acids into the blood stream leading to systemic and metabolic acidosis *(Radostits et al., 2007).* Clinical signs of acidosis are manifested by dullness, depression, anorexia, slight dehydration, ruminal stasis and pasty to semi-fluid intermittent diarrhea in sheep. The abdomen was slightly distended and on palpation it was doughy in consistency. Moreover, there was tachycardia and polypnea *(Nikolov, 2003),* while peracute clinical signs which comprised severe dehydration with sunken eyes and the animals had no diarrhea but showed blindness, salivation, grinding of teeth (*Pulina, 2004*). Lactic acidosis was associated with heamatological changes such as significant elevation in erythrocytes, leukocytes and hemoglobin concentration and packed cell volume *(Garry, 2002),* also lactic acidosis associated with biochemical changes such as decreased total protein, hyperglycemia *(Brown et al., 2000***),** hyponatremia, hyperkalemia, hypocalcemia, increase AST, ALT activity *(Jorg and Enemark, 2008),* increase urea nitrogen, creatinine level and serum lactic acid *(Patra et al., 1996).* Treatment of clinical acidosis may be difficult and the chances of success depend on the severity of the case. Sodium bicarbonate is an important buffer of ruminal pH *(Ding and Xu, 2006).* Additives or products as sodium bicarbonate that buffer rumen pH may prevent acidosis and improve the productive performance of feedlot animals that consume high-grain diets *(Wallace and Newbold, 1993).* Addition of yeast culture to the basal diet may alleviate the effect of acidosis that normally resulted in the depression in feed intake as live yeast and other bacterial cell species adhere to feed particles to support ruminal fermentation *(Kawas et al., 2007).* The main modes of action of yeast include supplementation of growth factors to rumen microorganisms; oxygen scavenging that creates more favourable conditions for the anaerobic communities and nutritional competition with autochtonous ruminal species for energy (*James, 2011*). Gentian root infusion, administered orally to sheep at a daily dose of 5 g, before feeding, produced a stimulant effect on secretion of enzymes in the small intestine and used as bitter stomachics (*Wichtl, 2002).* Gentian is stated to possess bitter, gastric stimulant, sialogogue and cholagogue properties. Traditionally, it has been used for anorexia, atonic dyspepsia and gastrointestinal atony. The German Commission approved use for digestive disorders such as loss of appetite, fullness and flatulence *(Schulz et al., 2000).*

This study aimed to follow up the main clinical signs, haematobiochemical changes, and ruminal juice examination associated with induced lactic acidosis in sheep. A further objective was to evaluate the effectiveness of sodium bicarbonate,

yeast and gentian root powder in treatment of such problems to evaluate the best one for veterinary uses.

Materials and methods

Animals and study design:

Experimental animals:

Five healthy sheep of both sexes, aged from 9-12 months and weighting 30-35 kg were used in this study in a crossover design with an interval of three weeks. They were kept in clean disinfected pens, fed on green fodder and concentrate. All sheep were dewurmed with anthelmentic. They were left for 2 weeks for acclimatization before the beginning of the experiment. During this period they were subjected to a clinical investigation to be ensured healthy and free from any clinical abnormality.

Experimental design:

The first experiment*:*

An average dose of 18 gm/kg b. wt sucrose was estimated to produce the classical clinical picture of the lactic acidosis according to *(Afshin et al., 2011).* All sheep received sucrose after being fasted for 12 h. The sucrose was mixed with 200ml warm tap water, to make a suitable suspension, and was given using stomach tube in a single dose and after the appearance of clinical signs they were treated with oral sodium bicarbonate at a dose of 1g/ Kg. Bwt. at 24,48,72 hours and oral fluid therapy every 12 hours in a form of sacrolyte.

The second experiment:

Lactic acidosis was induced by giving 18 g/kg b. wt of sucrose and treated by 5 g/ head yeast dissolved in 50ml water and was given using stomach tube at 24,48, 72 hours and oral fluid therapy every 12 hours in a form of sacrolyte.

The third experiment:

Lactic acidosis was induced by giving 18 g/kg b. wt of sucrose and treated 5g/ head gentian root dissolved in 50ml water and was given using stomach tube at 24,48,72 hours and oral fluid therapy every 12 hours in a form of sacrolyte. All samples were collected at 0 hr immediately before induction of acidosis, 12hr after induction of acidosis, then treatment begins at 24 hours and samples were taken at 24, 48, 72 and 96 hr after treatment.

2-3- Blood and serum analysis:

Two blood samples were drained from the jugular vein. The first sample was taken with anticoagulant (EDTA) for determination of blood picture using hematology analyzer (RBCs count, Hb content, PCV%, WBCs and differential leucocytic count)**.** The second sample was collected without anticoagulant for biochemical determination of glucose**,** urea nitrogen, creatinine**,** calcium**,** sodium**,**

postassium**,** chloride**,** AST**,** ALT *(Young, 1990),* lactic acid, total protein *(Pagana and Pagana, 2010),* albumin *(Fischbach and Dunning, 2009).* Globulin was determined by the differences between total protein and albumin *(Chernecky and Berger 2008).*

Ruminal juice analysis:

The ruminal juice was collected from all animals by using a simple ordinary stomach tube connecting with a suction plastic syringe 50 ml capacity. These samples were sieved and strained through a 2 folds of sterile gauze and examined immediately to estimate ruminal pH, physical characters *(Radostits et al., 2007*), protozoal activity, motility and numbers (*Abd El-Raof et al., 2007*). Ruminal fluid was preserved for further investigation. Preservation was adopted by the addition of 10% sulphuric acid, then the sample stored at -20◦C till analyzed for lactic acid (*Lorenz et al., 2003*) and rumen ammonia- nitrogen concentration *(Novozamsky et al., 1974*).

Statistical analysis:

The data were statically analysed by two-way analysis of variance (ANOVA) with Dunnet's as a post-hoc test as previously described (*Bailey, 2008*) using SPSS software (Ver. 16). Values (means±S.E.) were considered significantly different from control healthy when $P \leq 0.05$.

Results and Discussion

The clinical examination:

The common clinical signs appeared on the control group were normal appetite, shiny coat, shiny eyes, their tail were fatty and normal defecation in form of small hard pellets. Body temperature, respiratory rate, pulse rate and ruminal movement were within normal range as in (Table 1). Mucous membranes were light rosy red in color. The clinical examination of sheep after induction of lactic acidosis revealed that clinical signs started in sheep within few hours after administration of sucrose the affected sheep showed decrease feed intake, depression, weakness, semisolid feces and stand with their head held lowered. There was increase in pulse, respiratory rates, decrease in ruminal movement and the abdomen was slightly distended. The visible mucous membranes were light rosy red color. At the disease progresses, the classical signs of ruminal acidosis were observed at 12-24 hours after administration of sucrose, the affected sheep appeared dull, inactive and depressed. Pulse and respiratory rate increased while ruminal movements completely absent. Affected sheep showed diarrhea, dyspnea, in coordination and recumbency. Clinical symptoms were returned to the normal after treatment with sodium bicarbonate more rapidly than that treated with yeast and than that treated with gentian root as in (Table 1).

Table 1: Results of clinical examination in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Temp	39.13± 0.03 $^{3\,a}$	38.63± 0.12 $^{1\,a}$	39.13± 0.12 $^{2\,a}$	39.13± 0.03 $^{3\,a}$	38.63± 0.12 $^{2\,a}$	38.93± 0.12 $^{1\,2\,3\,a}$	39.13± 0.03 $^{2\,a}$	38.63± 0.12 $^{1\,a}$	38.9± 0.17 $^{1\,2\,a}$
Pulse rate /min	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	79.33± 1.52 $^{1\,a}$	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	80.35± 1.2 $^{1\,a}$	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	81.33± 1.76 $^{1\,a}$
Resp/min	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	25.66± 0.33 ^{1a}	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	28.66± 0.33 ^{2b}	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	29.66± 0.66 ^{2c}
Rumen mov/2 min	3.00± 0.31 ^{4a}	0.20± 0.20 ^{1a}	2.60± 0.40 ^{4a}	3.00± 0.31 ^{3a}	0.20± 0.20 ^{1a}	2.80± 0.37 ^{3ab}	3.00± 0.31 ^{3a}	0.20± 0.20 ^{1a}	2.80± 0.20 ^{3ab}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

Hematological examination:

There was a highly significant increase in Hb content, PCV%, and non significant increase in WBCs, lymphocyte, granulocyte and monocyte count while RBCs count was within the normal range, theses changes were more obvious at 24 hours, the hematological picture returned to the normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with gentian root but returned more rapidly after treatment by sodium bicarbonate as in (Table 2).

Table 2. Haematological picture in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Hb	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	10.76± 0.46 ^{1a}	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	11.16± 0.58 ^{1ab}	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	11.76± 0.08 ^{2b}
PCV %	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	29.03± 0.88 ^{1a}	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	29.13± 0.74 ^{1a}	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	30.03± 0.17 $^{1\,2b}$
RBCs. Count	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	11.39± 1.20 ^{1a}	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	11.71± 1.08 ^{1a}	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	12.36± 1.15 ^{1b}
WBCs count	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.65± 1.24 ^{1a}	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.72± 1.39 ^{1a}	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.85± 1.56 ^{1a}
Granuolcyte count	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	3.98± 0.62 ^{1a}	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	4.01± 0.67 ^{1a}	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	4.08± 0.72 ^{1a}
Lymphocyt count	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.14± 0.58 ^{1a}	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.16± 0.67 ^{1a}	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.18± 0.80 ^{1a}
Monocyte count	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.52± 0.05 ^{1a}	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.52± 0.06 ^{1a}	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.53± 0.08 ^{1a}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

The serum biochemical analysis:

There was a highly significant increase in serum levels of glucose, total protein, globulin, potassium, urea nitrogen, creatinine, ALT activity and lactic acid, while albumin level was within the normal range. There was a highly significant decrease in serum levels of sodium, chloride and calcium, while there was a non significant increase in the serum levels of AST activity, theses changes were more obvious at 24 hours. Serum biochemical changes returned to normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with Gentian root but returned more rapidly after treatment by sodium bicarbonate as in (Table, 3).

Table 3. Mean values of selected serum biochemical parameters in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Glucose (mg/ dL)	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	70.55± 1.41 ^{1a}	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	71.16± 1.08 ^{1a}	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	74.48± 1.17 ^{2b}
Total protein (gm/dL)	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.27± 0.09 $^{1\ 2a}$	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.31± 0.15 $^{1\ 2a}$	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.44± 0.12 ^{2ab}
Albumin (gm/dL)	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.28± 0.03 ^{1a}	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.26± 0.08 ^{1a}	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.19± 0.05 ^{1a}
Globulin (gm/dL)	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	3.99± 0.15 ^{12a}	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	4.04± 0.13 ^{2a}	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	4.25± 0.15 ^{2ab}
Sodium (mmol/L)	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	147.28± 2.04 ^{3c}	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	146.42± 1.44 $^{3\ 4b}$	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	143.84± 2.05 $^{2\ 3a}$
Chloride (mmol/L)	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	97.50± 1.33 $^{2\ 3b}$	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	96.80± 0.80 $^{2\ 3ab}$	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	95.53± 1.47 $^{2\ 3a}$
Potassium (mmol/L)	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	4.68± 0.16 ^{1a}	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	4.48± 0.10 ^{1a}	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	5.11± 0.12 $^{1\ 2ab}$
Calcium (mg/dL)	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	10.17± 0.14 ^{4ab}	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	10.03± 0.14 ^{3ab}	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	9.66± 0.16 $^{3\ 4a}$
Urea nitrogen (mg/dl)	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	34.44± 0.42 ^{1a}	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	35.37± 0.48 ^{1ab}	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	36.02± 0.71 $^{1\ 2b}$
Creatinine (mg/dl)	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.08± 0.04 ^{1a}	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.09± 0.06 ^{1a}	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.14± 0.04 ^{2a}
AST (I.U/L)	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	43.41± 2.47 ^{1a}	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	44.10± 2.18 ^{1a}	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	45.57± 1.82 $^{1\ 2\ b}$
ALT (I.U/L)	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	21.64± 0.96 ^{1a}	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	20.25± 0.95 ^{1a}	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	24.38± 0.1.21 ^{12b}
Lactic acid mmol/L	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	1.65± 0.04 ^{1a}	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	1.82± 0.07 ^{1a}	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	2.25± 0.14 ^{2b}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

Ruminal juice examination:

Colour, odour and consistency of ruminal juice were changed after induction of lactic acidosis while, sedimentation activity time showed a highly significant increased after induction of lactic acidosis. These changes were more obvious at 24 hours. There was a highly significant decrease in ruminal pH and ammonia level while, there was a highly significant increase in ruminal lactic acid

level after induction of acidosis. Microscopic examination of ruminal juice revealed that presence of few numbers of live protozoa and their number showed a highly significant decrease in sheep after induction of lactic acidosis. These changes returned to normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with gentian root but returned more rapidly after treatment by sodium bicarbonate.

Table 4. Examination of ruminal juice in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	**24h Post Induction**	**72h Post Treatment**	**0h**	**24h Post Induction**	**72h Post Treatment**	**0h**	**24h Post Induction**	**72h Post Treatment**
Color	Olive green	yellowish	Olive green	Olive green	yellowish	Olive green	Olive green	yellowish	Olive green
Odor	Aromatic	Soured	Aromatic	Aromatic	Soured	Aromatic	Aromatic	Soured	Aromatic
Consistency	viscous	watery	viscous	viscous	watery	viscous	viscous	watery	viscous
S.A.T	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	31.00 ± 1.15^{1a}	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	33.66 ± 1.45^{12b}	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	36.33 ± 1.45^{2c}
pH	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.66 ± 0.08^{4a}	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.63 ± 0.03^{4a}	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.50 ± 0.11^{3a}
Activity of ruminal protozoa	+++	---	+++	+++	---	+++	+++	---	+++
Protozoal count×10^5/ml	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	3.83 ± 0.16^{34a}	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	4.00 ± 0.28^{4ab}	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	3.66 ± 0.16^{3a}
ammonia	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	58.34 ± 1.58^{4c}	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	57.32± 1.764b	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	50.91± 1.72 3 4a
Lactic acid	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.75± 0.012a	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.71± 0.012a	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.81± 0.023 4a

Means with different superscript letters in the same raw are significantly different at $P\leq 0.05$.

Clinical examination of sheep following oral administration of sucrose in dose of 18 gm/kg bwt according to *(Afshin et al., 2011)* revealed that all animals showed signs of illness within 12-24 h., All these disturbances can be attributed to changes in the pH of the rumen under the effect of excessive lactic acid production, histamine, methanol and its action on the vital organs and nerve centres (*Radostits et al., 2007*). Clinical examination of the sheep after treatment by sodium bicarbonate and yeast revealed that animal began to feed 24h after treatment when pH began to increased, the results were in coincidence with (*Ding and Xu, 2006*)). On the other hand animal begin feeds 96h after treatment by freshly grated gentian root due to its stomachic properties as follows, promotion of saliva secretion, acceleration, inhibition of gastric juice secretion, promotion of viscous liquid secretion, bile secretion and enhancement of stomach motility (*Kohlein, 1991*).

Induced ruminal acidosis led to change in blood constituents due to systemic dehydration and degree of heamo concentration (*Radostits et al., 2007*). The haematological picture returned to the normal after treatment with the sodium bicarbonate, yeast and freshly grated gentian root but returned more rapidly after treatment by sodium bicarbonate due to correction of dehydration. The highly significant increase in serum levels of glucose after induction of lactic acidosis may be due to the fact that the absorbed lactic acid is used for the process of gluconeogenesis (*Garry, 2002*), while the significant increase in serum total protein and globulin at 24h after induction of lactic acidosis may be attributed to dehydration due to passage of water from the intravascular compartment into the rumen (*Brown et al., 2000*) and production of immunoglobulins (*Lomborg et al., 2008*) respectively. Decrease in serum sodium and chloride accompanied with ruminal lactic acidosis may be due to the shift of these electrolytes by osmolarity from the blood to hypertonic rumen or due to their losses (Na^+ and Cl^-) due to diarrhea (*Jorg and Enemark, 2008*). He also added that hyperkalemia may be attributed to heamoconcentration which occurred to the constituent of the blood due to dehydration, while hypocalcemia may be due to a temporary malabsorption of calcium due to damaged mucosa of intestine (*Radostits et al., 2007*). The significant increase in serum urea and creatinine are an index of decreased glomerular filtration rate in acidotic sheep, theses due to renal damage or reduction in effective renal flow and fall in arterial blood pressure which results in subnormal renal function as recorded by *(Lal et al., 1992*). Increased activity of ALT reflects hepatocellular damage which may be sublethal degeneration or necrosis, whereas non-significant rise in AST may be due to hepatocellular damage or released from degenerated skeletal muscles *(Kromer and Hoffman, 1997).* The concentration of lactic acid in serum and rumen was found to be directly related to each other, the excessive production of lactic acid in the rumen, less rapid metabolism and clearance causing its gradual accumulation and reach its peak level in the blood (*Ivany et al., 2002*). After treatment with alkalizing buffer sodium bicarbonate, lactic acid decreased significantly and more rapid than in animals treated with yeast and freshly grated gentian root. The changes in physical properties of ruminal juice and the prolonged period which was taken for complete the sedimentation activity test were attributed by (*Garry, 2002*) to poor microbial fermentation in the rumen. Physical properties of ruminal juice after treatment was improved and the SAT test take shorter time than before treatment this may attributed to decrease the level of lactic acid by alkalizing agent "sodium bicarbonate" and by activation of lactic acid utilizing bacteria leading the pH to increased and refreshment of microflora by yeast and gentian root (*Giger- Reverdin et al., 2004*). Fall in rumen pH is associated with increased production of lactic acid in the rumen due to increase the fermentation of starch by amylolytic bacteria in the rumino-reticular compartment (*Ding et al., 1997*). Also (*Owens et al., 1998*) recorded that pH drops because of the high rates of production and accumulation of TVFAs and lactic acid. When the

rumen pH is low, microbial diversity is reduced, as protozoa numbers may sharply decline and the bacterial population is altered (32). These indicate the inverse relationship between lactic acid concentration and PH as recorded by (*Martin et al., 2006*). The significant decreased of ruminal level of ammonia in sheep after induction of lactic acidosis were attributed by (*Henning et al., 2010*) due to death of microflora and microfuna in the rumen. Ruminal pH returned to the normal after treatment as sodium bicarbonate is considered alkalinizing agent or its neutralizing effect and yeast was efficient at stabilizing ruminal pH (James, 2011). Live yeast was efficient at stabilizing ruminal pH by stimulating ciliated protozoa, which are known to rapidly engulf starch granules and compete effectively with amylolytic bacteria for substrate *(Bach et al., 2007*). Moreover, ciliated protozoa are also able to take up some of the lactic acid and thus may prevent its accumulation in the rumen. Therefore, an increase in viable microbial cell numbers in the rumen promoted by live yeast supplementation may minimize the increase in ruminal concentrations of volatile fatty acids thereby avoiding a decrease in ruminal pH (*Giger- Reverdin et al., 2004*). Regarding lactic acid is decreased after treatment than in case of acidotic sheep due to the elevating effect of yeast on ruminal pH and lactic acid may be reduced due to reduced lactate concentrations in the rumen (*Williams and Coleman, 1997*), through the increase of activity of lactate-utilizing bacteria such and/or the decrease of activity of lactate producing bacteria (*Martin and Nisbet 1992*). Ammonia concentration was increased after treatment with sodium bicarbonate and yeast, in a study with adult ruminants, a similar effect on ammonia concentration occurred with daily yeast feeding (*Kumar et al., 1994*). They also suggested that some changes in the nitrogen metabolism of rumen microorganisms in the presence of yeast. Death of microflora may be due to decrease of ruminal pH and increase level of lactic acid as microflora accustoms the life in neutral media 6.2-7.2 (Steen, 2001). Microbial population was increased after treatment in all groups these results were similar to that obtained by (Chaucheyras- Durand *et al.,* 2008) and this may be due to increase of pH and decrease lactic acid, restore the normal ruminal function and the stomachic effect of gentian root (*Wichtl, 2002*).

Kliničke, hematobiohemijske i promene u rumenu kod nastanka i oporavak od indukovane mlečne acidoze kod ovaca

M.M. Zein-Eldin, M.M. Ghanem, Y.M. Abd El-Raof, H.M. El-Attar, H.M.El-khaiat

Rezime

Ukupno pet ovaca su korišćene u ogledu sa intervalom od tri nedelje za indukciju mlečne acidoze sa saharozom, i terapijom sa natrijum bikarbonatom kao antacidom, kvasacom kao probiotikom i prahom korena lincure kao lekovitog bilja. Acidotične ovce su pokazala značajno (P<0,05) smanjenje telesne temperature, značajno povećanje disanja, pulsa i smanjenje kretanja rumena sa depresijom, slabošću, polučvrsti izmet i držanjem oborene glave. Bilo je značajne promene u hematobiohemijskim parametrima, parametarima buraga, ove promene su bile očiglednije 24 sati po indukciji acidoze. Klinički, hematobiohemijski i parametari buraga kod indukovane mlečne acidoze su poboljšani ubrzo nakon tretmana sa natrijum-bikarbonatom i kvascom. Isti parametri su pokazali spor napredak makon tretmana sa korenom lincure u prahu. Zaključeno je da tretman indukovane mlečne acidoze ovaca sa natrijum-bikarbonatom i kvascem daju dobar rezultat i poboljšavaju opšte zdravstveno stanje životinje, ali poželjno je za lečenje mlečne acidoze koristiti kombinaciju natrijum-bikarbonata i kvasca, pošto natrijum-bikarbonat utiče na brzo povećanje pH buraga a kvasac ga stabilizuje. Tretman mlečne acidoze oralnom primenom sveže rendanog korena lincure pokazao je spor napredak, tako da dalje istraživanje mora da se uradi pre upotrebe ovog korena u lečenju mlečne acidoze.

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COMPARITIVE STUDY ON THE EFFICACY OF ELISA AND *IS900* PCR FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN GOATS

A. Abraham[1], N. Thomas[1], S. Joseph[2], K.C. Raghavan[1]

[1]Department of Animal Breeding, Genetics and Biostatistics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651, Kerala, India
[2]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651, Kerala, India
Corresponding author: asha05vet@gmail.com
Original scientific paper

Abstract: Paratuberculosis, one of the chronic granulomatous enteritis that predominantly affects ruminants worldwide, is caused by *Mycobacterium avium subsp. paratuberculosis* (MAP). It is most efficiently diagnosed by MAP from faeces by Polymerase Chain Reaction (PCR). Serological tests like Enzyme Linked Immuno Sorbent Assay (ELISA) also provides a rapid and cost-effective alternative diagnostic tool. Present study was carried out to directly evaluate the sensitivity and specificity of ELISA (ID vet innovative diagnostics; France) using *IS900* PCR as a gold standard. Serum and faecal samples were collected from 180 adult goats of either sex, from Malappuram and Thrissur districts of Kerala with unknown paratuberculosis status. Faecal samples were processed for direct *IS900* PCR and serum samples were tested for MAP antibodies using Indirect ELISA kit. *IS900* PCR detected 38 out of 180 confirmed to be shedding MAP. ELISA detected 22 out of 180 animals as positive. Overall, ELISA was 50 % sensitive and 97.9 % specific in comparison to *IS900* PCR. The *IS900* PCR outperformed ELISA in detecting animals potentially infected with MAP and is more sensitive than ELISA at detecting animals suspected of paratuberculosis. But, for early diagnosis of paratuberculosis in goats, ELISA can be done as easy and rapid farm level identification and *IS900* PCR as individual confirmatory test.

Key words: Paratuberculosis, goat, ELISA, *IS900* PCR, *Mycobacterium avium subsp. Paratuberculosis*

Introduction

Paratuberculosis or Johne's disease is considered to be one of the most serious, contagious, bacterial diseases of ruminants such as cattle, sheep, and goats. The disease is caused by *Mycobacterium avium subsp. paratuberculosis* (MAP)

that has also been implicated by many in the causation of human Crohn's disease. The disease is characterized by diarrhoea, rapid weight loss, reduced milk production, reproductive failure, and death in farm animals. Animals with paratuberculosis tend to waste away despite of a healthy appetite. Infections with MAP in caprine herds result in significant economic loss, through slow progressive wasting and the subsequent death of the infected animals *(Vidic et al., 2013).*

The ability to detect MAP accurately and rapidly is an integral part of herd management. However, detection and control of this bacterium is complicated due to its slow division time and its ability to persist in the environment. Although, MAP doesn't propagate in the environment, it survives for long period in different environmental conditions *(Raizmann et al., 2004).* Enzyme Linked Immunosorbent Assay (ELISA) is used to screen the herds for paratuberculosis. But, positive cases are confirmed by *IS900* PCR *(Vidic et al., 2010, 2011).* Present study was carried out to directly evaluate the sensitivity and specificity of ELISA (ID vet innovative diagnostics; France) in adult goats using *IS900* PCR as a gold standard.

Materials and Methods

Serum and Faecal samples were collected from 180 adult goats of either sex, from Malappuram and Thrissur districts of Kerala.

About 5 ml of blood was collected from jugular vein of 180 animals and serum was separated. It was then centrifuged at 2500 rpm for 10 minutes and stored at -20°C till use. Faecal samples are collected by rectal pinch method. Deoxyribonucleic acid (DNA) was isolated from faecal sample as per *Braunstein et al. (1993),* with some modifications. *IS900* is an insertion sequence or small mobile genetic element of MAP containing genes related to transposition. PCR of *IS900* was performed as per *Halldorsdottir et al. (2002)* with minor modifications. The final concentrations of PCR reagents used for the amplification of *IS900* gene were 10 pmol for primers, 1.5 mM for $MgCl_2$, 0.2 mM for dNTPs and 0.75 units for *Taq* DNA polymerase. Annealing temperature of 55 ^{0}C for 25 seconds was required for the amplification of expected product of 279 bp. PCR products were electrophoresed in 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet (UV) illuminator and photographed with gel documentation system.

ELISA kit developed by ID vet innovative diagnostics; France was used for this study. OD values measured at 450nm. For each sample and controls, corrected optical density (OD) was calculated.

$$\text{Corrected OD} = \text{OD}_{\text{even column}} - \text{OD}_{\text{odd column}}$$

Corrected OD values were then transformed to S/P ratio.

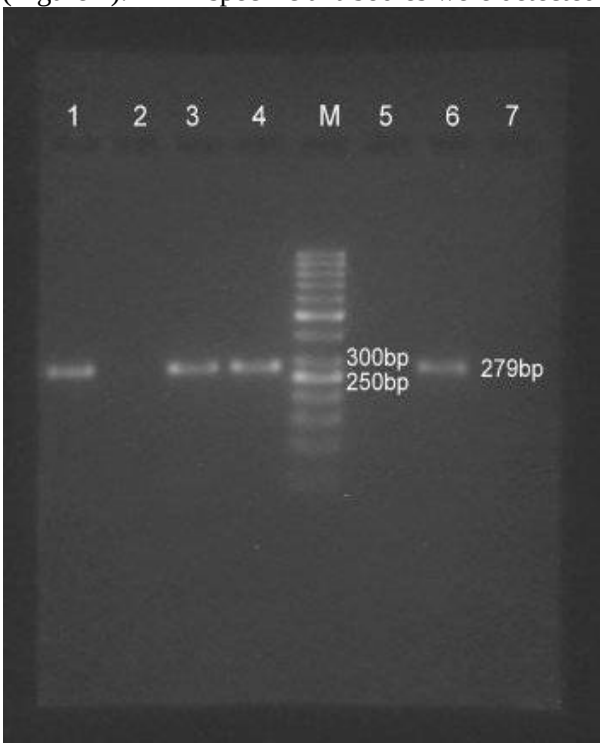
$$S/P = \frac{OD_{corrected\ sample}}{OD_{corrected\ positive\ control}}$$

Serum samples with S/P ratio above 60% were considered as positive for Paratuberculosis.

Detection of sensitivity, specificity and Chi-square values were estimated by statistical tests. *(www.statpages.org)*

Results and Discussion

Among the 180 goats subjected to study, *IS900* PCR yielded amplified products of an expected size of 279 bp, in 38 samples, suggestive of MAP infection (Figure 1). MAP-specific antibodies were detected by ELISA in 22 goats.



(Figure 1.)

Lane 1 - Positive control Lane 3, 4, 6 - MAP Positive samples
Lane 2 - Negative control Lane, 5, 7 - MAP Negative samples
M - 50 bp Marker

The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of MAP – ELISA when compared to *IS900* PCR were calculated and found to be 50%, 97.9%, 86.9% and 88% respectively, at 99% confidence intervals (Table 1).

Table 1.Evaluation of MAP – ELISA as compared to *IS900* PCR

			PCR			χ^2	***p*-value**	**C.I**
		Positive	Negative	Total	Uncorrected	64.078	0.000	
	Positive	19	3	22	Yates Corrected	59.692	0.000	
ELISA	Negative	19	139	158	Mantel-Haenzel	63.722	0.000	
	Total	39	142	180	Kappa	0.566	0.000	
					Sensitivity	50%		99%
					Specificity	97.9%		99%
					PPV	86.9%		99%
					NPV	88%		99%

The Chi-square and Mantel Haenzel test values shows that the results of ELISA and *IS900* PCR are statistically significant. The effect of Yates' correction is to prevent overestimation of statistical significance for small data. This formula is chiefly used when at least one cell of the table has an expected count smaller than 5. This reduces the chi-squared value obtained and thus increases its p-value. The kappa test can be used to measure the level of agreement beyond that which may be obtained by chance. Here, kappa value is above 0.4, which indicates a moderately good agreement between ELISA and *IS900* PCR for diagnosis of caprine paratuberculosis.

The ROC methodology provides an opportunity of identifying an optimum reporting cut-off value by identifying the point on the curve at which the sum of sensitivity and specificity is maximized *(Zweig and Campbell, 1993)*. An ROC curve is a graphical representation of the sensitivity (true positive rate (TPR)) as the y coordinates versus 1−Specificity (the true negative rate (TNR)) as the x coordinates *(Park et al., 2004)* of a diagnostic test across a variety of possible test thresholds. A good model performance is characterised by a curve that maximizes the sensitivity for low values of 1−Specificity, where the ROC curve passes close to the upper left corner of the plot *(Robertson et al., 1983; Schulzer, 1994)*. Here, the ROC curve for checking the sensitivity and specificity of ELISA and *IS900* PCR keeping *IS900* PCR as the gold standard test, give sensitivity 50% and 1-specificity 0.021% for ELISA (Figure 2).

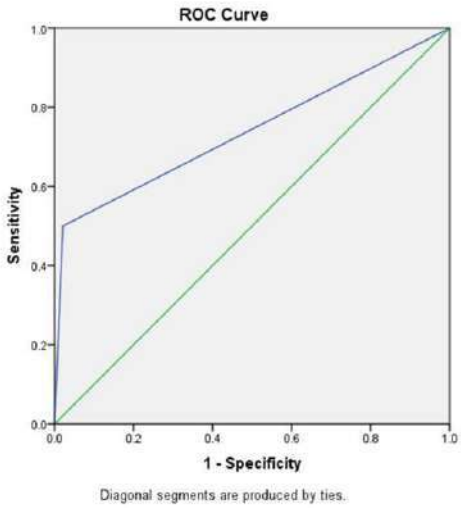


Figure 2. ROC curve for checking the sensitivity and specificity of ELISA and *IS900* PCR keeping *IS900* PCR as the gold standard test

The area under the curve (AUC) is a global (i.e. based on all possible cut-off values) summary statistic of diagnostic accuracy *(Greiner et al., 2000)*. The test gives AUC value of 0.739, which indicates moderately good performance than random chance. As a general rule of thumb, a test with at least 95% specificity and 75% sensitivity used best to rule in a disease *(Pfeiffer, 1998)*. Here, the ELISA test gives only 50% sensitivity, since MAP antibodies may not be detectable until late in infection.

The chronic nature of MAP infections requires prolonged therapy, with multiple drug regimens of low toxicity. Suitable drugs are therefore expensive, making the treatment of MAP infections economically unfeasible. As therapeutic measures proved inefficient, identification of sub-clinically infected animals and their eradication form the basis of treatment and control.

Conclusion

Eradication of paratuberculosis is hampered by the lack of accurate and sensitive diagnostic methods. The sub-clinically infected animals are difficult to identify by serological tests like ELISA, since animals do not produce measurable amount of antibodies until late stages of infection. Polymerase Chain Reaction is an accurate and reliable method for detecting paratuberculosis and can be used to identify samples that are culture negative and can detect fentogram amount of DNA *(Huntley et al., 2005).*

Results of present study revealed that *IS900* PCR was superior to ELISA for early diagnosis of paratuberculosis in goats, and serological tests like ELISA provides a rapid and easy alternative for herd level diagnosis. Hence, from the observations made in this study, it is concluded that ELISA can be done for farm level identification of paratuberculosis and after this, individual confirmation for paratuberculosis can be done with *IS900* PCR performed on faeces. Adoption of this strategy for early detection of paratuberculosis in goats will help to make effective culling and controlling of the disease.

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Uporedna studija efikasnosti ELISA i IS900 PCR u dijagnozi paratuberkuloze u koza

A. Abraham, N. Thomas, S. Joseph, K.C. Raghavan

Rezime

Paratuberkuloza, hronični granulomatozni enteritis koji pretežno pogađa preživare širom sveta, je uzrokovana *Micobacterium avium subsp. paratuberculosis* (MAP). Najefikasnije se dijagnostikuje MAP iz fekalija korišćenjem polimeraza lančane reakcije (PCR). Serološki testovi kao npr. enzimski imunološki sorbent analiza - Enzyme Linked Immuno Sorbent Assay (ELISA) takođe obezbeđuje brz i ekonomičan alternativni dijagnostički alat. Studija je sprovedena kako bi se direktno ocenila senzitivnost i specifičnost ELISA (ID vet innovative diagnostics; Francuska) koristeći IS900 PCR kao zlatni standard. Serum i fekalni uzorci prikupljeni od 180 odraslih koza oba pola, iz okruga Malappuram i Thrissur, Kerala, sa nepoznatim statusom paratuberkuloze. Fekalni uzorci su obrađeni za direktnu IS900 PCR a uzorci seruma su testirani na MAP antitela, koristeći indirektni ELISA kit. IS900 PCR je otkrila 38 od 180 potvrdio da rasturaju MAP. ELISA je otkrila 22 od 180 životinja kao pozitivne. Generalno, ELISA je bila 50% osetljiva i 97.9% specifična u odnosu na IS900 PCR. IS900 PCR je nadmašila ELISA u otkrivanju potencijalno MAP zaraženih životinja i osetljivija od ELISA na otkrivanju životinja za koje se sumnja da imaju paratuberkulozu. Ali, za rano

otkrivanje paratuberkuloze u koza, ELISA može da se uradi kao laka i brza, nivou farme, identifikacija a IS900 PCR kao individualni potvrdni test.

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EFFECTS OF DIETARY GARLIC ADDITION ON PRODUCTIVE PERFORMANCE AND BLOOD LIPID PROFILE OF BROILER CHICKENS

N. Puvača[1], Lj. Kostadinović[2], D. Ljubojević[3], D. Lukač[1], S. Popović[2], B. Dokmanovć[1], V. S Stanaćev[1]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Institute for Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[3]Scientific Institute of Veterinary Medicine Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia
Corresponding author: nikola.puvaca@stocarstvo.edu.rs
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Abstract: The aim of this study was to investigate the effect of dietary garlic powder addition on productive performance and blood lipid status of broiler chicken. At the beginning of experiment, three treatments of 150 one day old broiler chickens of hybrid line Hubbard per treatment, on a total of 450 chickens were formed. Every treatment was divided in four groups which represents four replicates of the experiment. Control treatment (T1) was fed with mixtures without addition of garlic powder, while experimental treatments were fed with addition of 0.5% (T2) and 1.0% (T3) of dietary garlic powder, respectively. Experiment lasted 42 days. After the completion of experimental period the highest achieved body weight of chicken was at treatment T2 (2371.1g) which was followed by treatment T3 (2336.1 g) with statistically significant differences ($p<0.05$) compared to control treatment. For the entire experimental period, feed conversion ratio was lowest in treatment T2 (1.8 kg/kg) and the highest in control treatment T1 (2.1 kg/kg), without statistically significant ($p>0.05$) differences. Addition of garlic powder led to a statistically significant ($p<0.05$) increase in values of EBI in compare to a control treatment T1. The highest mortality rate (5.1 %) and the lowest EBI (220.4 %) were recorded in control treatment. Addition of garlic powder in the amount of 1.0% (T3) significantly ($p<0.05$) decreased LDL concentrations in blood serum. The lowest concentration of total cholesterol was recorded at treatment T2 ($p<0.05$). The highest concentration of HDL (44.8 and 39.6 mg/dl) was recorded in treatments T3 and T2. It could be concluded that the addition of garlic has positive influence on chicken production and blood lipid status, but the further investigation of their mode of action is still necessary.

Key words: garlic, lipids, cholesterol, nutrition, broilers

Introduction

Drug resistance in bacteria and the drug residues in meat are important reasons for restriction and ban of in-feed antibiotics (*Demir et al., 2003*). Attempts were made to find other alternatives dietary supplements to overcome the poor performance and the increase susceptibility to diseases resulted from removal of antibiotics from poultry nutrition. Use of growth promoters of natural origin become of an interest in lasts decades (*Iji et al., 2001*). Garlic (*Allium sativum* L.) have bioactive substances like sulphur compounds such as alliin, diallylsulfides and allicin, that act as antibacterial, antifungal, antiphrastic, antiviral, antioxidant and antithrombotic.

Garlic has been found to lower serum and tissue cholesterol levels (*Issa and Omar, 2012; Stanaćev et al., 2012),* inhibit bacterial growth (*Cavallito et al., 1994*), inhibit platelet growth and reduce oxidative stress. In broilers, it was reported that garlic, as a natural feed additive, improved broiler growth and feed conversion ratio, and decreased mortality rate (*Tollba and Hassan, 2003; Puvača et al., 2014*).

Improvement of broilers performance, blood lipid profile and tissues can be achieved by supplementation of diets with garlic powder (*Amagase et al., 2001; Demir et al., 2003; Stanaćev et al., 2011*). It was reported that feeding garlic powder at levels of 1.5, 3 and 4.5% had no effect on poultry performance (*Konjufca et al., 1997*), but caused a significant reduction in poultry serum and liver cholesterol. However, *Horton et al.* (1991) concluded that triglyceride was not affected by dietary garlic addition in broiler chickens nutrition. Tissues cholesterol levels were decreased with feeding garlic powder to broiler chickens (*Stanaćev et al., 2012)* which showed that addition of garlic to broilers diet has effects on chicken performance and lipid profile. In research of *Puvača et al.* (2014), addition of various spice herbs and their mixture such as garlic, black pepper and hot red pepper led to an improved performance results and reduced mortality rate of chicken fed with addition of these spice herbs. Using garlic powder in broilers diet in research of *Horton et al.* (1991) had no significant effect on performance but had positive influence on meat quality and carcass yield. However, garlic effects on broiler performance and blood lipid status is still not enough researched and need further research investigation.

The aim of this study was to investigate the effect of dietary garlic powder addition on productive performance and blood lipid profile of broiler chicken.

Materials and Methods

Biological tests were carried out under production conditions at the experimental farm "Pustara" in property of the Faculty of Agriculture, Department of

Animal Science in Novi Sad. At the beginning of experiment, three treatments of 150 one day old broiler chickens of hybrid line Hubbard per treatment on a total of 450 chickens were formed. Every treatment was divided in four groups which represents four replicates of the experiment. For broilers nutrition three mixtures were used, starter, grower and finisher with 22, 20 and 18% of crude protein content, respectively. The first 14 days, during the preparatory period, chicks were fed with starter mixture. Following the preparation period, chicks were fed the next 21 days with grower mixtures, and then the last 7 days of fattening period with finisher mixtures according the experimental design given in Table 1. During the experiment, which lasted 42 days, chicks were fed and watered ad libitum, and microclimate conditions were regularly monitored. Chickens were on the floor holding system and control of body weight and feed consumption was performed weekly, every seven days.

Table 1. Experimental design

Experimental treatmens		Concetration of additives in chicken diets		
		In starter, %	In grower, %	In finisher, %
		1 – 14 days	15 – 35 days	36 – 42 days
T1	Control treatment	0.0	0.0	0.0
T2	Garlic powder	0.0	0.5	0.5
T3	Garlic powder	0.0	1.0	1.0

The European broiler index (EBI) was calculated for the entire feeding period according to the equation (Koreleski et al., 2010):

$$EBI = \frac{\text{average body weight (kg)} \times \text{survival rate (\%)}}{\text{age (days)} \times \text{feed convesrion ratio (kg feed/kg body weight gain)}} \times 100$$

At the end of 6th week, twelve birds were randomly chosen from each treatment and bled via wing vein puncture to obtain blood samples. Serum samples from blood were separated by centrifugation (4000 rpm for 5 min at 20°C). Commercially available kits (Randox Laboratories Limited - United Kingdom) were used to analyse the serum for triglycerides, total cholesterol, HDL and LDL on a biochemical autoanalyzer Cobas Mira Plus (Roche Diagnostics). Values were expressed as mg/dl. Statistical analyses were conducted using the statistical package program Statistica 12. Significant effects were further explored using analysis of variance (ANOVA), LSM and Fisher's LSD post-hoc multiple range test to ascertain differences among treatment means. A significance level of $p<0.05$ was used.

Results and Discussion

Based on the obtained results it can be noted that the addition of garlic in the diet of broiler chickens led to a statistically significant ($p>0.05$) differences in body weight. From the preparatory period chickens have exit with uniform body weight with no statistical significant differences ($p>0.05$).

Table 2. Body weight of chickens, g

Experimental treatments		Age of chickens						
		1 day	7 days	14 days	21 days	28 days	35 days	42 days
T1	LSM	42.8[a]	162.7[a]	388.6[a]	785.6[b]	1162.4[b]	1643.8[b]	2075.8[b]
	SE_{LSM}	0.47	1.52	3.64	8.38	11.84	12.2	24.23
T2	LSM	42.1[a]	160.2[a]	389.7[a]	818.5[a]	1202.3[a]	1743.1[a]	2371.1[a]
	SE_{LSM}	0.47	1.63	3.84	8.41	11.8	12.16	23.96
T3	LSM	42.2[a]	159.7[a]	386.4[a]	804.6[ab]	1204.9[a]	1737.2[a]	2336.1[a]
	SE_{LSM}	0.47	1.64	3.79	8.5	11.75	11.94	23.43

Treatments with different letter indexes in the same column are statistically significantly different ($p<0.05$)

At the end of the third week, chickens in treatment T2 achieved the highest body weight (818.5 g) with statistically significant differences ($p<0.05$) compared to the treatment T1. The same tendency was observed at the end of fourth week where the highest body masses was recorded in chickens with dietary addition of 0.5 and 1.0 g/100g of garlic powder (1202.3 and 1204.9 g) with statistically significant differences ($p<0.05$) compared with T1. After the completion of experimental period the highest achieved body weight of chicken was at treatment T2 (2371.1g) which was followed by treatment T3 (2336.1 g) with statistically significant differences ($p<0.05$) compared to control treatment.

Table 3. Chicken feed conversion ratio, kg/kg

Experimental treatments		Periods of nutrition			
		Starter phase	Grower phase	Finisher phase	Entire period
		1 – 14 days	15 – 35 days	36 – 42 days	1 – 42 days
T1	LSM	1.3[a]	1.8[a]	3.0[a]	2.1[a]
	SE_{LSM}	0.01	0.05	0.14	0.15
T2	LSM	1.4[a]	1.7[a]	2.3[b]	1.8[a]
	SE_{LSM}	0.01	0.05	0.14	0.15
T3	LSM	1.4[a]	1.8[a]	2.5[b]	1.9[a]
	SE_{LSM}	0.01	0.05	0.14	0.15

Treatments with different letter indexes in the same column are statistically significantly different ($p<0.05$)

The feed conversion ratio is given in table 3. In preparation period of chicken feed conversion ratio was uniform and ranged between 1.3 and 1.4 kg of feed per kg of gain, without statistically significant ($p>0.05$) differences. In the grower phase the lowest achieved feed conversion ratio was in treatment T2 (1.7 kg/kg). Feed conversion ratio in finisher phase was highest in control treatment T1

(3.0 kg/kg) with statistically significant ($p<0.05$) differences compare to the treatments T1 and T2. For the entire experimental period, feed conversion ratio was lowest in treatments T2 (1.8 kg/kg) and the highest in control treatment T1 (2.1 kg/kg), without statistically significant ($p>0.05$) differences. Lowest feed conversion ratio in treatment T2 shows that addition of garlic in amount of 0.5% had positive influence on feed utilization and efficiency.

Table 4 gives overview in European broiler index (EBI) and chicken mortality rate. Addition of garlic powder led to a statistically significant ($p<0.05$) increase in values of EBI in compare to a control treatment T1. The highest mortality rate (5.1 %) and the lowest EBI (220.4 %) were recorded in control treatment.

Table 4. European broiler index and chicken mortality, %

Experimental treatments		EBI	Mortality
T1	LSM	220.4^{c}	5.1^{a}
	SE_{LSM}	2.77	0.96
T2	LSM	295.1^{a}	3.2^{b}
	SE_{LSM}	2.77	0.96
T3	LSM	283.7^{b}	1.3^{c}
	SE_{LSM}	2.77	0.96

Treatments with different letter indexes in the same column are statistically significantly different ($p<0.05$)

The highest recorded EBI value was 295.1 % in treatment T2 with significant ($p<0.05$) differences with control T1 and T3 experimental treatment. Addition of garlic powder as feed additive to a broiler chicken nutrition led to a high improvement of blood lipid profile. From the results given in Table 5 can be noticed that the highest amount of triglycerides (65.9 mg/dl), total cholesterol (97.2 mg/dl) and LDL (36.7 mg/dl) was in treatment T1 with statistically significant ($p<0.05$) differences in compare with experimental treatments. Addition of garlic powder in the amount of 1.0% (T3) significantly ($p<0.05$) decreased LDL concentrations in blood serum. The lowest concentration of total cholesterol was recorded at treatment T2 ($p<0.05$). The highest concentration of HDL (44.8 and 39.6 mg/dl) was recorded in treatments T3 and T2.

Table 5. Biochemical blood parameters and lipid profile, mg/dl

Experimental treatments		Triglycerides	Total cholesterol	HDL	LDL	non HDL	HDL/LDL
T1	LSM	65.9^{a}	97.2^{a}	19.2^{c}	36.7^{a}	78.0^{a}	0.5^{c}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T2	LSM	19.3^{c}	54.1^{b}	39.6^{b}	5.8^{b}	14.5^{b}	7.7^{b}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33
T3	LSM	22.4^{b}	55.7^{b}	44.8^{a}	0.9^{c}	10.9^{c}	48.9^{a}
	SE_{LSM}	0.8	0.9	1.16	1.01	1.03	2.33

Treatments with different letter indexes in the same column are statistically significantly different ($p<0.05$)

The significant effect of garlic powder on the mean values of HDL compared to control treatment can be explained by the hypocholesterolaemic mechanism and the hypolipidemic action of garlic powder. The compound allicin combines with the -SH group that is important in activation of Acetyl CoA which is essential for the biosynthesis of cholesterol. Results of this study indicate the significant ($p<0.05$) increase of HDL by addition of garlic powder. Both levels of garlic powder decreased LDL levels compared to the level in chickens of the control treatment. This effect can be explained by the possible mechanism of antioxidant and antiperoxide lowering action on LDL or the decrease in hepatic production of very low density lipoprotein (VLDL) which serves as the precursor of LDL in the blood circulation (*Kim et al., 2009*).

Conclusion

Based on the obtained results, it can be concluded that the addition of garlic powder in broiler chicken nutrition has positive effect on production performances and blood lipid status. Addition of garlic in amount of 0.5% has led to highest final body weight, lower feed conversion ratio and higher feed utilization, with the highest percentage of European broiler index. Also in conclusion, significantly lowering of plasma cholesterol, triglycerides, LDL and increase of HDL in broiler diet indicate that garlic is very effective in regulation of lipid metabolism in a favourable manner with the aim for prevention of atherosclerosis or coronary heart diseases of people who consumed meat products from this type reared chickens. Therefore the general conclusion would be that the addition of garlic has positive influence on chicken production and blood lipid status, but the further investigation of their mode of action is still necessary.

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Efekti belog luka na proizvodne performanse i lipidni status krvi brojlerskih pilića

N. Puvača, Lj. Kostadinović, D. Ljubojević, D. Lukač, S. Popović, B. Dokmanović, V. S. Stanaćev

Rezime

Cilj ovog rada je bio da se ispita efekat belog luka u prahu u ishrani brojlerskih pilića na proizvodne performanse i lipidni status krvi. Na početku tova formirana su tri tretmana sa po 150 jednodnevnih pilića linijskog hibrida Hubbard po jednom tretmanu, sa ukupno 450 pilića u eksperimentu. Svaki tretman je podeljen u četiri grupe koje su predstavljale četiri ponavljanja ogleda. Pilići na kontrolnom tretmanu (T1) su hranjeni smešama bez dodatka belog luka, dok je u eksperimentalnim smešama za ishranu pilića bilo uključeno dve koncentracije belog luka 0,5% (T2) i 1,0% (T3). Eksperiment je trajao 42 dana. Na kraju eksperimentalnog perioda najveća zabeležena telesna masa je bila kod pilića na tretmanu T2 (2371,1 g) a potom na tretmanu T3 (2336,1 g) sa statistički značajnim razlikama ($p<0,05$) u poređenju sa kontrolnim tretmanom. Najmanja konverzija hrane za ceo eksperimentalni period zabeležena je na tretmanu T2 (1,8 kg/kg), a najveća na tretmanu T1 (2,1 kg/kg), bez statistički značajnih ($p>0,05$) razlika. Uvođenje belog luka u ishranu pilića je dovelo do statistički značajnih razlika ($p<0,05$) u povećanju vrednosti EBI u poređenju sa kontrolnim tretmanom T1. Najveći stepen mortaliteta (5,1 %) i najmanji EBI (220,4 %) je zabeležen kod pilića kontrolnog tretmana. Beli luk u prahu u koncentraciji od 1,0% (T3) je značajno uticao ($p<0,05$) na smanjenje sadržaja LDL u krvnom serumu. Najmanje koncentracije ukupnog holesterola u krvi pilića su zabeležene na tretmanu T2 ($p<0,05$). Najveće koncentracije HDL (44,8 i 39,6 mg/dl) su zabeležene na eksperimentalnim tretmanima T3 i T2. Na osnovu dobijenih rezultata može se zaključiti da je dodatak belog luka u prahu ispoljio pozitivan uticaj na proizvodne performanse pilića i lipidni status krvi, ali su dalja ispitivanja njegovih mehanizama delovanja još uvek neophodna.

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EFFECTS OF DIETARY SOYBEAN, FLAXSEED AND RAPESEED OIL ADDITION ON BROILERS MEAT QUALITY

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

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]Institute for Animal Husbandry, Auto put 16, 11070 Beograd-Zemun, Serbia
[3]VinFeed d.o.o., Neimarova 28, 21000 Novi Sad, Serbia
Corresponding author: vidica.stanacev@stocarstvo.edu.rs
Original scientific paper

Abstract: The aim of this paper is was to investigate the effects of soybean oil, flax and rapeseed oil on the body weight, fatty acid composition of lipids and sensory characteristics of chicken breast meat. At the beginning, six groups with 40 one day old chicks Cobb 500 hybrid line, with five replications was formed. Chickens were fed with three mixtures of 21, 20 and 18% protein, respectively. The experiment lasted 42 days. The use of different types of oils in the diet did not exhibited statistically significant ($P>0.05$) differences in body weight of chickens. The control group achieved final body weight of 2704 g and 2695 g, and the experimental groups in a row 2735, 2645, 2735 and 2670g. The use of flax oil and rapeseed oil changes the fatty acid composition of lipids. Replacing rapeseed with soybean oil reduces the percentage of palmitic, stearic and linoleic acids, and increases the share of oleic and linolenic acids in the abdominal fat pad. The inclusion of flax oil in the diet of chickens in an amounts of 4% and 8% increase the amount of linoleic acid to 63% and 203%, which was statistically highly significant ($P<0.01$) difference compare to the control groups I and II, whereas the amount of linoleic acid is reduced by 14% and 33%. Dietary addition of vegetable oils in this experiment did not show any improvement of chicken breast meat sensory quality, but lipids of meat was improved with the higher levels of PUFAs which contributes to a higher quality of gained chicken meat.

Key words: oils, meat, quality, PUFA, nutrition, broilers

Introduction

Quality may be defined as the sum of demands of the consumer concerning foodstuffs. According to *Wrick (1995),* the expectation of the consumer for meat is

that it should be healthy, rich in protein, low in fat, tender, and have a typical flavour. Currently, dietary recommendations favour the consumption of less saturated fat. For this reason, an increase in unsaturated fat in meat would be of direct nutritional benefit to the consumer (*Ruiz et al., 2001*).

The content of polyunsaturated fatty acids (PUFA) in poultry meat depends on their content in the diet to a great extent. Enrichment of poultry products with n-3 PUFA may provide an excellent alternative source of these acids in the human diet (*Zelenka et al., 2008*). Unsaturated lipids readily undergo oxidation to produce peroxides and aldehydes. The oxidative stability of unsaturated lipids decreases as their degree of unsaturation increases. Poultry meat with enhanced linolenic acid content is more susceptible to oxidative damage than meat with a similar concentration of linoleic acid. The balance of volatile compounds resulting from an oxidative breakdown of n-3 PUFA causes the occurrence of fishy aroma and off-taste characteristic of the meat of poultry fed a higher level of n-3 PUFA (*Rymer and Givens, 2005*).

Lipid oxidation is a major cause of quality deterioration in meat and meat products and can give rise to rancidity and the formation of undesirable odours and flavours, which affect the functional, sensory, and nutritive values of meat products (*Gray et al., 1996*).

The positive effect of replacing n-6 rich soybean oil with n-3 rich linseed oil and rapeseed oil on the nutritional value of chicken meat has been documented (*Haug et al., 2007; Pappas et al., 2012*).

The use of vegetable oils rich in n-3 fatty acids in chicken diets to increase the deposition of PUFA in tissues has led to concerns related to the increased liability of these fatty acids to oxidation and the effect this may have on organoleptic properties. In the study of *Nyquist et al. (2013)* there were no differences in flavor or taste for the chicken breast meat from the different dietary groups fed with addition of linseed and rapeseed oil and other vegetable oil combination, after six months of storage at -20°C. *Betti et al. (2009)* reported no effect on perceivable sensory characteristics when enriching a chicken diet with n-3 rich linseed meal for less than 16 to 20 days. *Haug et al. (2007)* found no differences in antioxidant status or organoleptic properties of meat from chickens fed low and high Se levels combined with rapeseed and linseed oil.

The aim of this study was to investigate the effect of dietary soybean, flaxseed and rapeseed oil addition on chicken body weight, abdominal fat pad fatty acid composition and sensory properties of breast meat.

Materials and Methods

Tests were conducted in production condition on the experimental estate »Pustara" in Temerin, in the floor system posture. At the beginning of experiment, six groups of the 40 one day old chicks Cobb 500 hybrid line, were formed. The

experiment was performed in five replicates, in total of 200 chickens per treatment. Chickens were fed with three mixtures of 21, 20 and 18% protein, respectively. The first 14 days was a preparatory period of chicken in which all groups were fed with starter mixture of standard composition and quality. Next 21 days of fattening period the grower mixtures were used with the different source and the amounts of oils. Last 7 days chicks were fed with finisher diets with the same addition of oil (Table 1). The control group was fed a mixtures based on 4 and 8% soybean oil, and in the experimental groups was included 4 and 8% flax and rapeseed oil. In the mixtures with low levels of oil tocopherol acetate as antioxidant was added in amount of 100mg/kg, and the mixtures with a higher level of oil is supplemented received 200mg/kg of antioxidant to prevent oxidation and to maintain the quality of the oil. During the experiment, which lasted 42 days, the chicks were fed and watered *ad libitum*, and microclimate conditions was regularly monitored. Control of body weight and feed consumption was performed every seven days.

Table 1. Experimental design

Group and Treatment	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
Source of oil	Soy	Soy	Flax	Flax	Rapeseed	Rapeseed
Grover	4%	8%	4%	8%	4%	8%
Finisher	4%	8%	4%	8%	4%	8%

At the end of the experiment, after 12 hours of fasting, from each group was collected 10 chickens (5 males and 5 females) of mean body weight, marked with stamps and sacrificed for the purpose of testing the fatty acid composition of lipid in abdominal fat pad. Analyses of fatty acid composition of abdominal fat pad were performed by gas chromatography (GC). The scoring for the sensory characters such as smell, taste, juiciness and gentleness was done on a 7 point hedonic scale (Table 2).

Table 2. Sensor analysis of thermally processed chicken meat

Mark	Thermal processed meat			
	Smell	Taste	Juiciness	Gentleness
1	Extremely bad (weak, not prominent, strange, overemphasized)	Extremely bad (weak, not prominent, strange, overemphasized)	Extremely bad (very dry or very juicy)	Extremely bad (very rough or very soft)
2	Very bad	Very bad	Very bad	Very bad
3	Bad	Bad	Bad	Bad
4	Not good, not bad	Not good, not bad	Not good, not bad	Not good, not bad
5	Good	Good	Good	Good
6	Very good	Very good	Very good	Very good
7	Very good (optimal)	Very good (optimal)	Very good (optimal)	Very good (optimal)

Analysis of variance (ANOVA) was applied to the data sets comprising the investigated traits. The experiments were repeated five times and the data were analysed using Statistica 12 and the differences between the means were compared with in Tucky post-hoc test at the significance levels of 0.05 and 0.01.

Results and Discussion

Based on the obtained results it can be concluded that the introduction of various types and levels of vegetable oil in the diet of broilers did not affect the intensity of growth (Table 3).

Table 3. Body weight of chickens, g

Age of chickens (weeks)	Group, the treatment and the amount of oil					
	I (T5)	II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-flax	8%-flax	4%-rapeseed	8%-rapeseed
	Preparatory period of chickens without experimental oil addition					
Initial weight	42±2.08	42±3.20	42±1.41	42±2.50	42±2.08	42±2.16
1	185±7.16	185±7.25	183±10.68	190±6.07	187±7.16	190±6.02
2	468 ± 35.3	469 ± 38.1	468 ± 28.3	468 ± 33.2	469 ± 42.5	469 ± 33.6
Index,%	*100*	*100*	*100*	*99.78*	*100.21*	*100*
	Periods with experimental nutrition of chickens with oil addition					
3	986 ± 57.2	967 ± 58.3^{b}	989 ± 52.8	997 ± 54.7^{B}	995 ± 64.5	977 ± 55.4
4	1457 ± 155.3^{BD}	1422 ± 134.1^{ABCD}	1523 ± 127.3^{A}	1532 ± 125.6^{B}	1515 ± 154.1^{C}	1575 ± 90.8^{D}
5	2122 ± 231.5^{E}	2053 ± 212.0^{ae}	2164 ± 260.2^{A}	2094 ± 231.5	2121 ± 255.1	2081 ± 223.7^{A}
Index,%	*100*	*100*	*101.97*	*101.99*	*99.95*	*101.36*
6	2704 ± 310.6	2695 ± 308.8	2735 ± 336.9	2645 ± 311.5	2735 ± 309.5	2670 ± 303.7
Index,%	*100*	*100*	*101.14*	*98.14*	*101.15*	*99.07*

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

During the preparatory period, chicks had a uniform body weight in all groups. However, in the due course of the experimental period, in the third and fourth week statistically significant (P<0.05) and highly significant (P<0.01) difference in the body weight between the control and experimental groups were recorded. In the fifth week of age very small depression in the treatment with 4% of rapeseed oil (V) was observed, while the other groups (III, IV, and VI) were superior to the control groups (I and II). In the six week of age, body weight of chicks exhibited significant differences (P>0.05) between the groups, but body weight in the groups with 8% of oil in the diet was lower, relative to the weight of

chickens that were on treatment with lower oils amounts in the diet. Similar results when the chicken growth performance is in question were reported by Lopez-*Ferrer et al. (1999)* with use of fish oil and rapeseed oil in the diet, followed by Nguyen et al. (2003) with the use of flaxseed and rapeseed oil, as well as in the investigation of *Kavouridou et al. (2008)* with a mixture of different vegetable oils.

Based on the data on the content of fatty acids in abdominal fat pad, as shown in Table 4, it can be noted that the use of flax oil and rapeseed oil changes the fatty acid composition of lipids.

Table 4. Fatty acid composition of chicken abdominal fat pad

Fatty acid	Treatments and fatty acid composition of abdominal fat,%					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-flax	8%-flax	4%-rapeseed	8%- rapeseed
C14: 0	0.04 ± 0.01 ^{d}	0.01 ± 0.01 ^{ABD}	0.07 ± 0.03 ^{A}	0.07 ± 0.05 ^{B}	0.03 ± 0.00 ^{C}	0.10 ± 0.02 ^{CD}
C16: 0	17.75 ± 0.87^{DE}	14.66 ± 1.12 ^{AcE}	18.39 ± 0.94^{A}	16.35 ± 0.75	16.87 ± 1.00 ^{C}	14.59 ± 1.50^{ACD}
C16: 1	3.28 ± 0.81	2.83 ± 1.41	3.83 ± 0.35	3.46 ± 0.39	3.41 ± 0.53	2.79 ± 0.61
C18: 0	5.23 ± 0.53	4.75 ± 0.93	5.06 ± 0.37	4.72 ± 0.23	4.67 ± 0.51	5.34 ± 1.85
C18: 1	35.45 ± 0.98	33.57 ± 1.93	35.72 ± 1.54	34.37 ± 3.50	37.07 ± 1.15	37.46 ± 2.69
C18: 2	29.22 ± 0.76 ^{E}	37.07 ± 2.73^{ABCDE}	25.13 ± 0.38^{A}	24.81 ± 3.49 ^{B}	27.48 ± 1.72^{aC}	26.52 ± 2.46 ^{cD}
C18: 3	5.89 ± 0.46 ^{B}	4.75 ± 0.35 ^{aB}	9.61 ± 0.72 ^{A}	14.54 ± 3.63^{aB}	6.83 ± 2.60 ^{B}	9.18 ± 3.46 ^{b}
C20: 0	0.07 ± 0.04	0.10 ± 0.01	0.07 ± 0.05	0.09 ± 0.09	0.09 ± 0.02	0.08 ± 0.03
C20: 1	0.46 ± 0.16	0.41 ± 0.03	0.42 ± 0.02	0.33 ± 0.02	0.49 ± 0.05	0.56 ± 0.13
C22: 0	0.06 ± 0.04	0.00 ± 0.00 ^{d}	0.01 ± 0.03	0.09 ± 0.08	0.08 ± 0.02	0.09 ± 0.05 ^{D}
C24: 0	0.01 ± 0.01 ^{D}	0.00 ± 0.00 ^{cD}	0.00 ± 0.00 ^{A}	0.00 ± 0.00 ^{B}	0.05 ± 0.03^{ABC}	0.08 ± 0.05 ^{ABD}

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

Replacing soybean oil with rapeseed oil in an amount of 4%, in the chickens diet, reduces the percentage of palmitic, stearic and linoleic acids, and increases the share of oleic acid and linolenic acid in the abdominal fat. These changes are directly correlated with the fatty acid composition of the oil. An increase in the linoleic acid in V group, from 5.89% to 6.83% was statistically highly significant (P<0.01). By increasing the amount of oil in the diet mixtures to 8%, the same acids tendency is maintained except stearic acid, whose participation is increased in the VI group. Reduction of linoleic acid was significantly higher (P<0.01) as compared to the second (control) group, while an increase in linoleic acid had significant difference (P<0.05) compared to II (control) group. The inclusion of flaxseed oil in the diet of chickens in amount of 4% and 8% increase

the amount of linoleic acid to 63% and 203%, which is a statistically significant difference ($P<0.01$) compared to control group I and II, while the amount of the other acid were reduced. From the above it can be determined that linoleic acid is reduced by 14% and 33%, with statistically significant difference ($P<0.01$) compared to the second (control) group. Decreased feed consumption in groups with 8% of the oil had an impact on the amount of fatty acids in the abdominal fat. All groups with a higher level of oil had a lower proportion of acid in the same oil, except for the dominate acid. Analysis of variance and Tucky post-hoc test showed significant differences ($P<0.05$) between I and V groups; II and IV of linolenic acid, followed by a highly significant difference ($P<0.01$) between the II and IV; II and VI group of linoleic acid. Chickens fed a diet supplemented with flaxseed and rapeseed grain with significantly different concentrations of monounsaturated fatty acids and polyunsaturated fatty acids had no significant effect on the deposition of fatty acids in chicken breast meat (*Rahimi et al., 2011*). It has been found that the flaxseed oil is an excellent source of polyunsaturated fatty acids of the n-3 family, which can be very efficiently converted from phospholipids in tissues lipids of poultry (*Ferrer-Lopez et al., 2001*).

Table 5 shows the results of sensory characteristics of thermally processed chicken meat. From the given results it can be seen that addition of soybean oil in chicken diet led to a good smell of chicken meat ranged from scores of 4.87 (T5) and 4.69 (T6), with the similar scores of the taste. Introduction of flaxseed and rapeseed oil led to adverse effects of smell and taste, but when the gentleness is in question, treatment T1 with the addition of 4% of flaxseed oil achieved the highest score of 6.80 what have classified it as very good. Dietary addition of vegetable oils in this experiment did not show any remarkable improvement of chicken breast meat quality, but lipids of meat was improved with the higher levels of PUFAs which contributes to a higher quality of chicken meat.

Table 5. Marks of sensory characteristics of thermal processed chicken breast meat

	Treatments and marks of thermal processed chicken breast meat					
	Control, I (T5)	Control, II (T6)	III (T1)	IV (T2)	V (T3)	VI (T4)
	4%-soy	8%-soy	4%-flax	8%-flax	4%-rapeseed	8%-rapeseed
Smell	4.87 ± 0.22	4.69 ± 0.51	4.40 ± 0.18	3.83 ± 0.26	3.28 ± 0.29	3.35 ± 0.24
Taste	4.60 ± 0.26	4.54 ± 0.42	3.75 ± 0.28	3.65 ± 0.36	3.23 ± 0.21	3.17 ± 0.23
Juiciness	5.30 ± 0.61	5.32 ± 0.71	6.65 ± 0.54	4.14 ± 0.21	3.51 ± 0.05	3.01 ± 0.04
Gentleness	6.27 ± 0.25	6.37 ± 0.22	6.80 ± 0.41	6.02 ± 0.06	6.03 ± 0.06	6.08 ± 0.09

In the study of *Nyquist et al. (2013)* there were no differences in taste for the chicken breast meat fed with red palm oil, palm oil or rendered animal fat in combinations with flaxseed oil, rapeseed oil and two levels of selenium. The same authors reported, however, some differences when it came to odour. The high rapeseed and flaxseed oil dietary group had the highest intensity for acidulous odour, and lowest intensity for stale odour. Acidulous taste and smell relates to a fresh, sweet and sour experience while a stale taste and smell relates to lack of freshness, low aromatic, nauseous or oversweet experience. In their research *Betti et al. (2009)* reported no effect on perceivable sensory characteristics when enriching a chicken diet with n-3 rich flaxseed meal for less than 16 to 20 days.

Conclusion

Based on the obtained results it can be concluded that the use of 4% and 8% flax oil and rapeseed oil did not showed significant differences in body weight compared to the control group. Groups with lower oil addition had a higher body weight at the end of the experiment. The use of flax oil and rapeseed oil changes the fatty acid composition of lipids. Replacing soybean oil with rapeseed oil in an amount of 4%, reduces the percentage of palmitic, stearic and linoleic acids, and increases the share of oleic acid and linolenic acid in the abdominal fat pad. Addition of these types of oil did not improved sensor quality of chicken breast meat but has led to a significant improvement of lipids fatty acid composition.

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Efekat sojinog, lanenog i repičinog ulja u ishrani brojlera na kvalitet mesa

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

Rezime

Cilj rada je bio da se ispitaju efekti sojinog, lanenog i repičinog ulja u ishrani brojlerskih pilića na telesnu masu, masnokiselinski sastav lipida i senzorni kvalitet mesa grudi. Na početku eksperimenta je formirano šest grupa od po 40 jednodnevnih pilića hibrine linije Cobb 500 u pet ponavljanja. Za ishranu pilića su

korišćene tri različite smeše sa 21, 20 i 18% sirovih proteina. Eksperiment je trajao 42 dana. Upotreba različitih vrsta biljnih ulja u ishrani nije ispoljila statistički značajne razlike (P>0,05) u završnoj telesnoj masi pilića. Završna telesna masa pilića u kontrolnim grupama je iznosila 2704 i 2695 g, dok je u eksperimentalnim iznosila za redom 2735, 2645, 2735 i 2670 g. Upotreba lanenog i repičinog ulja je uticala na promenu sastava lipida. Zamena sojinog ulja repičinim je dovela do smanjenja udela palmitinske, stearinske i linolne masne kiseline i povećanja udela oleinske i linolenske kiseline u abdominalnoj masti. Uvođenje lanenog ulja u ishranu pilića u količini od 4 i 8% je dovelo do povećanja sadržaja linolenske kiseline za 63 i 203%, sa statistički visoko značajnom razlikom (P<0,01) u poređenju sa kontrolnim grupama I i II, dok se sadržaj linolne kiseline smanjio za 14 i 33%. Upotreba biljnih ulja u ovom eksperimentu nije dovela do poboljšanja senzornih karakteristika mesa grudi, ali je dovela do značajnog poboljšanja masnokiselinskog sastava mesa u vidu povećanog sadržaja PUFA kiselina, što doprinosi značajnom poboljšanju nutrtivnog kvaliteta mesa pilića.

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VARIATION OF TRAITS OF FATTENERS UNDER THE IMPACT OF VARIOUS FACTORS

M. Gogić[1], M. Petrović[2], Č. Radović[1], B. Živković[1], D. Radojković[2], N. Stanišić[1], R. Savić[2]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina, 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: gogic.marija@gmail.com
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Abstract: The aim of this study was to determine the effect of the sire breed, sire within sire breed, genotype of fatteners, gender of fatteners, gender within sire breed, season of birth of fatteners and mass of warm carcass side on the following traits: back fat thickness - middle of the back (DSL), back fat thickness – lower back (DSK), meat yield of carcass sides (JUSKG) and percentage/share of meat in carcass sides (JUSPRO). The research was conducted in the experimental slaughterhouse and laboratory of the Institute for Animal Husbandry, Belgrade-Zemun, and included females and castrated male animals. Sires of fatteners were pure breeds: Swedish Landrace (SL, n = 10), Large White (LW, n = 3) and Pietrain (P, n = 3), while the offspring belonged to the following genotypes: pure breed - Swedish Landrace (SL, n=252), and crosses of Large White × Swedish Landrace (LW × SL) (n=170), Pietrain × Swedish Landrace (P × SL) (n=13), [Pietrain × (Large White × Swedish Landrace)] P × (LW × SL) (n=35), [Swedish Landrace × (Large White × Swedish Landrace)] SL × (LW × SL) (n=33) and [Large White × (Large White × Swedish Landrace)] LW × (LW × SL) (n=33). The study included total 536 offspring of which 276 are male castrated and 260 female animals. In the winter 24 piglets were born, in the spring 95, in the summer 148 and autumn 269 piglets. It was established that the sire within sire breed Pietrain (S:P) does not affect the variation of the studied traits of fattening pigs ($P>0.05$); sire within sire breed Swedish Landrace (S:SL) does not affect the varying of the trait JUSPRO ($P>0.05$); season of birth within the Model 1 does not affect the traits yield and share of meat ($P>0.05$); the offspring gender within genotype (Gender : Genotype) does not affect the variation of fat thickness at the centre of the back ($P>0.05$). All other factors (sire breed, sire within the sire breed - Large White, gender and genotype of fattening pigs, gender within sire breed, the mass of warm carcass side, and also birth season of fattening pigs in the Model 2) included in the models showed statistically significant impact on the variability of traits of fattening pigs ($P<0.05$; $P<0.01$ and $P<0.001$).

Key words: sire, genotype, gender, season of birth, fattener

Introduction

Production of pigs and pork depends on many factors, the most important are the market and economic efficiency of production. The following factors which affect the quantity and quality of the pork carcass are price of fattened pigs, method of evaluation of breeding pigs (i.e. whether fatteners are paid per kilogram of live weight or meatiness established on the slaughter line), genetic and environmental factors (breed, origin, method of breeding, age and weight at slaughter, castration, nutrition, pig procedures before, during and after slaughter, etc.) *(Radović et al., 2009).* The total work in the field of genetics, selection, nutrition, reproduction and health care *(Radović et al., 2007)* are evaluated with the evaluation/assessment of the carcass side quality. Quality of meat within species can be influenced by the origin of the animals, the feed materials, slaughter and chilling procedures, storage conditions, muscle type, etc. (Stanišić et al., 2013). Indicators of lean meat are indicators of quality of pig carcasses and include various types of measurements, on different locations and carried out in different ways *(Kosovac et al., 2009).* The task of breeding/selection is to provide high-quality breeding animals and the quality pigs for slaughter industry and meat processing industry, based on a clearly defined breeding goal, which is always necessary to improve depending on customer preferences. The profitability of production depends on the success of the selection, which is particularly evident on large farms. The fundamental requirements for economical production of pigs are: increase of the annual production of fattening pigs per sow, reduced feed per kilogram of gain and increase of meat yield of fatteners *(Radović, 2012a).* Intensity of growth, food utilization and meat yield are of great importance in breeding and selection *(Radović et al., 2013).* Sire breed and sires within the same genotype influence the variability of carcass side quality in offspring *(Kosovac et al., 1998; Petrović et al., 2002; Radović et al. 2003; Pušić and Petrović, 2004).* Sires and offspring gender affect the variability of traits of offspring *(Petrović et al., 2006a).* By crossing of pigs the aim is to achieve heterosis effect for important production traits. Finding the best combination of crossing is a continuous process, given that the frequency of particular genes changes continuously through the selection *(Senčić et al., 2003).* Timely obtaining of feedback from the slaughter line and/or from the processing industry enables the breeder to evaluate the effects of breeding and selection work and make changes in the future work if there are such requirements of the meat industry and to achieve greater genetic gain medium and high heritability traits *(Petrović et al., 2004).*

Materials and Methods

Measuring of carcass quality traits was done on animals of both genders - the males were castrated, total of 536 pigs. The research included all four seasons of the birth of offspring in a single herd. The pigs come from 16 pure breed sires, namely: Swedish Landrace (SL) 10, Large White (LW) 3 and Pietrain (P) 3. Genotypes of fattening pigs that were included in this study were as follows: Swedish Landrace (SL) as a pure breed, and crosses Large White × Swedish Landrace, Pietrain × Swedish Landrace, [Pietrain × (Large White × Swedish Landrace)], [Swedish Landrace × (Large White × Swedish Landrace)] and [Large White × (Large White × Swedish Landrace)]. Pre-slaughter mass (weight at the end of fattening period) and warm carcass side weight were measured on a scale with an accuracy of ± 0.5 kg. Fat thickness was measured at the middle of the back (where the fat is the thinnest between the 13th and 15th thoracic vertebra) and the lower back above *m. gluteus medius* (measures taken at the spot where the *m. gluteus medius* penetrates into fat tissue). The sum of back fat thickness in the middle of the back and lower back represents the back fat thickness (*OG SFRY, 1985*). Fat thickness was measured by a steel ribbon, with a precision of ± 1 mm. To determine the yield (JUSKG) and the share of meat (JUSPRO) in carcass sides, based on realized measurements, the tables for meaty pigs were used, which are an integral part of the Rulebook on the quality of slaughtered pigs and pork categorization (*OG SFRY, 1985).*

Data analysis was performed using adequate computer package "LSMLMW and MIXMDL, PC-2 VERSION" *(Harvey, 1990)* i.e. the procedures of least squares method in order to determine the significance ($P<0.05$) of systematic influences on the traits of fat thickness on the lower back (DSK) back fat thickness at the middle of the back (DSL), yield and percentage of meat (JUSKG and JUSPRO). The models included: sire breed, sires within sire breed, genotype of the fattener, gender, gender within genotype, season of birth and weight of warm carcass sides (linear regression influence).

To test the variation of traits of fattening pigs the following models were used:

Model 1: $Y_{ijklm} = \mu + R_i + O_{j:i} + P_k + P_{k:i} + S_l + b_1 (x_1 - \square) + \varepsilon_{ijklm}$
where: Y_{ijklm} - μ = general population average, R_i – impact of sire breed (i=1, 2, 5); $O_{j:i}$ – impact of sires within the breed ($j{:}i_1$ =1, 2, 3, 7, 8, 9, 15, 16, 17, 18; $j{:}i_2$ =4, 5, 6; $j{:}i_3$=14, 19, 20); P_k – impact of gender (k =1,2); $P_{k:i}$ – impact of gender within breed; S_l – impact of birth season of progeny (l =1, 2, 3, 4); expression of trait of individual animal m, of boar breed i, of j sire within i breed, k gender and l birth season; b_1 – linear regression impact of weight of warm carcass side (x_1); ε_{ijklm} – random error (residue).

Model 2: $Y_{ijkl} = \mu + G_i + P_j + P_{j:i} + S_k + b_1 (x_1 - \square) + \varepsilon_{ijkl}$
gde je: Y_{ijkl} - μ = general population average; G_i – impact of genotype (i =1, 2, 5, 6, 8, 9); P_j – impact of gender (j=1,2); $P_{j:i}$ - impact of gender within breed; S_k - impact of birth season of progeny (k =1, 2, 3, 4); expression of trait of individual animal l, i genotype, j gender, j –gender within i- genotype, k-birth season; b_1 - linear regression impact of weight of warm carcass side (x_1); ε_{ijkl} - random error (residue).

Results and Discussion

All tested traits are adjusted to the same mass of warm carcass side (MTP), which is 81.20 kg. The average values and standard deviations of corrected properties are shown in Table 1.

Table 1. Mean values and standard deviations of growth traits and carcass quality of progeny

Trait		$\overline{X}$±SD
MTP	Weight of warm carcass side, kg	81.20 ±8.60
MPK	Final body mass of fatteners (pre-slaughter), kg	101.04±9.61
DSL	Back fat thickness – centre, mm	17.22 ±5.55
DSK	Back fat thickness – lower back, mm	15.96 ±6.15
JUSKG	Meat yield in carcass sides (OG SFRY, 1985), kg	35.39±3.70
JUSPRO	Meat percentage/share in carcass sides (OG SFRY, 1985), %	43.61±1.80

Pušić and Petrović (2004), have established in their study higher MTP (+10.65 kg), higher meat yield (+3.14 kg), but lower percentage of meat (41.95%), while *Petrović et al. (2006b)* have reported on a farm A MTP of 85.78 kg and on farm B MTP of 82.59 kg, and the percentage/share of meat on the farms of 43.28 and 43.91%, respectively, which is very similar to our results. Also, *Petrović et al. (2006a)* have reported value for JUSPRO of 43.27%, and that the sum of back fat thickness on the back and lower back, with MTP - 85.59 kg, of 37.20 mm, which is contrary to our results, where the sum of fat thickness was 33.18 mm, but at a lower MTP.

Table 2 shows the values of the fat thickness of bacon that are necessary in order to obtain the values for the yield (JUSKG) and the share of meat in the carcass side (JUSPRO) from the tables for meaty pigs, which are the integral part of the Rulebook on the quality of slaughtered pigs and pork categorization *(OG SFRY, 1985).* The difference between fat thickness at the middle of the back and lower back is 0.13 mm. Analysis of the data showed that the lowest values for fat thickness occur in the progeny of Pietrain sires (13.20 and 14.31 mm), which resulted in the highest yield (36.22 kg) and the share of meat (44.25%) of the

carcass side. The highest values for fat thickness wer recorded in animals that originated from Large White sires (20.51 and 19.12 mm), and therefore the lowest values for yield and share of meat in carcass sides (34.59 kg and 42.73%). Within sire breed SL, the lowest value for trait DSL was recorded in progeny of boar No. 17 (11.78 mm), however with the highest percentage of meat (44.69%), while the progeny of boar No. 2 had the lowest value for the trait DSK (12.19 mm). By contrast, animals that come from the Sire No. 5, LW breed, had the thickest fat (22.25 and 21.37 mm), and the lowest yield and share of meat (33.92 kg and 41.94%). The regression effect of the mass of warm carcass side shows that the studied traits statistically very highly varied ($P<0.01$ and $P<0.001$). With the increase in body weight at the end of fattening by 1 kg, the fat thickness at the middle of the back and lower back increased by 0.246 mm and 0.244 mm, respectively, and meat yield of carcass sides by 0.382 kg. Contrary to the above traits, share/percentage of meat decreased by 0.019% per kilogram of increased mass of fatteners.

Table 2. The effect of sire breed, sires within the breed and MTP (Model 1) on fat thickness, yield and share/percentage of meat in carcass side (LSMean ±S.E.)

Sources of variation		DSL[2)], mm	DSK, mm	JUSKG, kg	JUSPRO, %
μ ± S.E.		16.21 ±0.43	16.08 ±0.46	35.54 ±0.14	43.65 ±0.15
RO[1)]	Sire No.				
Swedish Landraces	1	15.76±0.62	14.50±0.68	35.84±0.20	44.23±0.22
	2	14.52±0.63	12.19±0.69	36.04±0.21	44.46±0.22
	3	17.53±0.80	14.80±0.87	35.46±0.26	43.86±0.28
	7	15.70±1.61	16.40±1.75	35.29±0.53	43.34±0.56
	8	17.31±1.52	17.04±1.65	34.61±0.50	42.98±0.52
	9	14.98±1.62	13.30±1.76	35.27±0.53	43.80±0.56
	15	14.34±1.50	15.88±1.63	36.30±0.49	44.05±0.52
	16	14.43±1.40	16.45±1.51	36.09±0.46	44.14±0.48
	17	11.78±1.38	12.83±1.50	36.35±0.45	44.69±0.48
	18	13.04±1.30	14.73±1.41	36.92±0.42	44.10±0.45
	Average	14.94±0.56	14.81±0.61	35.82±0.18	43.96±0.19
Large White	4	20.07±0.62	18.16±0.67	34.82±0.20	43.00±0.21
	5	22.25±0.71	21.37±0.77	33.92±0.23	41.94±0.24
	6	19.20±0.66	17.84±0.71	35.04±0.21	43.25±0.23
	Average	20.51±0.48	19.12±0.52	34.59±0.16	42.73±0.17
Pietrain	14	15.24±0.89	12.48±0.97	36.54±0.29	44.64±0.31
	19	12.22±1.70	15.63±1.84	36.18±0.56	44.24±0.59
	20	12.14±1.62	14.83±1.76	35.94±0.53	43.86±0.56
	Average	13.20±1.01	14.31±1.09	36.22±0.33	44.25 ±0.35
MTP (b)		0.246[3)]***	0.244***	0.382***	-0.019**

[1)]RO-sire breed, MTP (b)- linear impact of the mass of warm carcass side(MTP=81,20 kg); [2)] DSL-fat thickness at the middle of back; DSK- fat thickness at the lower back; JUSKG- meat yield of

carcass side; JUSPRO- share/percentage of meat in carcass side; [3] NS=P>0.05;*=P<0.05;**=P<0.01; ***=P<0.001

Considering of the progeny gender as a source of variation of investigated traits (Table 3), it was established that females had a lower value for DSL (-3.39 mm) and DSK (-3.52 mm) compared to male castrated animals and thus greater value of the yield (+0.83 kg) and the share of meat (+ 1.10%). The study of the progeny gender within the sire breed showed that males within sire breed LW had the highest value for DSL (22.99 mm) and DSK (22.12 mm), thus these animals had the lowest yield (33.71 kg) and the share of meat (41.67 %) in the carcass sides. Female animals within the breed P had the lowest value for trait DSL (12.19 mm), but they had the highest yield and the percentage of meat (36.38 kg and 44.47%). Looking at the season of birth of progeny, it was found that offspring born in the winter had the thinnest back fat (14.16 and 14.59 mm), and the highest values for yield and share/percentage of meat (35.89 kg and 44.01%).

Table 3. The average values of the properties DSL, DSK, JUSKG and JUSPRO within factors gender, gender within sire breed and season of birth (Model 1)

Sources of variation		DSL[2], mm	DSK, mm	JUSKG, kg	JUSPRO, %
Gender	M[1]	17.91±0.48	17.84±0.52	35.13±0.16	43.10±0.17
	Ž	14.52±0.51	14.32±0.55	35.96±0.17	44.20±0.18
Gender within sire breed	M:ŠL	16.54±0.59	16.70±0.64	35.62±0.19	43.60±0.20
	Ž:ŠL	13.34±0.64	12.93±0.70	36.02±0.21	44.33±0.22
	M:VJ	22.99±0.57	22.12±0.62	33.71±0.19	41.67±0.20
	Ž:VJ	18.02±0.56	16.13±0.61	35.47±0.18	43.79±0.19
	M:P	14.21±1.15	14.72±1.25	36.06±0.38	44.02±0.40
	Ž:P	12.19 ±1.22	13.91 ±1.32	36.38 ±0.40	44.47±0.42
Season	Winter	14.16±1.16	14.59±1.26	35.89±0.38	44.01±0.40
	Spring	18.88±0.68	18.75±0.74	35.09±0.22	43.21±0.24
	Summer	16.00±0.69	15.10±0.75	35.73±0.23	43.76±0.24
	Autumn	15.82±0.64	15.89±0.70	35.47±0.21	43.60±0.22

[1]M- castrated males, Ž–females; [2]DSL- fat thickness at the middle of back; DSK- fat thickness at the lower back; JUSKG- meat yield of carcass side; JUSPRO- share/percentage of meat in carcass side

Table 4 shows the average values for the traits of fattening pigs (DSL, DSK, JUSKG and JUSPRO) within the factors - genotype, gender, season of birth and MTP (Model 2). Thinnest fat - DSL was recorded in fatteners of genotype 6 (13.71 mm) i.e. three breed crosses with Pietrain as a terminal breed [Px (LWxSL)]. The thinnest fat - DSK (12.59 mm), and the highest yield and the share/percentage of meat (36.63 kg and 44.82%) were recorded in animals of genotype 5 (two breed crosses with 50% of genes of breed P). Contrary to them, fatteners of genotype 9 [LWx (LWxSL)] had the highest values of fat thickness (20.54 and 19.61 mm) and the lowest yield (34.45 kg) and the share of meat (42.50%) at the same average mass of warm carcass sides. The gender of fattening pigs and season of birth displayed the same tendency as in Model 1. Females had

more meat in the carcass sides (36.09 kg and 44.36%) and thinner back fat than castrated males, which is in line with the research of *Petrović et al. (2006b)*. Fatteners born during the winter had the thinnest fat and the most meat in the carcass side. Contrary to them, animals born during the spring, had the highest mean values for fat thickness and the lowest for meat yield of carcass sides. The regression effect of the mass of warm carcass side showed statistically significant and very high varying of traits (P<0.05 and P<0.001). With the increase in body weight at the end of fattening by 1 kg, the fat thickness at the middle of the back and lower back increased by 0.229 mm and 0.246 mm, respectively, and meat yield of the carcass sides by 0.378 kg. Contrary to the above traits, share/percentage of meat decreased by 0.020% per kilogram of increased mass of fatteners.

Table 4. The effect of genotype, gender, season and MTP (Model 2) on fat thickness, yield and share/percentage of meat in carcass side (LSMean ±S.E.)

Source of variation		DSL[3)], mm	DSK , mm	JUSKG, kg	JUSPRO, %
μ ± S.E.		16.93±0.36	15.67±0.39	35.61 ±0.12	43.76± 0.12
Genotype	1[1)]	14.94±0.36	13.80±0.39	35.94±0.12	44.28± 0.12
	2	19.77±0.45	19.13±0.49	34.75±0.15	42.85± 0.16
	5	15.22±1.25	12.59±1.36	36.63±0.41	44.82± 0.43
	6	13.71±0.87	12.95±0.95	36.46±0.29	44.61± 0.30
	8	17.42±0.82	15.92±0.90	35.44±0.27	43.50± 0.28
	9	20.54±0.80	19.61±0.87	34.45±0.26	42.50± 0.27
Gender	M[2)]	18.58±0.49	17.52±0.54	35.13±0.16	43.16 ±0.17
	Ž	15.29±0.46	13.82±0.50	36.09±0.15	44.36 ±0.16
Season	Winter	14.86±0.99	13.28±1.08	36.17±0.33	44,46 ±0,34
	Spring	19.13±0.54	19.74±0.59	34.91±0.18	42,86 ±0,19
	Summer	16.75±0.48	14.63±0.52	35.89±0.16	43,99 ±0,16
	Autumn	16.99±0.39	15.01±0.42	35.49±0.13	43,73 ±0,13
MTP (b)		0,229[4)]***	0.246***	0.378***	-0.020*

[1)]1-ŠL, 2- VJxŠL, 5-PxŠL, 6- Px(VJxŠL), 8- ŠLx(VJxŠL), 9- VJx(VJxŠL); [2)]M-castrated males, Ž–females, MTP(b)- linear effect of the mass of warm carcass side; [3)] DSL- fat thickness at the middle of back; DSK- fat thickness at the lower back; JUSKG- meat yield of carcass side; JUSPRO-share/percentage of meat in carcass side; [4)] NS=P>0.05;*=P<0.05;**=P<0.01; ***=P<0.001

The statistical significance of the factors included in the Models 1 and 2, when analysing the traits of fat thickness, yield and share of meat in the carcass sides, are shown in Table 5.

Table 5. Statistical significance (significance level) of the impact of factors included in the models (Model 1 and 2) when analyzing the traits of fat thickness and meat yield (OG SFRY,1985)

Sources of variation (impact)[1)]		DSL[2)]	DSK	JUSKG	JUSPRO
Model 1	RO	***[3)]	***	***	***
	O:ŠL	**	**	**	NS
	O:VJ	***	***	***	***
	O:P	NS	NS	NS	NS
	Gender	***	***	***	***
	Season	*	*	NS	NS
	Gender:RO	*	***	***	***
	MTP (b)	***	***	***	**
	R^2	0,442	0,465	0,865	0,366
Model 2	Genotype	***	***	***	***
	Gender	***	***	***	***
	Season	***	***	***	***
	Gender:Genotype	NS	*	***	***
	MTP (b)	***	***	***	*
	R^2	0,395	0,416	0,852	0,316

[1)]RO-sire breed, O:ŠL-sires within Swedish Landrace sire breed, O:VJ-sires within Large White sire breed, O:P- sires within Pietrain breed, Gender:RO-gender of progeny within sire breed, Gender:Genotype- gender of progeny within genotype; [2)] DSL- fat thickness at the middle of back; DSK- fat thickness at the lower back; JUSKG- meat yield of carcass side; JUSPRO- share/percentage of meat in carcass side; MTP (b)- linear effect of the mass of warm carcass side; [3)] NS=P>0.05;*=P<0.05;**=P<0.01; ***=P<0.001

From Table 5 (Model 1) it can be seen that the sire breeds, siress within the LW breed and gender of fatteners influenced statistically high (P<0.001) variation of all traits of offspring. However, variation of any traits (P>0.05) was not determined between the progeny from different sires of breed P. Sires of SL breed showed statistically highly significant effect (P<0.01) on all traits except for JUSPRO (P>0.05). Season of birth of progeny significantly affected fat thickness (P<0.05), but had no influence on the properties of yield and share of meat, while by applying the Model 2, season showed statistically high varying of all the studied traits (P<0.001), as well as the genotype (P<0.001). The obtained differences in the mean values for all traits between females and male castrated animals were statistically highly significant (P<0.001) in both models used. The values of the coefficient of determination for Model 1, were in the range of 0.366 (JUSPRO) to 0.865 (JUSKG). The coefficient of determination R^2 showed that the effects included in Model 1 (sire breed, sires within the breed, gender, season and gender within sire breed) explained 44.2% of variation of DSL, 46.5% of the variation of DSK, 86.5% of the variation of JUSKG, and 36.6% of the variation of

JUSPRO. The coefficient of determination R^2 showed that the effects included in Model 2 (genotype, gender, season and gender within genotype) explained 39.5% of variation of DSL, 41.6% of the variation of DSK, 85.2% of the variation of JUSKG, and 31.6% of the variation of JUSPRO. So, by using the effect of factors the variation of JUSKG is explained the most, in both applied models, whereas variation of trait JUSPRO is explained the least in both models. *Pušić and Petrović (2004)* have reported that the sire breed, gender of progeny and MTP significantly affect the variation of all traits of fattening pigs, which is in line with our research. Sire breed affects all traits of offspring on one farm, while on the other farm, sire breed has no influence on the properties of JUSKG and JUSPRO *(Petrović et al., 2006b* which is contrary to our results. The results of this research showing highly statistically significant impact of gender on carcass side quality traits are consistent with a greater number of researchers *(Petrović et al., 2004; Pušić and Petrović, 2004; Petrović et al., 2006a; Petrović et al., 2006b; Radović et al., 2007; Radović et al., 2012b)*, but not in line with the research *Kosovac et al. (1998)* and *Radović et al. (2009)* who have found no statistically significant differences between the genders. Contrary to our research *Radović et al. (2009)* suggest that sire breed has no influence on the variability of traits DSK and JUSPRO.

Conclusion

On the basis of the obtained results it was established that sire breed, gender of fatteners and genotype highly significantly affected all studied traits of fattening pigs ($P<0.001$). Sires within LW statistically highly influenced the variation of all traits of fattening pigs ($P<0.001$), and contrary to them, sires within the breed P had no impact on the variability of traits of their offspring ($P>0.05$). Sires within SL significantly affected the variation of all traits ($P<0.01$), with the exception of the trait JUSPRO. Applying the Model 1, season of birth of offspring exerted influence only on the properties of fat thickness ($P<0.05$), while the application of the Model 2 resulted in very high statistical impact on variation of all traits of fattening pigs ($P<0.001$). By using both models, MTP showed very high statistically significant impact on the variability of traits of fattening pigs ($P<0.001$), except for the trait JUSPRO which had a lower impact ($P <0.01$ and $P <0.05$). Increasing the MTP by 1 kg resulted in an increase in values for all traits of fattening pigs, except for the trait JUSPRO which decreased.

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Variranje osobina tovljenika pod uticajem različitih faktora

M. Gogić, M. Petrović, Č. Radović, B. Živković, D. Radojković, N. Stanišić, R. Savić

Rezime

Cilj ovog istraživanja je da se utvrdi uticaj rase oca, oca unutar rase oca, genotipa tovljenika, pola tovljenika, pola unutar rase oca, sezone rođenja tovljenika i mase tople polutke na sledeće osobine tovljenika: debljina slanine na sredini leđa (DSL), debljina slanine na krstima (DSK), prinos mesa u polutkama (JUSKG) i udeo mesa u polutkama (JUSPRO). Istraživanje je obavljeno u eksperimentalnoj klanici i laboratoriji Instituta za stočarstvo, Zemun-Beograd, kojim su obuhvaćena ženska grla i muška kastrirana grla. Očevi tovljenika pripadaju čistim rasama: švedski landras (ŠL, n=10), veliki jorkšir (VJ, n=3) i pijetren (P, n=3), dok potomci pripadaju sledećim genotipovima: od čistih rasa zastupljen je švedski landras (ŠL), a od meleza javljaju se veliki jorkšir × švedski landras (VJ×ŠL), pijetren×švedski landras (P×ŠL), [pijetren×(veliki jorkšir×švedski landras)] P×(VJ×ŠL), [švedski landras×(veliki jorkšir×švedski landras)] ŠL×(VJ×ŠL) i [veliki jorkšir×(veliki jorkšir×švedski landras)] VJ×(VJ×ŠL). Utvrđeno je da otac unutar rase oca pijetren (O:P) ne utiče na variranje ispitivanih osobina tovljenika (P>0,05); otac unutar rase oca švedski landras (O:ŠL) ne utiče na variranje osobine JUSPRO (P>0,05); sezona rođenja tovljenika ne utiče u okviru Modela 1 na osobine prinos i udeo mesa (P>0,05); pol potomaka unutar genotipa (Pol:Genotip) ne utiče na variranje debljine slanine na sredini leđa (P>0,05). Svi ostali faktori uključeni u modele su pokazali statistički značajan uticaj na variranje osobina tovljenika (P<0,05; P<0,01 i P<0,001).

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BIOCHEMICAL CHARACTERISTICS OF *STREPTOCOCCUS SUIS* STRAINS ISOLATED FROM HEALTHY AND DECEASED PIGS

A. Stanojković[1], M. M. Petrović[1], Z. Škrbić[1], V. Mandić[1], N. Stanišić[1], M. Gogić[1], A. Stanojković-Sebić[2]

[1]Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[2]Institute of Soil Science, Belgrade, Republic of Serbia
Corresponding author: izs.aleksandar@gmail.com
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Abstract: The aim of this study was to determine the biochemical properties of *Streptococcus suis* strains isolated from healthy and deceased pigs. For this research we tested 34 *S. suis* strains isolated from deceased pigs that had clinical signs of septicemia and meningitis, as well as from clinically healthy pigs. The strains that have been already confirmed with specific antisera were tested using commercial battery of biochemical tests (API 20 Strep and ID 32 Strep) to determine the dominant biochemical characteristics that can be used in diagnosis of bacterial infection if specific *S. suis* antisera are not available. The main results showed that all *S. suis* strains were postive in esculine, trehalose, glycogen, lactose, sacharose, starch, leucine aminopeptidase, alanine-phenyl-alanine-proline arylamidase tests, while negative in Voges-Proskauer, hipurate, ribose, arabinose and sorbitol tests. *S. suis* strains were in high percentage positive in arginine dihydrolase, β-glucoronidase, α-galactosidase, β-galactosidase, methyl-β-d-glucopyranoside, glycyl-tryptophan arylamidase and inulin tests. Athough *S. suis* is in highly positive in some tests, it can be concluded that Voges-Proskauer, hipurate, trehalose, esculine tests, along with β-glucoronidase (βGUR) and α-galactosidase (αGAL), were significant in differentiation of this bacteria from other similar streptococci, along with some other crucial features (α hemolysis on blood sheep agar, absence of growth in 6,5% NaCl broth).

Key words: *Streptococcus suis*, biochemical characteristics, pigs

Introduction

Streptococcus suis is a facultative anaerobic, coccoid, gram-positive bacterium with the ability to synthesize capsule and secrete hemolysin. It has components of the cell wall antigens similar to those displayed by group D

streptococci. However, *S. suis* is not genetically associated with group D streptococci.

S. suis is a very heterogeneous species. So far, 35 of *S. suis* serotypes have been described on the basis of the composition of the capsular polysaccharide (1-34 and ½). During the last 20 years, *S. suis* has been considered to be one of the main pathogen that causes severe economic losses in countries with developed pig industries. *S. suis* is a normal inhabitant of the pigs respiratory system, mostly of the tonsils and nasal cavities, and can often be isolated from the genital and gastrointestinal systems in healthy animals *(Higgins and Gottschalk, 2005)*. Since it is a very good colonizer of the mucosal surfaces, clinically healthy pigs are the main reservoir of infection, and the most important link in the epidemiology of human infections caused by *S. suis (Gottschalk et al., 2010)*. *S. suis* can be also easily isolated from noses and tonsils of live pigs, as well as from pig carcasses and butchers' knives (*Stanojkovic et al., 2012*).

All age categories, including suckling piglets, older piglets and pigs are prone to disease caused by *S. suis*. Animals at different production stages harbored isolates with similar phenotypic and genetic profiles, highlighting the importance of healthy animals in the maintenance of strains responsible for outbreaks of clinical disease (*Luque et al., 2010*). Even if pigs are infected with *S. suis*, the emergence of a clinically apparent disease varies periodically and is generally below 5% (*Sihvonen et al., 1988*).

The most prominent feature of *S. suis* infection in pigs and humans is meningitis. Infections caused by this species may also manifest as arthritis, endocarditis, pneumonia, rhinitis, vaginitis, and abortion (*Sanford and Tilker, 1982; Sihvonen et al., 1988*) . Human infections caused by *S. suis* are considered to be sporadic, mostly in people who come in contact with pigs and their products (*Arends and Zanen, 1988*). However, in China in 2005, the outbreak caused by *S. suis* affected more than 200 people, with almost 20% mortality rate. This epidemic has completely changed the perception of the danger which this pathogen presents to human health.

A preliminary diagnosis of *S. suis* infection in pigs is usually made on the basis of clinical signs and macroscopic lesions. However, the diagnosis is confirmed by bacteria isolation and detection of microscopic lesions in tissues. It is demonstrated that *S. suis* accumulates in the kidney during *S. suis* infection. These findings might be useful for diagnosis of streptococcal infection (*Nakayama et al., 2011*).

S. suis is α hemolytic on sheep blood agar plates with variable biochemical properties. *Kilper-Balz and Schleifer (1987)* presented and described the following biochemical characteristics of *S. suis*: acid from fermentation of D-glucose, sucrose, lactose, maltose, salicin, trehalose, and inulin, no fermentation of L-arabinose, D-mannitol, D-sorbitol, glycerol, melezitose, or D-ribose, positive hydrolysis of L-arginine, esculin, salicin, starch, and glycogen; no hydrolysis of hippurate; no production of acetoin (Voges-Proskauer - VP negative). According

to these authors *S. suis* is acid phosphatase and alkaline phosphatase negative, L-ornithine decarboxylase, N-cetylglucosaminidase, α-galactosidase, β-glucuronidase, and leucine arylamidase positive; β-alactosidase variable, resistant to optochin and doesn not grow in 6.5% NaCl or 0.04% tellurite.

According to *Tarradas et al. (1994), S. suis* can be confirmed using only a few tests: no growth in broth with 6.5% NaCl, postive esculine and trehalose reactions and negative VP test. *Higgins and Gotsschalk (1990)* and *Gottschalk et al. (1991)* proposed the following indicators as specific for *S. suis*: VP negativity, negativity for growth in the presence of 6,5% NaCl, salicin and trehalose positivity. There is an opinion of many authors *(Facklam et al, 2002; Princivalli et al., 2009*; *Gottschalk et al. 2010)* that it is sometimes very difficult to distinguish *S. suis* from viridans streptococci. Although serotyping and PCR are at the moment the only definitely methods to determine *S. suis* infections, these methods are available only to small number of diagnostic laboratories.

The aim of this study is to determine dominant biochemical characteristics of *S. suis* that can be used by large number of laboratories to be able to diagnose infection caused by this bacterium in high rate of precision.

Materials and methods

The material analysed in this study included 186 tonsil and nose swabs of clinically healthy pigs and 40 meningeal, renal and joint samples (swabs and parts of organs) of deceased pigs that had symptoms resembling those associated with *S. suis* infection. Swabs and samples were transported in trypton soy broth (Oxoid, England) within 2 h of sampling. All samples were inoculated on Columbia agar with added 5% sheep blood (CBA) (bioMérieux, France), and incubated for 24 h aerobically at 37 °C. Parts of diseased organs of pigs were homogenized, inoculated on CBA and incubated aerobically for 24 h at 37 °C. Bacterial strains were selected on the basis of colony morphology, hemolytic characteristics that they produce on blood agar, absence of growth in 6.5% NaCl broth and their microscopic appearance.

In order to definitely determine isolated strains, serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used. Strains already confirmed with specific antisera were tested using salicin fermentation test in the tube (peptone water with 1% salicine and indicator added) and commercial battery of biochemical tests (API 20 Strep and ID 32 Strep) to determine dominant biochemical characteristics.

Results and discussion

From the 226 tested samples, 34 strains of *S. suis* were isolated and thus confirmed with specific antisera for bacterial capsular antigens. All strains were

tested with the battery of commercial biochemical tests API 20 Strep and ID 32 Strep.

S. suis in this study showed very variable results in most of the tests and only a few test were indicator of *S. suis* infection. Table 1 shows dominant biochemical characteristics of *S. suis*.

In the present study all of *S. suis* strains were α hemolytic on sheep blood agar plates, showed no growth in broth with 6,5% NaCl, esculine and trehalose positive and VP negative. These results are in agreement with those described by most of the autors *(Kilper-Balz and Schleifer, 1987*; *Tarradas et al., 1994)*. Also, all strains were positive in glycogen (GLYG), lactose (LAC), sacharose (SAC), starch (AMD), leucine aminopeptidase (LAP), alanine-phenyl-alanine-proline arylamidase tests (APPA), and negative in hipurate (HIP), ribose (RIB), arabinose (ARA) and sorbitol (SOR) tests. *S. suis* strains were in high percent positive in arginine dihydrolase (ADH), β-glucoronidase (βGUR), α-galactosidase (αGAL), β-galactosidase (βGAL), methyl-β-d-glucopyranoside, glycyl-tryptophan arylamidase (MβDG) and inulin (INU) tests. These results are similar to those presented by *Kilper-Balz and Schleifer (1987)* and *Facklam et al. (2002)*.

Table 1. The dominant biochemical characteristics of *S. suis* strains

S. suis	6,5% NaCl	VP	HIP	*GLYG*	ADH	*AMD*	MβDG	βGAL	SAC	*RIB*	*AR*
	0	0	0	100	↑70	100	↑90	↑80	100	0	0
	Hemolysis	ESC	*TRE*	βGUR	αGAL	*INU*	*LAC*	APPA	GT	LAP	SO
	α	100	100	↑80	↑80	↑90	100	100	↑90	100	0

Absence of growth in 6.5% NaCl broth was the test that excluded *Enterococcus* species that have sometimes very similar biochemical patterns as *S. suis*. It was noticed that VP negativity was test that distinguishes *S. suis* from *S. bovis* and *S. salivarius* and that sometimes hipurate negativity and especially esculine and trehalose positive test were critical for distinguishing *S. suis* from some other streptococi (viridans group streptococci).

Only 38.2% of strains fermented salicin which is not in accordance with results presented by *Higgins and Gotsschalk (1990)* and *Gottschalk et al. (1991)* which proposed that positive salicin fermentation test is characteristic for *S. suis*.

Athough *S. suis* dominant features in this study were negativity in sorbitol, ribose and arabinose test, positivity in APPA, LAP, glycogen,sacharose and lactose test, it is noticed that these tests were also in high percentage a feature of other

similar bacteria and thus not critical in diagnosis. On the contrary, tests in which *S. suis* was highly positive, like β-glucoronidase (βGUR) and α-galactosidase (αGAL) were sometimes critical in diagnosis of *S. suis* infection.

Bearing all this in mind, we acknowledge that these results are similar to those of other authors *(Kilper-Balz and Schleifer, 1987*; *Tarradas et al., 1994)*. Despite that, we found that some other biochemical characteristics may be critical in diagnosis of *S. suis* infection, and can be used in all laboratoires that are not specialized in diagnosis of this pathogen.

Conclusion

The results of this study showed that except known growth and biochemical features of *S. suis* (α hemolytic on sheep blood agar, abscence of growth in 6,5% NaCl, Voges-Proskauer and hipurine negativity, esculine and trehalose positivity) some other features may be important and critical for *S. suis* diagnosis if specific antisera or PCR are not available.

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Biohemijske karakteristike sojeva *Streptococcus suis* izolovanih iz zdravih i obolelih svinja

A. Stanojković, M. M. Petrović, Z. Škrbić, V. Mandić, , N. Stanišić, M. Gogić, A. Stanojković-Sebić

Rezime

Cilj ovog istraživanja je bio da se utvrde biohemijske osobine sojeva S. suis. U ovom istraživanju biohemijski smo testirali 34 soja *S. suis* koji su izolovani kod uginulih svinja koje su prethodno pokazivale kliničke znake septikemije i meningitisa kao i od klinički zdravih svinja. Sojevi koji su potvrđeni specifičnim antiserumima su testirani komercijalnim nizom testova (API 20 Strep and ID 32 Strep) da bi se definisale njihove biohemijske osobine koje se mogu koristiti pri dijagnozi ukoliko specifični antiserumi nisu na raspolaganju. Svi sojevi *S. suis* u ovom istraživanju su bili pozitivni u testovima razlaganja eskulina, trehaloze, glikogena, laktoze, saharoze, skroba, u leucin aminopeptidaza, alanin-fenil-alanin prolin arilamidaza testovima, i negativni u Voges-Proskauer, hipurat, riboza,

arabinoza i sorbitol testovima. Takođe, *S. suis* je u visokom procentu pozitivan u arginin dihidrolaza, β-glukoronidaza, α-galaktozidaza, β-galaktozidaza, methil-β-d-glukopiranozid, glicil-triptofan arilamidaza i inulin testovima. Iako je *S. suis* često pozitivan u nekim testovima može se zaključiti da su osim već poznatih karakteristika ove bakterije (α hemoliza na krvnom agaru sa ovčijom krvi, odsustvo rasta u bujonu sa 6,5% NaCl) Voges-Proskauer, hipurat , trehaloza i eskulin testovi zajedno sa β-glukoronidaza i α-galaktozidaza testovima najznačajniji u diferencijaciji ove od drugih sličnih streptokoka.

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CHANGES IN CHEMICAL AND PHYSICOCHEMICAL CHARACTERISTICS DURING THE PRODUCTION OF TRADITIONAL SREMSKA SAUSAGE

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

[1] Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
[2] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia
[3] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of Serbia
Corresponding author: nikola0135@yahoo.com
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Abstract: The aim of this trial was to investigate changes in chemical and physico-chemical parameters during the production of traditional Sremska sausage (dry fermented sausage) from pork of three pig breeds: Mangalitsa (MA), Moravka (MO) and Swedish Landrace (SL). Analyses of all variants of sausages were carried out after stuffing (day 0) and on production days 3, 7, 14 and 21. The reduction in moisture during production caused the increase in protein, fat and ash contents ($p<0.001$) in all three variants of sausages, were found to be within the range for this type of sausages. Higher fat content in MA and MO sausages compared to SL variant was most likely a result of the different chemical composition of the meat from pigs of autochthonous breeds. All three sausage variants had a similar final pH value, but the mildest drop of pH was determined in MA sausages. Pig breed significantly affected ($p<0.05$) all three indicators of oxidative changes (thiobarbituric acid value, peroxide value and free fatty acid content). It was found that they were higher in SL compared with MA and MO sausages and to significantly increase during the production process.

Keywords: Sremska sausage, pig breed, chemical properties, physico-chemical properties

Introduction

Today, dry fermented sausages are produced from meat of modern pig breeds and manufacturing technology is based on the use of controlled ripening rooms and rapid curing techniques, resulting in lower production time and improved product safety *(Flores et al., 1997; Marco et al., 2008)*. The sausages obtained are excellent in appearance, but their typical sensory characteristics are

poor and most often they have a vigorous acidic taste that is not accepted by the consumer *(Sanz et al., 1998)*. Opposed to them, traditionally produced dry fermented sausages are made from meat of late maturing breeds of pigs and by spontaneous meat fermentation at low temperatures, without additives (nitrate, nitrite, glucono-γ-lactone, etc) and starter cultures *(Marcos et al., 2007)*. Sausages produced in this way have usually very high sensory quality.

Sremska sausage is popular dry fermented sausage in Serbia and all Balkan region. It is characterized by specific hot taste, aromatic and spicy flavour, dark red colour and hard consistency *(Stanišić et al., 2012)*. Traditionally, it was produced from the meat of autochthonous pig breeds such as Mangalitsa and Moravka and in traditional smoking house. Today, however, it is produced from modern pig breeds in controlled ripening rooms and with the use of rapid curing techniques, which gives them a slightly modified characteristics in comparison to traditional products.

The scientific knowledge of traditional produced Sremska sausage is limited and its quality is very variable, because there is very little uniformity in the production by different homemade producers and meat industries. In order to preserve the quality of traditionally Sremska sausage and to describe production process, this trial was set to investigate changes in chemical and physico-chemical parameters of Sremska sausage manufactured in traditional manner from pork of three pig breeds: Mangalitsa, Moravka and Swedish Landrace. Mangalitsa and Moravka breeds were selected as autochthonous Serbian pig breeds *(Petrović et al., 2010)*, while Swedish Landrace, was chosen as the most represented commercial pig breed in Serbia.

Materials and Methods

All pigs used in the study were bred at the farm of the Institute for Animal Husbandry (Belgrade, Serbia). The diet of animals consisted of concentrated commercial feed administered "*ad libitum*". Water was provided using automatic feeding troughs. Pigs were slaughtered when they attained their target slaughter weight of 105 ± 5.0 kg. Sremska sausages were prepared in a meat processing plant of the Institute for Animal Husbandry (Belgrade, Serbia). Three different types of Sremska sausages were produced, each from the meat of different breeds of pig: Swedish Landrace (SL), Mangalitsa (MA) and Moravka (MO).

For the production of Sremska sausage, meat from shoulder was used and back fat in the ratio of 75:25. All three variants were produced on the same day and in an identical manner: meat and fat were chopped and minced to 8 mm particle size and mixed with in a cutter (Seydelman K60, Germany), whereupon they were transferred to a mixer and the same amounts of ingredients were added: 2.2% NaCl, 0.3% glucose, 0.17% garlic (powder), 0.55% hot red paprika (powder) and 0.5% sweet red paprika (powder). The sausage mixture was stuffed into natural

casings (pig small intestines) of around 32 mm diameter. After stuffing (day 0) the sausages were drained in a cold store (4 ± 1°C) for 12 h, for the surface to dry, after which they were hung to dry in a traditional smoking house. The ripening was as follows: the first stage lasted 14 days in a traditional smoking house at 10-15°C with 75–90% relative humidity (RH), where they were smoked for 6 h each day; last 7 days (from day 15 to day 21) sausages were processed in a drying room at 14–16°C with about 75% RH, to reach about 35.0% moisture content. The total processing time was 21 day. Sampling of all variants of sausages was carried out after stuffing (day 0) and on production days 3, 7, 14 and 21.

The proximate composition of sausages was determined in the following manner: moisture content by drying samples at 105°C *(ISO 1442, 1997)*; protein content by Kjeldahl method and multiplying by factor 6.25 *(ISO 937, 1978)*; total fat content by Soxhlet method *(ISO 1443, 1973)*, and ash content by mineralization of samples at 550 ± 25°C *(ISO 936, 1998)*.

pH value was measured by pH-meter Hanna, HI 83141 (Hanna Instruments, USA), equipped with an puncture electrode. The pH meter was calibrated using standard phosphate buffers *(ISO 2917, 1999)*.

The 2-thiobarbituric acid (TBA) method was performed according to *Tarladgis et al. (1960)*. The absorbance was measured on a spectrophotometer (Spekol 1300, Analytic Jena, Germany) at 532 nm. TBA values were calculated against standard curve of malonaldehyde (MDA) and expressed as mg MDA/kg sample.

Peroxide value was determined by method described in the *ISO 3960 (1977)*, and peroxide values were expressed as milliequivalents of active oxygen per kg of fat (mEq O_2/kg).

Free fatty acid content was expressed as g oleic acid/100 g fat, after the titration with 0.1N NaOH and determining the total acidity, as described in *ISO 660 (2011)*.

An analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the SPSS 20.0 software (IBM SPSS Statistics, Version 20, IBM Corp, USA) was performed for all variables considered. If the effect of main factor (breed or time) was found significant, LSD test was used to evaluate the significance of difference at $p<0.05$.

Results and Discussion

The changes in proximate composition during ripening of three variants of Sremska sausages are shown in Table 1. The reduction in moisture during ripening caused the increase in protein, fat and ash contents ($p<0.001$) in all three variants of sausages. The fat contents of the sausages on the day of preparation were close to, but did not exactly match between variants. Sausages made from the meat of

Mangalitsa (MA) and Moravka (MO) at the beginning (day 0) had the lower moisture content ($p<0.05$), but also the higher fat content ($p<0.01$), compared with SL sausages. This discrepancy was most likely a result of the different chemical composition of the meat from pigs of autochthonous breeds, which have been found to have a higher intramuscular fat content, compared with modern pig breeds *(Petrović et al., 2010)*.

At the end of ripening, moisture content decreased to the level from 27.16% (MA) to 30.21% (SL). Such low moisture content is typical for similar products in Greece, Hungary and Croatia *(Kozacinski et al., 2008)*. Although a SL sausages had significantly the highest water content at the beginning ($p<0.05$), at the end of the production process (day 21) no significant difference in water content between SA and MA variants were establish. This could be explained by higher share of intramuscular fat in MA sausages, which was reported to extent the dehydration process by decreasing moisture diffusivity coefficient *(Arnau et al., 1997)*.

Some studies have indicated the occurrence of lower protein content in meat from autochthonous pig breeds compared to meat from commercial pig breeds *(Kim et al., 2008; Parunović et al., 2012)*, thus partly explaining the slightly lower protein content at the end of the ripening (day 21) in MA and MO sausages compared with SL variants ($p<0.05$), in the current study.

The ash values significantly decreased with time in all three variants of sausages ($p<0.001$), which is in disagreement with findings of *(Salgado et al., 2005)*, who have reported that ash content remained constant during the ripening process of Chorizo, a traditional Spanish fermented sausage.

Table 1. Changes in the proximate composition of the three variants of Sremska sausage during the production process (means ± standard deviation)

(%)	Day					
	0	3	7	14	21	p
Water						
SL	58.13 ± 1.06^{aA}	55.93 ± 0.80^{bA}	49.22 ± 1.9^{cA}	38.45 ± 0.88^{dA}	30.21 ± 0.96^{eA}	***
MA	55.74 ± 1.12^{aB}	54.06 ± 1.00^{aA}	45.74 ± 2.12^{bB}	33.72 ± 0.91^{cB}	29.31 ± 0.47^{dAB}	***
MO	55.45 ± 1.59^{aB}	52.65 ± 1.98^{aB}	47.28 ± 0.87^{bAB}	34.44 ± 0.55^{cB}	27.16 ± 0.45^{dB}	***
p	*	*	*	**	*	
Fat						
SL	21.23 ± 1.43^{aA}	21.69 ± 0.98^{aA}	24.50 ± 1.02^{bA}	30.98 ± 2.54^{cA}	38.42 ± 1.43^{dA}	***
MA	25.14 ± 1.33^{aB}	24.37 ± 1.13^{aB}	31.29 ± 1.87^{bB}	38.38 ± 1.60^{cB}	42.09 ± 0.42^{dB}	***
MO	24.28 ± 1.12^{aB}	26.02 ± 1.04^{abB}	$27.48 \pm 2.19b^{AB}$	35.36 ± 0.78^{cC}	40.73 ± 0.30^{dAB}	***
p	**	**	*	***	*	
Protein						
SL	17.65 ± 0.98^{a}	19.36 ± 1.53^{ab}	21.79 ± 0.28^{b}	25.23 ± 1.52^{cA}	25.59 ± 2.21^{cAB}	***
MA	16.16 ± 1.75^{a}	18.36 ± 1.71^{b}	19.25 ± 0.80^{b}	22.69 ± 1.04^{cB}	23.26 ± 1.06^{cA}	***
MO	17.38 ± 1.44^{a}	18.19 ± 0.44^{ab}	21.04 ± 0.57^{b}	25.04 ± 1.13^{cA}	26.60 ± 0.67^{cB}	***
p	ns	ns	ns	*	*	
Ash						
SL	2.85 ± 0.05^{a}	3.04 ± 0.13^{a}	4.41 ± 0.07^{bA}	5.31 ± 0.22^{c}	5.67 ± 0.09^{dA}	***
MA	2.94 ± 0.07^{a}	3.22 ± 0.09^{b}	3.70 ± 0.12^{cB}	5.21 ± 0.20^{d}	5.31 ± 0.14^{dB}	***
MO	2.91 ± 0.18^{a}	3.12 ± 0.09^{b}	4.20 ± 0.16^{cA}	5.18 ± 0.14^{d}	5.49 ± 0.11^{eAB}	***
p	ns	ns	**	ns	*	

$^{a-e}$ Different letters within the same row denote significant differences between means
$^{A-C}$ Different letters within the same column denote significant differences between means
^{ns} ($p \geq 0.05$); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

During the fermentation lactic acid is formed, and the pH is reduced *(Lücke, 1994)*. In this trial pH dropped moderately and reached the minimum on day 14 of the process, and remained approximately the same until the end of the production process (day 21), for sausage variant MO and SL (Figure 1). As contrast to them, the variant MA had a significant decline in the pH value from the fourteenth to the twenty-first day when the minimum pH is determined. As the fat content was highest in the MA sausages (Table 1), this results is in agreement with the trend reported in similar studies *(Olivares et al., 2010; Lorenzo and Franco, 2012)*, where a greater decrease of pH values were obtained for low fat sausages. However, some authors found no significant effect of fat level on pH *(Liaros et al., 2009)*. At the end of the production process, all variants of sausages had similar pH value, which ranged from 5.0 to 5.1, which is lower than values reported by other authors (5.2 to 6.4), for naturally fermented dry sausages *(Comi et al., 2005)*. These

established lower pH values are probably the result of added sugar in the sausage stuffing and the possible presence of sugar in spices, such as paprika *(Oberdick, 1988)*.

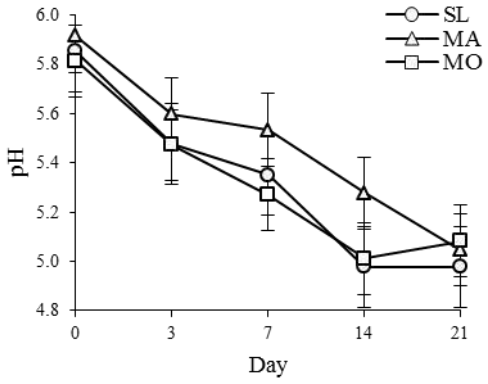

Figure 1. Changes in pH of the three variants of Sremska sausage during the production process. Each bar represents the mean value ± standard deviation.

The oxidative process during ripening of Sremska sausages was evaluated by the TBA values, peroxide index and FFA content in order to determine how it was affected by the different meat type (Figure 2).

Initial TBA values were approximately the same in all three variants of sausages, however, by the end of the production process SL sausages had the highest TBA value compared with MA and MO variants (Figure 2a). TBA values increased in all three variants of sausages during production through the manufacture process and this was also reported by other authors *(Fanco et al., 2002; Lorenzo and Franco, 2012)*. In this trial, the values for this parameter, both in the initial phase and in the final product, are lower than those found in the literature for other dry-fermented sausage varieties *(Lorenzo et al., 2000)*. The relatively low TBA values at the end of the process (0.12-0.19 mg MDA/kg) were similar to those described by *Fanco et al. (2002)*.

Peroxide values increased significantly ($p<0.05$) up to 14 days and then slightly decreased at day 21, in all three sausage variants (Figure 2b). The peroxide values in this trial were lower than one alleged by *Fanco et al. (2002)* of 16 and 28 meq O_2/kg in Androlla (a Spanish dry-cured pork sausage) at 0 and 14 days, respectively, and *Salgado et al. (2006)* who found that the average peroxide value in homemade Chorizo was 12.85 meq O_2/kg of fat. Pig breed had a significant effect on the value of peroxide, which was higher in the SL sausages compared to MO and MA sausages ($p<0.05$).

The FFA (expressed as % of oleic acid) during the production of Sremska sausages (Figure 2c), increase slightly in the first seven days of ripening and no

statistically significant difference were established between the groups of sausages. However from day 7, there was the increase in the FFA content, which was very similar in MA and MO sausages, as opposed to SL variant where the increase of FFA content was more rapidly until the end of ripening ($p<0.05$). These results are in agreement with the results of *Bañón et al. (2010)*, that sausages made from meat of indigenous breeds of pigs had significantly lower acidity values ($p<0.001$) compared to those made from the meat of modern pig breed.

As shown in Figure 2 pig breed significantly affected ($p<0.05$) all three indicators of oxidative changes. It was found that they were higher in SL compared with MA and MO sausages. This results, are in disagree with findings of *Liaros et al. (2009)* and *Lorenzo and Franco (2012)*, that the higher degree of oxidative changes are established in sausages with higher fat content. However, a different fatty acid composition of meat from autochthonous pig breeds may be one of the reasons for this inconsistency.

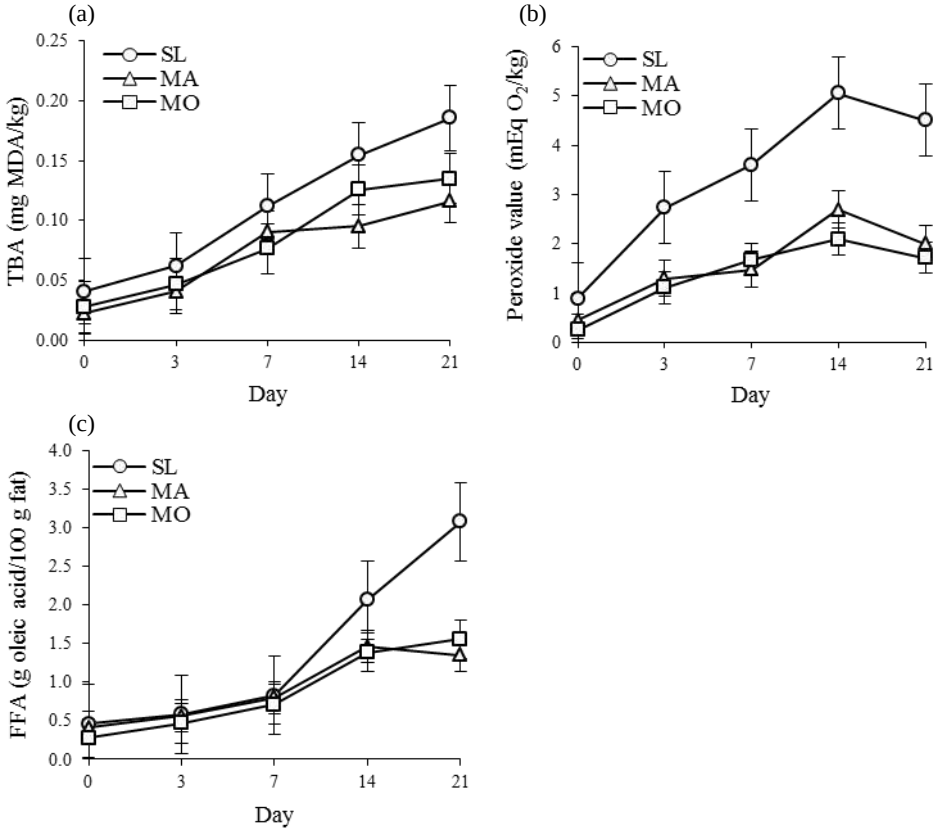

Figure 2. Changes in TBA (a), peroxide (b) and FFA (c) value of the three variants of Sremska sausage during the production process. Each bar represents the mean value ± standard deviation.

Conclusion

Traditionally produced Sremska sausage made from meat of autochthonous pig breeds (Mangalitsa nad Moravka), compared to the one produced from meat of Swedish Landrace breed, is characterised by higher fat content and consequently lower water content throughout the production process. The reduction in moisture during ripening caused the increase in protein, fat and ash contents in all three variants of sausages.

The mildest drop of pH was determined in MA sausages, although the final pH values were approximately the same in all variants. Oxidative changes, evaluated by the TBA values, peroxide index and FFA content, were significantly increasing during the production process, but were higher in SL compared with MA and MO sausages.

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Promene hemijskih i fizičko-hemijskih karakteristika tokom proizvodnje tradicionalne Sremske kobasice

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

Rezime

Cilj ovog ogleda bio je da se ispitaju promene tokom proizvodnje Sremske kobasice (suva fermentisana kobasica) na tradicionalan način od mesa tri rase svinja: Mangulica (MA), Moravka (MO) i Švedski Landras (SL). Analize svih varijanti kobasica su rađene nakon punjenja (dan 0) i nakon 3-, 7-, 14- i 21-og dana proizvodnje. Smanjenje udela vode tokom proizvodnje imalo je za posledicu povećanje udela proteina, masti i pepela u svim grupama ($p<0,001$), i bilo je karakteristično za ovaj tip kobasica. Utvrđen veći udeo masti kod MA i MO kobasica u poređenju sa SL varijantom, verovatno je posledica različitog hemijskog sastava mesa autohtonih rasa svinja. Sve tri varijante kobasica su imale sličnu finalnu pH vrednost, međutim, najblaži pad pH vrednosti tokom proizvodnje utvrđen je kod MA kobasica. Rasa svinja je imala značajan uticaj na parametre oksidativnih promena (broj tiobarbiturne kiseline, peroksidni broj i sadržaj

slobodnih masnih kiselina). Utvrđeno je da su bili veći kod SL u odnosu na MA i MO grupu kobasica i da se značajno povećavaju tokom procesa proizvodnje.

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GENOTYPE, GESTATION LENGTH, SEASON, PARITY AND SEX EFFECTS ON GROWTH TRAITS OF TWO RABBIT BREEDS AND THEIR CROSSES

S.S.A. Egena[1], G.N, Akpa[2], I.C. Alemede[1], A. Aremu[1]

[1]Department of Animal Production, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria
[2]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
*Corresponding author's email: achenejesirisky.egena@futminna.edu.ng
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Abstract: One hundred and thirty rabbits were used to evaluate the effect of genotype, gestation length, season, parity and sex on growth traits of two breeds of rabbit and their crosses. The rabbit used for the experiment were breeds of the New Zealand White (NZW) and Chinchilla (CH) breed. Six breeding bucks (three/breed) and eighteen breeding does (nine/breed) served as the foundation stock. Traits measured include: body weight (BW), nose to shoulder length (NTS), shoulder to tail length (STL), heart girth (HG), trunk length (TL) and length of ear (LE). Results revealed that, BW of the rabbits were influenced ($p<0.05$) by genotype, gestation length and season. CH x (CH x NZW) progenies had better BW at 35-d and 49-d of age while NZW x CH progenies had better BW at 21-d of age. Kittens born late (32-34 days) had better BW at 21-d, 35-d and 49-d while kittens kindled during early dry season had better BW at 21-d, 35-d and 49-d. Genotype affected ($p<0.05$) all the body measurements at 21-d, 35-d and 49-d. Gestation length affected ($p<0.05$) all the body measurements except for NTS at 21-d and HG at 49-d respectively. Season of birth also influenced ($p<0.05$) all the body measurements except for LE 21-d. Parity and sex had no effect ($p>0.05$) on BW, NTS, STL, HG, TL and LE. It was concluded that genotype, gestation length and season influenced BW and body dimensions of the two breeds of rabbit and their crosses while parity and sex had no effect.

Key words: Genotype, gestation, season, parity and sex.

Introduction

There is no doubt that the Nigerian population is fast growing. The recent human population figure of Nigeria was put at over 140 million (*NPC*, 2006). This means that urgent action needs to be taken if the animal protein need of the people

is to be met. Livestock have a big role to play in ensuring food security through increased output of their products in the form of meat, egg and milk. Livestock products are generally rich in nutrients and are of high biological value and hence very suitable for man. They are a good source of micronutrients difficult to obtain in adequate quantities from plant sources (*Adama*, 2008). One of the animals likely to play a key role in alleviating protein deficiency in the population is the rabbit. The rabbit is prolific, with excellent meat quality (*Fielding*, 1991). The meat is white, low in fat, rich in protein and in some minerals and vitamins (*Aduku and Olukosi*, 1990). There is no known taboo (cultural or religious) against the consumption of its meat (*Biobaku*, 1992). This makes it a veritable source of easily available protein. It has fast growth, short gestation period, ability to utilize feeds that are not-competed for by man and small body size. The potential for genetic improvement is a characteristic that makes the rabbit suitable as a source of cheap and readily available animal protein.

For the rabbit to meet its full potential as a source of meat and possibly fur, its genetic improvement in Nigeria must be undertaken. One of the prerequisite for genetic improvement is the knowledge of genetic parameters related to important economic traits (*Akanno and Ibe*, 2005). With such knowledge, it becomes easier for breeders to cross different breeds of rabbits with the hope of tapping into hybrid vigour that may arise as a result of such crosses. There are many breeds of rabbit; therefore, this study aims at evaluating the influence of genotype on growth traits of two breeds of rabbit and their crosses. The effect of other factors such as gestation length, season, parity and sex was also investigated.

Materials and Methods

The experiment was conducted at the Rabbitry section of the Teaching and Research Farm of the Department of Animal Production, Federal University of Technology, Minna, Niger State, Nigeria. Minna is located between latitude 9^0 37' north and longitude 6^0 32' east of the equator. The altitude is 853 feet (260 m) above sea level. Annual precipitation averages 1312 mm with a mean temperature of between 19^0C and 37^0C. The mean relative humidity is between 21 – 73 % (*Climatetemp*, 2011). Animals used for this study were NZW and CH rabbits obtained from the National Veterinary Research Institute Vom, Plateau state, Nigeria. This represents the main rabbit types found in the country. Twenty four rabbits made up of 3 bucks per breed, and 9 does per breed served as the foundation animals. The animals were housed in groups (according to breed) in well ventilated and shaded hutches. Feed (16 % CP; 2776 Kcal/Kg ME formulated concentrate, Mango leaves, *Tridax procumbens* as well as legume hay supplement) and water were given *ad libitum* throughout the experimental period. Other routine management practices were observed. Mating began when the rabbits were

between 4-5 months of age (120-150 days) and weighing between 1.45-1.50 Kg. Pregnancy was monitored via palpation of the abdominal region between the thighs. Nesting boxes were placed in the doe's hutch five days pre kindling.

Traits measured include BW, NTS, distance from the nose to the point of the shoulder; STL, distance from the point of the shoulder to the pin bone or the end of coccygeal vertebrate; HG, body circumference measured just behind the fore limbs; TL, the longitudinal distance from the point of the shoulder to the tuberosity of the ischium and LE, distance from the point of attachment of the ear to the tip of the ear. The genetic types were form from the mating of the breeds' i.e, purebred, crossbred and its reciprocal, and backcrossing (Table 1). Statistical analysis was performed by means of the PROC GLM procedure of SAS (*SAS*, 1993) with the model

Table 1. Description of genetic group of sires, dams and progenies

Genetic group of sire	Genetic group of dam	Genetic group of progeny
NZW	NZW	NZW x NZW
CH	CH	CH x CH
NZW	CH	NZW x CH
CH	NZW	CH x NZW
NZW	NZW X CH	NZW x (NZW x CH)
CH	CH x NZW	CH x (CH x NZW)
NZW = New Zealand White; CH= Chinchilla.		

$$Y_{ijklmn} = \mu + B_i + C_j + D_k + E_l + F_m + G_n + e_{ijklmn}$$

Where Y_{ijklmn} = record of dependent variable (BW, NTS, STL, HG, TL and LE), μ = overall mean, B_i = effect of the i^{th} genotype (1,…,6), C_j = effect of the j^{th} litter size, D_k = effect of the k^{th} gestation length (1,…,3), E_l = effect of the l^{th} parity (1,2), F_m = effect of the m^{th} sex (Male, Female), G_n = effect of the n^{th} season (1,…,4) and e_{ijklmn} = random error effect.

Results and Discussion

The body weight data for 21, 35 and 49-d of age are presented in Table 2. Mean weights at 21, 35 and 49-d for the various genotypes were significantly ($p<0.05$) different. The NZW x NZW kittens had heavier body weight at 21-d (227.06 g). NZW x NZW and CH x (CH x NZW) kittens had similar body weights at 35-d but CH x (CH x NZW) kittens were superior at 49-d (602.00 g). The mean weights of rabbits by gestation length significantly ($p<0.05$) differed at 21, 35 and 49-d. Kittens born 32-34 days (late gestation) were consistently superior in body weight over those kindled earlier or later (223.97; 386.36; 531.92). Kittens kindled very late (>34 d) were generally poorer than the others in body weight. The mean weights by season were significantly ($p<0.05$) different at 21, 35 and 49-d. Kittens born at the onset of the dry season were better than those born during early rain,

late rain and late dry season (270.00; 533.33; 773.33). Kittens born during late dry season were poorer in terms of body weight except at 49-d when kittens born during late rain were poorer. Parity and sex means did not differ significantly ($p<0.05$) for body weight at various ages. Kittens kindled at 1st parity were superior in body weight over those kindled at 2nd parity. Females were only heavier at 21-d while males predominated at 35 and 49-d respectively. The crossbred recorded lower performances when compared to the NZW x NZW pure breed crosses. The lower values observed for the crossbred may be because; heterosis is generally low for growth traits (about 5 %) and even lower for anatomical traits (e.g. body shape). Even in the presence of substantial heterosis, the better F1 crossbred may not necessarily exceed the better parent; unless both parents have similar performance which clearly was not the case observed in this study. The better performance of the pure breeds over the crossbred contradicts the report of *Chineke et al.* (2003). The mean values obtained for 21, 35 and 49-d were not too far from values earlier reported by some authors (*Adesina*, 2000; *Alokan*, 2000; *Babatunde et al.*, 2000; *Sanni and Dada*, 2001).

Table 2. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d body weight (g) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	227.06 ± 11.85^{a}	420.59 ± 19.07^{a}	544.67 ± 35.42^{ab}
CH x CH	187.63 ± 11.20^{b}	342.11 ± 18.04^{b}	429.68 ± 31.47^{c}
NZW x CH	192.24 ± 9.07^{b}	319.93 ± 14.86^{b}	393.70 ± 26.40^{c}
CH x NZW	212.32 ± 9.23^{ab}	354.07 ± 15.14^{b}	492.92 ± 27.99^{bc}
NZW X (NZW x CH)	188.68 ± 11.20^{b}	340.00 ± 18.04^{b}	485.88 ± 33.27^{bc}
CH x (CH x NZW)	216.92 ± 13.55^{ab}	416.00 ± 24.87^{a}	602.00 ± 43.37^{a}
Gestation length			
Early (29-31 days)	189.35 ± 6.22^{b}	339.70 ± 11.22^{b}	446.68 ± 19.46^{b}
Late (32-34 days)	223.97 ± 6.01^{a}	386.35 ± 10.51^{a}	531.92 ± 18.50^{a}
Very late (35days +)	164.23 ± 12.69^{c}	290.85 ± 21.99^{c}	355.39 ± 36.99^{c}
Season			
Early rain	205.47 ± 6.13^{b}	377.05 ± 9.81^{b}	502.18 ± 17.59^{b}
Late rain	198.33 ± 6.67^{b}	325.35 ± 10.63^{b}	425.83 ± 19.17^{b}
Early dry	270.00 ± 28.31^{a}	533.33 ± 44.23^{a}	773.33 ± 76.69^{a}
Late dry	170.00 ± 24.52^{b}	317.50 ± 38.31^{b}	497.50 ± 66.41^{b}
Parity			
1st	203.90 ± 5.59	363.10 ± 8.52	483.30 ± 14.71
2nd	200.60 ± 7.71	355.00 ± 17.58	468.90 ± 28.87
Sex			
Male	202.60 ± 6.09	371.30 ± 12.02	491.80 ± 22.60
Female	203.70 ± 6.72	347.40 ± 10.51	464.40 ± 16.16

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

NTS length by genotype differed significantly ($p<0.05$) at 21, 35 and 49-d (Table 3). CH x NZW and NZW x CH kittens were superior over other genotypes in NTS measurements (9.96 and 9.70). The least performance in NTS measurement was observed for NZW x (NZW x CH) kittens at 21-d (8.86), NZW x NZW kittens at 35-d (10.70) and, CH x CH kittens at 49-d (11.55) respectively. There was no difference ($p>0.05$) in NTS measurement by gestation length at 21 and 49-d. However, gestation length significantly ($p<0.05$) affected NTS at 35-d. Late gestated kittens (11.57) were superior in NTS measurement than those born either early or very late. Season significantly ($p<0.05$) affected NTS at 21, 35 and 49-d. Kittens born during early dry season and late rain were statistically superior in NTS measurements at 21 and 35-d. Kittens born during late dry season were poorer at 21-d NTS while those kindled during early rain were poorer at 35 and 49-d. Mean NTS length by parity were not significantly ($p>0.05$) different at 21, 35 and 49-d. However, kittens born during 2nd parity consistently had longer NTS length compared to those born at 1st parity. Mean NTS length by sex were not significantly ($p>0.05$) influenced. Male kittens however had longer NTS length than females.

Table 3. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d NTS (cm) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	9.20 ± 0.18^{b}	10.70 ± 0.19^{b}	12.12 ± 0.23^{ab}
CH x CH	8.87 ± 0.17^{b}	10.76 ± 0.18^{b}	11.55 ± 0.21^{b}
NZW x CH	9.70 ± 0.14^{a}	11.46 ± 0.15^{a}	12.49 ± 0.17^{a}
CH x NZW	9.96 ± 0.14^{a}	11.51 ± 0.15^{a}	12.60 ± 0.18^{a}
NZW X (NZW x CH)	8.86 ± 0.17^{b}	11.04 ± 0.18^{ab}	12.14 ± 0.22^{ab}
CH x (CH x NZW)	9.19 ± 0.20^{b}	10.84 ± 0.25^{b}	12.26 ± 0.28^{a}
Gestation length			
Early (29-31 days)	9.07 ± 1.11	10.63 ± 0.10^{c}	11.95 ± 0.13
Late (32-34 days)	11.26 ± 1.07	11.57 ± 0.10^{a}	12.49 ± 0.13
Very late (35days +)	9.21 ± 2.26	11.17 ± 0.20^{b}	12.25 ± 0.26
Season			
Early rain	9.01 ± 0.09^{b}	10.82 ± 0.10^{b}	11.90 ± 0.12^{c}
Late rain	9.82 ± 0.10^{ab}	11.46 ± 0.11^{ab}	12.48 ± 0.13^{bc}
Early dry	9.93 ± 0.43^{a}	12.03 ± 0.45^{a}	13.53 ± 0.51^{a}
Late dry	8.98 ± 0.37^{b}	11.18 ± 0.39^{ab}	13.00 ± 0.44^{ab}
Parity			
1st	9.21 ± 0.09	10.91 ± 0.10	12.05 ± 0.11
2nd	9.79 ± 0.12	11.66 ± 0.12	12.63 ± 0.13
Sex			
Male	9.41 ± 0.11	11.21 ± 0.10	12.36 ± 0.12
Female	9.36 ± 0.11	11.07 ± 0.10	12.13 ± 0.14

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

The mean of STL by genotype differed significantly ($p<0.05$) at 21, 35 and 49-d respectively (Table 4). NZW x NZW, CH x NZW and CH x (CH x NZW) kittens were similar in STL measurement at 21-d. NZW x NZW and CH x NZW kittens were similar at 35-d, while at 49-d, no statistical difference was observed between NZW x NZW and CH x (CH x NZW) kittens in STL measurement. CH x CH kittens showed poor performance in STL measurements at 21 and 35-d respectively (15.86; 20.01). Gestation length means differed significantly ($p<0.05$) for STL at various ages. Kittens born during late gestation were superior in STL measurement compared to those born at early and very late gestation (16.96; 21.86; 24.88). Season means for STL at all ages also differed significantly ($p<0.05$) with kittens kindled during early dry season having superior STL measurements (18.67; 25.00; 28.67) while those kindled during late dry season were no different statistically in STL measurement at all ages from those kindled at early and late rain. Parity had no significant ($p>0.05$) effect on all STL measurements. Longer STL was however observed for kittens born at 2nd parity compared to those born at 1st parity. Sex equally had no significant ($p>0.05$) influence on STL measurements at all ages. However, males had consistently better STL measurements compared to their female counterparts.

Table 4. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d STL (cm) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	17.28 ± 0.36^{a}	22.61 ± 0.45^{a}	24.63 ± 0.58^{a}
CH x CH	15.86 ± 0.34^{c}	20.01 ± 0.42^{c}	22.06 ± 0.51^{b}
NZW x CH	16.07 ± 0.27^{bc}	20.98 ± 0.35^{bc}	23.63 ± 0.43^{ab}
CH x NZW	17.00 ± 0.28^{ab}	20.96 ± 0.35^{bc}	24.75 ± 0.45^{a}
NZW X (NZW x CH)	15.99 ± 0.34^{bc}	20.01 ± 0.42^{c}	23.34 ± 0.54^{ab}
CH x (CH x NZW)	16.62 ± 0.41^{abc}	21.62 ± 0.58^{ab}	25.00 ± 0.70^{a}
Gestation length			
Early (29-31 days)	16.10 ± 0.20^{b}	20.23 ± 0.26^{b}	22.87 ± 0.32^{b}
Late (32-34 days)	16.96 ± 0.19^{a}	21.86 ± 0.24^{a}	24.88 ± 0.30^{a}
Very late (35days +)	15.85 ± 0.41^{b}	19.69 ± 0.50^{b}	23.08 ± 0.61^{b}
Season			
Early rain	16.48 ± 0.19^{b}	21.01 ± 0.24^{b}	23.57 ± 0.30^{b}
Late rain	16.41 ± 0.21^{b}	20.74 ± 0.26^{b}	23.88 ± 0.33^{b}
Early dry	18.67 ± 0.87^{a}	25.00 ± 1.09^{a}	28.67 ± 1.31^{a}
Late dry	15.50 ± 0.76^{b}	19.88 ± 0.95^{b}	23.00 ± 1.14^{b}
Parity			
1st	16.38 ± 0.16	20.74 ± 0.21	23.42 ± 0.25
2nd	16.66 ± 0.27	21.57 ± 0.39	24.70 ± 0.42
Sex			
Male	16.61 ± 0.21	21.23 ± 0.28	24.22 ± 0.36
Female	16.36 ± 0.19	20.70 ± 0.23	23.46 ± 0.28

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

The means of heart girth by genotype, gestation length and season of birth, parity and sex at 21, 35 and 49-d of age are presented in Table 5. HG values differed significantly ($p<0.05$) by genotype at all ages. The CH x (CH x NZW), NZW x (NZW x CH) and CH x NZW kittens had similar HG measurements than the others at 21-d (14.00; 13.59; 13.31) while CH x (CH x NZW) kittens were superior at 35-d (17.85) when compared to the other genotypes. At 49-d, HG measurements for CH x (CH x NZW) and NZW x (NZW x CH) kittens were comparable statistically. CH x CH kittens had poorer HG measurement at 21, 35 and 49-d (12.42; 14.20; 15.58) although not statistically different from that of NZW x NZW and NZW x CH (21-days), and NZW x CH (35 and 49-d). The means of HG by gestation length also differed significantly ($p<0.05$) at 21 and 35-d with kittens born during late gestation having improved HG measurements at all ages (13.82; 16.08). Mean HG measurement was significantly ($p<0.05$) affected by season of birth with kittens born during early dry season performing better than the others at 21 and 35-d (14.83; 18.67). Mean HG measurements were comparable for kittens kindled at early rain, early dry and late dry season. Parity and sex means did not differ significantly ($p>0.05$) for HG measurements at various ages. However, better performance was observed for kittens born at 1st parity. Females were better than males only at 21-d post-partum.

Table 5. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d HG (cm) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	13.14 ± 0.30^{bc}	15.09 ± 0.30^{cd}	16.90 ± 0.43^{b}
CH x CH	12.42 ± 0.28^{c}	14.20 ± 0.29^{e}	15.58 ± 0.38^{c}
NZW x CH	12.80 ± 0.23^{bc}	14.75 ± 0.23^{de}	16.63 ± 0.32^{bc}
CH x NZW	13.31 ± 0.23^{ab}	15.68 ± 0.24^{bc}	17.15 ± 0.34^{b}
NZW X (NZW x CH)	13.59 ± 0.28^{ab}	16.05 ± 0.29^{b}	18.77 ± 0.40^{a}
CH x (CH x NZW)	14.00 ± 0.34^{a}	17.85 ± 0.39^{a}	19.83 ± 0.52^{a}
Gestation length			
Early (29-31 days)	12.69 ± 0.15^{b}	14.86 ± 0.21^{b}	17.00 ± 2.61
Late (32-34 days)	13.82 ± 0.15^{a}	16.08 ± 0.20^{a}	21.30 ± 2.48
Very late (35days +)	12.00 ± 0.31^{c}	14.62 ± 0.42^{b}	15.77 ± 4.96
Season			
Early rain	13.21 ± 0.16^{b}	15.55 ± 0.20^{b}	17.44 ± 0.26^{ab}
Late rain	12.97 ± 0.17^{b}	15.08 ± 0.22^{b}	16.70 ± 0.29^{b}
Early dry	14.83 ± 0.73^{a}	18.67 ± 0.89^{a}	19.67 ± 1.14^{a}
Late dry	13.25 ± 0.63^{b}	15.38 ± 0.77^{b}	18.25 ± 0.99^{ab}
Parity			
1st	13.13 ± 0.15	17.00 ± 1.64	17.26 ± 0.25
2nd	13.16 ± 0.19	15.84 ± 0.28	17.20 ± 0.31
Sex			
Male	13.02 ± 0.17	17.16 ± 1.78	17.34 ± 0.29
Female	13.28 ± 0.16	15.41 ± 0.20	17.13 ± 0.26

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

Trunk lengths by genotype, gestation length, season, parity and sex are presented in Table 6. TL measurement was significantly ($p<0.05$) affected by the genotype of the kittens. NZW x NZW, CH x (CH x NZW), CH x NZW, and NZW x (NZW x CH) had comparable TL measurements at 21-d. CH x (CH x NZW) kittens were superior to other genotypes at 35-d (18.35) while there were similarities in TL measurements of CH x (CH x NZW), NZW x NZW and CH x NZW kittens at 49-d. CH x CH kittens were poorer in TL measurements at 21-d (13.30). Gestation length means differed significantly ($p<0.05$) at all ages with kittens born during late gestation outperforming those born during very late and early gestation at all ages (14.44; 18.19; 20.28). Kittens born during very late and early gestation were comparable in TL measurements. Season significantly ($p<0.05$) affected TL at all ages. Kittens born during early dry, early and late rainy season had similar (longer) TL measurements at 21-d. At 35 and 49-d however, kittens kindled during early dry season were consistently better (20.00; 23.00) than those kindled at other seasons. The means of TL by parity and sex at 21, 35 and 49-d were not significantly ($P>0.05$) different.

Table 6. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d TL (cm) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	14.55 ± 0.31^{a}	18.19 ± 0.42^{ab}	20.43 ± 0.54^{ab}
CH x CH	13.30 ± 0.29^{c}	17.02 ± 0.40^{bc}	17.87 ± 0.48^{c}
NZW x CH	13.71 ± 0.24^{ab}	17.23 ± 0.33^{abc}	19.28 ± 0.40^{bc}
CH x NZW	14.32 ± 0.24^{a}	17.09 ± 0.34^{bc}	20.17 ± 0.42^{ab}
NZW X (NZW x CH)	13.70 ± 0.29^{ab}	16.68 ± 0.40^{c}	19.53 ± 0.50^{b}
CH x (CH x NZW)	14.40 ± 0.35^{a}	18.35 ± 0.55^{a}	21.25 ± 0.66^{a}
Gestation length			
Early (29-31 days)	13.60 ± 0.17^{b}	16.56 ± 0.22^{b}	19.03 ± 0.32^{b}
Late (32-34 days)	14.44 ± 0.17^{a}	18.19 ± 0.21^{a}	20.28 ± 0.30^{a}
Very late (35days +)	13.39 ± 0.35^{b}	16.23 ± 0.44^{b}	18.85 ± 0.60^{b}
Season			
Early rain	13.98 ± 0.16^{ab}	17.49 ± 0.22^{b}	19.52 ± 0.29^{b}
Late rain	13.94 ± 0.18^{ab}	17.00 ± 0.24^{b}	19.49 ± 0.32^{b}
Early dry	15.33 ± 0.76^{a}	20.00 ± 0.99^{a}	23.00 ± 1.27^{a}
Late dry	13.25 ± 0.66^{b}	16.25 ± 0.86^{b}	19.25 ± 1.10^{b}
Parity			
1st	13.94 ± 0.15	17.21 ± 0.20	19.35 ± 0.26
2nd	14.06 ± 0.20	17.63 ± 0.30	20.19 ± 0.36
Sex			
Male	13.88 ± 0.24	17.49 ± 0.24	20.02 ± 0.32
Female	13.91 ± 0.16	17.14 ± 0.22	19.20 ± 0.27

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

The means of length of ear by genotype, gestation length and season of birth, parity and sex are presented in Table 7. Means of LE by genotype differed significantly ($p<0.05$) at all ages with NZW x NZW, CH x NZW and NZW x CH kittens having similar (longer) ears at 21-d. NZW x NZW kittens were superior at 35-d (8.25) while comparable LE measurements were observed for NZW x NZW, CH x (CH x NZW) and NZW x (NZW x CH) kittens at 49-d. Kittens born as a result of CH x CH mating had shorter LE measurement at 21-d (5.19). Gestation length means differed significantly ($p<0.05$) at all ages with kittens born during late and very late gestation outperforming those born during early gestation at 21-d. At 35 and 49-d however, very late (>34 d) gestated kittens were inferior to their peers in LE measurements. Parity and sex had no significant ($P>0.05$) effect on LE measurements of kittens. It was however observed that kittens kindled at 1st parity had slightly longer LE at 35 and 49-d. Females equally had longer but no significant LE at 21 and 35-d.

Table 7. Effect of genotype, gestation length, season, parity and sex on 21-d, 35-d and 49-d LE (cm) of rabbit

Factor	21-d (**N=125**)	35-d (**N=120**)	49-d (**N=112**)
Genotype			
NZW x NZW	6.01 ± 0.121^{a}	8.25 ± 0.13^{a}	8.90 ± 0.15^{a}
CH x CH	5.19 ± 0.11^{c}	7.47 ± 0.13^{b}	8.15 ± 0.13^{b}
NZW x CH	5.70 ± 0.09^{ab}	7.48 ± 0.11^{b}	8.32 ± 0.11^{b}
CH x NZW	5.88 ± 0.10^{ab}	7.36 ± 0.11^{b}	8.25 ± 0.12^{b}
NZW X (NZW x CH)	5.60 ± 0.11^{b}	7.51 ± 0.13^{b}	8.52 ± 0.14^{ab}
CH x (CH x NZW)	5.65 ± 0.14^{b}	7.62 ± 0.17^{b}	8.57 ± 0.18^{ab}
Gestation length			
Early (29-31 days)	5.44 ± 0.07^{b}	7.52 ± 0.08^{a}	8.30 ± 0.09^{ab}
Late (32-34 days)	5.88 ± 0.07^{a}	7.72 ± 0.08^{a}	8.58 ± 0.08^{a}
Very late (35days +)	5.85 ± 0.14^{a}	7.18 ± 0.16^{b}	8.15 ± 0.16^{b}
Season			
Early rain	5.60 ± 0.07	7.74 ± 0.09^{ab}	8.51 ± 0.08^{ab}
Late rain	5.77 ± 0.07	7.38 ± 0.08^{b}	8.24 ± 0.08^{b}
Early dry	6.17 ± 0.32	8.07 ± 0.34^{a}	9.03 ± 0.34^{a}
Late dry	5.63 ± 0.27	7.33 ± 0.29^{b}	8.40 ± 0.29^{ab}
Parity			
1st	5.61 ± 0.06	7.63 ± 0.07	8.44 ± 0.07
2nd	5.84 ± 0.08	7.51 ± 0.11	8.38 ± 0.09
Sex			
Male	5.67 ± 0.07	7.61 ± 0.08	8.44 ± 0.09
Female	5.69 ± 0.07	7.71 ± 0.19	8.39 ± 0.08

Means denoted by different superscripts within the same column differ ($p<0.05$) significantly; ± SEM; NZW = New Zealand White; CH = Chinchilla.

The body measurements were generally observed to show increase with age of the rabbits. This is expected in normal growing and healthy rabbits. The differences observed in body traits, could not be linked solely to breed differences

since no particular trend was observed in the superiority of the genotypes as per the traits studied. However, CH x NZW kittens were superior in nose to shoulder measurements at all ages, followed by NZW x CH kittens. This trend repeated itself in almost all the body traits. This difference could be tied to maternal effects since kittens produced from the two mating (CH x NZW and NZW x CH) basically have the same genes (supposing that the gene pool of the NZW males was the same as that of the NZW dams). This seems to indicate the superiority of NZW does over their CH peers. NZW does have been reported to exhibit an outstanding maternal ability related to behaviour, fecundity and lactation (*Lukefahr et al.*, 1983a, 1983b; *Ozimba and Lukefahr*, 1991; *McNitt et al.*, 2000). Nose to shoulder length measurements at all ages were lower than that reported by *Chineke et al.* (2006). Breed differences observed in all body traits agrees with earlier findings (*Chineke et al.*, 2003; *Chineke et al.*, 2006; *Oke et al.*, 2010).

Body weights did not increase with gestation length. However, kittens born within 32-34 d had better body weights at all ages although 32-34 d does not fall within the 30±2 days gestation length reported for rabbits by *Omoikhoje et al.* (2008). Gestation was an important source of variation for body measurements considered in the study. Kittens born within 32-34 d had better body measurements compared to those born earlier or lately. This effect of gestation length on body measurements agrees with the findings of *Chineke et al.* (2003). Season of kindling contributed significantly to the variations in the body weights of the rabbits. The differences observed in body weight cannot be tied to season alone though but also feed availability especially roughage. The variation could possibly be linked to the pattern of seasonality of weather conditions and its effect on roughage quality. According to *Khalil et al.* (1987), seasonality can exert their effects on weaning weights of rabbits in the amount of milk produced by the suckling dams and, on growth performance at later ages (through the quantity and quality of the directly ingested feed, the appetite of the young and food utilization during the post-weaning months). *Orengo et al.* (2004) also reported that season had a great effect on all weaning traits. Season also influenced growth-related traits at different ages except 21-d LE. In all the measurements, kittens born during early dry season were superior. This period likely was the most conducive period for the rabbits leading to enhanced feed intake and hence, better growth. The differences observed in growth-related traits in different season might also be attributed to health and the nutritional status of the does and kittens. According to *Orengo et al.* (2004), hot season has a depressor effect on live weight of rabbits.

Parity effect was found to be not significant for body weight and growth-related traits although kittens born during 1st parity showed the best performance in terms of body weight at all ages. This is in disagreement with earlier reports (*Abdel-Ghany et al.*, 2000; *Garcia*, 2001; *Prayaga and Eady*, 2003; *Chineke et al.*, 2006; *Abou Khadiga et al.*, 2008) as they reported parity to be a significant source of variation of body weight in rabbits. Sex effect was also found not to be

significant for body weight and growth-related traits at all ages and this agrees with the reports of *Chineke et al*. (2003) and *Abou Khadiga et al*. (2008).

Conclusion

From the results of this study, it could be concluded that genotype, gestation length and season of birth influenced body weight and growth-related traits of the rabbit breeds and their crosses while parity and sex had no effect. Maternal additive effect appears to be more important than paternal additive effect in influencing body traits in the rabbits.

Uticaj genotipa, trajanja gestacije, sezone, pariteta i pola na osobine porasta dve rase zečeva i njihovih meleza

S.S.A. Egena, G.N, Akpa, I.C. Alemede, A. Aremu

Rezime

Sto trideset zečeva je korišćeno za evaluaciju efekata genotipa, dužine gestacije, sezone, pariteta i pola na osobine porasta dve rasa zečeva i njihovih meleza. Zečevi korišćeni u ogledu su rase novozelandski beli zec (NZV) i činčila (CH). Šest priplodnih mužjaka (tri/rasa) i osamnaest priplodnih ženki (devet/rasa) su predstavljali matično stado. Merene su sledeće osobine: telesna masa (BW), dužina od nosa da ramena (NTS), dužina od ramena do repa (STL), obim srca (HG), dužina tela (TL) i dužine uha (LE). Rezultati su pokazali da je BW zečeva pod uticajem ($p<0,05$) genotipa, dužine gestacije i sezone. CH x (CH x NZV) potomci imali su bolju BW u uzrastu od 35 i 49 dana starosti, dok su potomci NZV x CH imali bolju BW u uzrastu od 21dana. Zečevi rođeni kasno (32-34 dana) imali su bolju BW 21., 35. i 49. dana, dok su zečevi rođeni tokom ranog perioda sušne sezone imali bolju BW 21., 35. i 49. dana. Genotip je uticao ($p<0,05$) na sve telsne mere 21., 35. i 49. dana. Dužina gestacije je uticala ($p<0,05$) na sve telesne mere osim NTS 21., i HG 49. dana, respektivno. Sezona rođenja je takođe uticala ($p <0.05$) na sve telesne mere osim LE 21. dana. Paritet i pol nisu imali uticaj ($p>0,05$) na BW, NTS, STL, HG, TL i LE. Zaključeno je da su genotip, dužina gestacije i sezona uticali na BW i dimenzije tela dveju rasa zečeva i njihovih meleza, dok paritet i pol nisu imali efekta.

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NATURAL MYCOBIOTA AND AFLATOXIN B_1 PRESENCE IN BEE POLLEN COLLECTED IN SERBIA

T. Petrović[1], N. Nedić[1], D. Paunović[1], J. Rajić[1], K. Matović[2], Z. Radulović[1], V. Krnjaja[3]

[1]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
[2]Veterinary Specialist Institute, Žička 34, 36000 Kraljevo, Serbia
[3]Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Serbia
Corresponding author: tpetrovic@agrif.bg.ac.rs
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Abstract: Total fungal count, incidence of fungi and aflatoxin B_1 (AFB_1) concentration were studied in 33 samples of bee pollen randomly collected from beekeepers in Serbia. The total number of fungi was determined by dilution method whereas AFB_1 was detected using the Enzyme-Linked Immuno-Sorbent Assay (ELISA). The mycological estimation showed the presence of nine genera of fungi as followed: *Acremonium, Alternaria, Aspergillus*, *Cladosporium, Epiccocum*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*, with total number ranging from 1×10^3 to 1×10^5 CFU g^{-1}. The results have shown the predominance of the fungi from the genera *Aspergillus* and *Alternaria*. Among *Aspergillus* species it was observed that the most frequent species was *A. flavus* with incidence of 27.27 %. Mycotoxin AFB_1 was detected as 100% positive in all samples (100%) with an average concentration of 8.61 μg kg^{-1}. The obtained results indicated that honey bee pollen must be strictly controlled during its manipulation in the harvesting and manufacturing. Therefore, the implementation of good manufacturing (beekeeping) practice to define procedures for honeybee products could be crucial to reduce the risk of possible contamination and provide natural and safety product without risk on the human health**.**

Key words: bee pollen, fungi, aflatoxin B_1

Introduction

Serbia posess excellent prerequisities for the development of beekeeping, due to heterogeneous relief and climatic conditions and various honey bee pasture *(Nedić et al., 2011)*. Bee pollen as one of bee product is considered as the most complete food in the nature. It is made of natural flower pollen homogenized with small quantities of nectar and bee saliva and collected at the hive entrance. The consumption of bee pollen is constantly increased due to grooving consumers

demand for the healthier and nutritious diet *(Linskens and Jorde, 1997)*. Nowadays, it is used less as a crude product, but more as dietary supplement added to the honey mixture.

Bee pollen consists of proteins, lipids, sugars, dietary fibers, trace elements, enzymes, fatty acids, vitamins and minerals *(Bonvehí and Jordà, 1997)*. Pollen has also high contents of biological active substances such as polyphenols, mainly flavonoids which possess high antioxidant capacity *(Carpes et al., 2009; Leja et al., 2007)*.

In the fresh collected bee pollen the water content is about 20-30% *(Bogdanov, 2012)* which combined with highly nutritional ingredients represent a suitable substrate for the growth of variety of microorganisms especially yeasts, moulds, spore forming bacteria and cocci *(Brindza et al., 2010)*. Therefore, the pollen loads has to be dried immediately after harvesting in order to extend its shelf life and prevent deterioration. In the dried bee pollen the moisture content should be in the range of 4 to 8% *(Mutsaers, 2005; Melo et al., 2011)*. The quality of bee pollen is highly depending on the applied methods of its preservation. Unfortunately, a part of beekeepers in Serbia traditionally carry out the draying process outdoors, on the natural flow of air, in the shadow, which could be the reason for the contamination of pollen loads with mycotoxigenic fungi.

Mycotoxins can occur in wide variety of products including maize, rice, cereals, nuts, dried food, green and roasted coffee, cacao beans and spices as a result of fungal contamination before and after harvest *(Magan and Olsen, 2004)*. Human could be exposed to mycotoxins directly by consumption of contaminated foods, or indirectly by consuming animal foods previously exposed to mycotoxins in feed *(Tarr, 2006; Krnjaja et al., 2012)*.

Mycotoxins with the most detrimental impact on animal and human health are aflatoxins. They are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites mainly by the species *Aspergillus flavus* and *Aspergillus parasiticus* which are ubiquitous in air and soil *(Rustom, 1997)*. Among aflatoxins B_1 (AFB_1) is consider as the most toxic and can cause liver cancer in humans *(Groopman et al., 1988)*.

The maximum recommended values of mycotoxins have been established in many countries for the number of food products, but there are no permissible limits for the mycotoxins in bee pollen. The scientific literature about the occurrence of mycotoxins in bee pollen is scarce. It was reported that 28.6% of *A. flavus* and *A. parasiticus* isolated from Spanish bee pollen was able to produce AFB_1 *(González et al., 2005)*. In the study of *Medina et al. (2004)* it was performed that bee pollen could be a suitable substrate for the production of ochratoxin A (OTA). Moreover, it was observed that the species from the genera *Aspergillus* and *Penicillium* isolated from Slovakian pollen should be consider as the most important producers of mycotoxins *(Kačaniová and Fikselová, 2007)*. AFB_1 was not detectable in the batch samples of Greek bee pollen with natural

mycobiota, but if the bee pollen substrate was inoculated with *A. parasiticus,* AFB_1 was detected *(Pitta and Markaki, 2010).*

Considering that the consumption of bee pollen could be a potential risk for the human health if it is contaminated with the mycotoxigenic fungi, the main objective of this study was to determine the occurrence of natural mycobiota in bee pollen originated from Serbia, as well as to estimate the level of AFB_1 in those samples. As far as we know, this is the first study about natural mycobiota and AFB_1 presence in bee pollen from Serbia.

Materials and Methods

Bee pollen samples. Thirty three samples of bee pollen were randomly purchased from the beekeepers from Serbia. The samples originated from different region in Serbia (regional geographic map used by *Lazarević et al. (2012)*: 4 samples being from Region Vojvodina, 9 samples originated from Belgrade Region, 3 sample being from Western Region, 2 samples being from Eastern Region, 9 samples being from Central Region and 6 samples being from Southern Region were collected during the period of 2010-2012 (Table 1). After collection the bee pollen samples were stored frozen, prior to the analyses.

Determination of moisture content and water activity. The moisture content was determined by drying 3-5 g of samples in an oven at 105ºC, until a constant weight (AOAC, 1997). Water activity was performed at 22ºC using a Instrument Testo 650, Germany.

Isolation and identification of fungi. Mycological analysis was performed according to the standard methods. For each bee pollen, 10 g of sample were homogenized into 90 ml of saline solution (NaCl, 8.5 g/l). A serial dilution method was done and 1 ml of dilution of 10^{-3} and 10^{-4} were poured over the surface of Sabouraud maltose agar. After 5-7 days of incubation at 25°C, the total fungal count were identified and expressed as colony-forming units per gram of bee pollen (CFU g^{-1}). The morphological characteristics of isolated colonies were identified based on macroscopic (colony appearance) and microscopic (spores forming) investigations *(Watanabe, 1994)* to the genera level, except for potent producers of AFB1, *A. flavus* which was determined to the species level.

The mycotoxins analyses. The presence of AFB_1 was detected by ELISA according to the instructions Tecna S.r.l. (Italy) ELISA kits on an ELISA reader (Biotek EL x 800TM, USA) with detection limit of 1 μg kg^{-1} for AFB_1.

Statistical analysis. The incidence of fungal species (%) was calculated as number of samples with fungal species x 100/ total number of samples. Correlation between individual values obtained for moisture content, total fungal count and AFB_1 was determined using Pearson's correlation coefficient.

Results and Discussion

Moisture content and water activity. The water content is consider as an important parameter for the quality control of dehydrated foods like bee pollen because it play an important role in organoleptic properties and maintaining shelf life. The parameters officially established for the maximum water content in dry pollen are 10, 8, 6 and 4% in Bulgaria, Argentina, Switzerland and Poland and Brazil, respectively *(Melo et al., 2011)*. According to Serbian Official Gazette the maximum established value for water content of bee pollen is 8% *(Official Gazette, 2003)*. In the tested samples the water content varied in the ranged from 7.00 to 10.56%, of which 51.51% of the samples had content above the limit set by Serbian legislation. However, these samples were within the established limits of Bulgaria (10%), for commercial bee pollen with an exception of thre samples (18, 25 and 30) that exceeded this level (Table 1).

The most important environmental factors for the fungal growth are the water activity (measures of amount of free water) and temperature *(Lacey and Magan, 1991)*. It has been recommended that the water activity should be lower than 0.30 for the good storage stability of dehydrated foods like bee pollen *(Bonvehí and Jordà, 1997)*. The mean water activity in the tested samples was 0.34 a_w which could be considered as low enough to provide the microbiological stability of the product.

Microbial contamination of bee pollen. The total fungal count was in the range of $1x10^3$ to $1x10^5$ CFU g^{-1} (Table 1). According to morphological appearance by microscopic examination nine genera of fungi were determined, such as: *Acremonium, Alternaria, Aspergillus, Cladosporium, Epiccocum, Fusarium, Mucor, Penicillium* and *Rhizopus*. The predominant fungi were from the genera *Alternaria* (48.48) and *Aspergillus* (39.39%), followed by *Penicillium* spp. (24.24%) and *Mucor* spp. (21.21%) while the most common isolated species was *A. flavus* with an incidence of 27.27% (Table 2). These results are similar to those obtained by *González et al. (2005)* and indicated that the common fungi occurred in bee pollen are related to those usually presented in the grains and cereals before and after harvest, which are also known as “field” and “storage” fungi *(Logrieco et al., 2003)*. Also, in the work of *Brindza et al. (2010)* the most of the isolated species were from the genera *Mucor, Aspergillus, Alternaria* and *Rhizopus*. According to these authors the great majority of isolated fungi from bee pollen

represented the saprophytic microorganisms inhabiting soil and plant residues, indicating that these fungi originated from the microenvironment. On the other hand, *Snowdon and Cliver (1996)* performed that the species from the genera *Aspergillus* and *Penicillium* have also been associated with the intestines of honey bee.

Table 1. The moisture content (W), water activity (a_w) and total fungal count (CFU g^{-1}) in tested bee pollen samples collected in different regions in Serbia during 2010-2012

No. of sample	Year	Sample origin	W (%)	a_w	CFU g^{-1}
1	2010	Central Region	9.21	0.33	$1.8x10^4$
2	2010	Central Region	7.98	0.30	$1.3x10^4$
3	2010	Region Vojvodina	7.42	0.31	$7.x10^3$
4	2010	Central Region	7.96	0.32	$6x10^3$
5	2010	Belgrade Region	8.56	0.33	$2x10^3$
6	2010	Central Region	8.49	0.33	$1x10^3$
7	2012	Central Region	7.92	0.30	$1.1x10^4$
8	2011	Region Vojvodina	8.01	0.30	$1x10^3$
9	2011	Belgrade Region	7.52	0.29	$8x10^3$
10	2011	Eastern Region	7.93	0.31	$2x10^3$
11	2011	Southern Region	9.07	0.33	$1x10^3$
12	2011	Belgrade Region	7.94	0.31	$6x10^3$
13	2011	Belgrade Region	8.00	0.31	$1.3x10^4$
14	2011	Belgrade Region	7.91	0.33	$2x10^3$
15	2011	Eastern Region	7.57	0.30	$1.7x10^4$
16	2011	Belgrade Region	9.47	0.35	$4x10^3$
17	2011	Central Region	9.91	0.32	$1x10^5$
18	2011	Belgrade Region	10.56	0.40	$3x10^3$
19	2010	Region Vojvodina	9.01	0.34	$8x10^3$
20	2012	Region Vojvodina	7.58	0.30	$8x10^3$
21	2012	Southern Region	8.77	0.31	$1x10^4$
22	2012	Belgrade Region	8.76	0.31	$2x10^4$
23	2012	Central Region	7.94	0.30	$2x10^4$
24	2012	Central Region	8.38	0.31	$4x10^3$
25	2012	Central Region	10.19	0.36	$2x10^3$
26	2012	Southern Region	8.44	0.44	$1x10^4$
27	2012	Western Region	9.03	0.43	$2x10^4$
28	2012	Western Region	9.83	0.46	$6x10^3$
29	2012	Belgrade Region	9.29	0.41	$3x10^3$
30	2012	Southern Region	10.26	0.40	$7x10^3$
31	2010	Southern Region	7.01	0.31	$3x10^3$
32	2010	Southern Region	7.00	0.29	$3.2x10^3$
33	2010	Western Region	7.93	0.32	$2x10^3$

Table 2. The incidence (%) of fungal species isolated from tested bee pollen samples

Fungal species	Incidence (%)
Acremonium spp.	3.03
Alternaria spp.	48.48
Aspergillus spp.	39.39
Aspergillus flavus	27.27
Cladosoprium spp.	6.06
Epiccocum spp.	3.03
Fusarium spp.	9.09
Mucor spp.	21.21
Penicillium spp.	24.24
Rhizopus spp.	9.09

Mycotoxicological analysis. Mycotoxicological analysis by ELISA revealed the presence of AFB_1 in all 33 samples, with an average concentration of 8.61 μg kg^{-1} (Table 3). High level of total aflatoxins were found in Slovakian poppy bee pollen (up to 16.20 μg kg^{-1}) and rape bee pollen (up to 5.40 μg kg^{-1}), while this concentration was lower in the samples of sunflower bee pollen *(Kačaniová et al., 2011).* In Greece in the study of *Pitta and Markaki (2010)* AFB_1 was not found in a batch sample of bee pollen with natural mycobiota throughout the 20 days of incubation period, but when bee pollen was used as a substrate for inoculation of *A. parasiticus*, AFB_1 was detected in inoculated samples after the 3rd day of incubation. According to the results of *González et al. (2005)* toxigenic potential of toxigenic *Aspergillus* isolates was in range from 3.5 to 9.3 μg AFB_1 kg^{-1}.

Table 3. The concentration of aflatoxin B_1 (AFB_1) in tested bee pollen samples

Item	AFB_1
Sample size[a]	33/33
Incidence %	100
Range (μg kg^{-1})	3.49-14.02
Mean[b] (μg kg^{-1})	8.61

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

The positive correlation found between the moisture content and level of AFB_1 (r = 0.05), between the water activity and level of AFB_1 (r = 0.01), as well as between the moisture content and total fungal count (r = 0.23) was not significant. In addition, the established negative correlation between the water activity and total fungal count (r= - 0.07) and between the total fungal count and level of AFB_1 (r = - 0.18) was also not significant.

It is usually accepted that the fungal growth and mycotoxins production are related with an interaction occurring among the strain, substrate and environmental conditions. Likewise, it has already been reported that the presence of fungi in products does not necessarily mean the presence of mycotoxins *(Harley, 1997)* as well as mycotoxins content are not always related to the number of fungi presented *(Tarr, 2006)*.

Bee pollen was a suitable medium for the proliferation of fungi and AFB_1 production, probably not only because it is a rich source of free amino acids, sugars, minerals etc., which could stimulate the production of AFB_1, but also because of improperly handle and store during production. Concerning that the a_w value of the tested samples was in the limit that ensure the microbial stability of the product it could be assumed that the production of AFB_1 was occurred in the period from the gathering of the pollen loads to drying and packaging stages. Growth of fungi and subsequent production of mycotoxins depend on climatic conditions and cultivation techniques or systems. Therefore, the presence of AFB1 in the tested samples could be explained by the drought and high ambient temperatures which contributed the production of AFB_1 *(Bruns, 2003)*. According to Annual Reports by Republic Hydro-meteorological Service of Serbia on agro-meteorological conditions in Serbia during the period from 2010 to 2012 quoted on drought during the period of June-August. The specified period largely coincides with the beekeepers season and collection of honeybee pollen. It has been assumed that the drought in 2012 had also been the reason for the occurrence of mycotoxigenic fungi and high level of AFB_1 in the maize grains, whereas *A. flavus* was isolated as the most common species from the *Aspergillus* genus *(Krnjaja et al., 2013; Lević et al., 2013)*.

The potential reason for the fungal contamination of bee collected pollen could also be the impurity of pollen traps if they are not disinfected before upcoming beekeeping season *(Nedić et al., 2008)*. In the study of *Snowdon and Cliver (1996)* it was discussed that the primary sources of microbial contamination are very difficult to control because they included the epiphyte microflora of pollen, digestive tracts of honey bees, dust, air, etc. On the other hand, the second (after harvest) sources have the strongest effect on the contamination of bee pollen like higienic condition during cleaning, drying and storage of the product, equipment and buildings.

The application of good manufacturing practice is the best way to control contamination of bee pollen. It has been recommended that pollen has to be collected no later than 48 hours after the installation of pollen trap *(Bonvehí and Jordà, 1997)* and should be dried as quickly as possible, to reduce the levels of microbial contamination. The outdoors drying, which is in common practice in one part of beekeepers in Serbia, must be avoided in order to obtain the natural product without the risk on the human health. The drying process should be carried out for 2 h, by artificial heating at the air temperature of 40°C *(Bonvehí and Jordà, 1997)*.

Bee collected pollen could also be frozen and sold in vacuum-sealed packaging, which enable the preservation of more sensitive ingredients. In contrast to this recommendation, bee pollen in Serbia is mainly repacked in polyethylene bags, with the net weight of 100 g of dried pollen and sold on the beekeeping retail exhibitions that are held outdoors.

Consumers, especially in developing countries, continue to use bee pollen, usually as a dietary supplement, despite the fact that currently there are no international microbiological criteria and regulations for its production. More studies are needed about the microbial quality and safety of pollen as well as establishment of international quality standards, although in commercial transactions an analysis of aflatoxins is usually included *(Hani et al., 2012; Nogueira et al., 2012).*

Conclusion

The results obtained in this study confirmed that bee pollen was a suitable substrate for the fungal growth and AFB1 production. The relatively high level of AFB1 in the tested samples indicated that the hygienic condition during processing and storage of bee pollen was not at a satisfactory level. Therefore, the implementation of good manufacturing practice and HACCP approach in all stages of bee pollen production will certainly ensure the high quality of the product. For that purpose it is very important that governments set legal limits for mycotoxins in bee pollen. The information about the presence of mycotoxins in honeybee products could be very important for risk assessment on the human health.

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Prirodna mikobiota i prisustvo aflatoksina B1 u polenu prikupljenom u Srbiji

T. Petrović, N. Nedić, D. Paunović, J. Rajić, K. Matović, Z. Radulović, V. Krnjaja

Rezime

Ukupan broj gljiva, učestalost (incidenca) gljiva i koncentracija aflatoksina B_1 (AFB_1) ispitivani su u 33 uzoraka polena sakupljenih od pčelara iz različitih

regiona u Srbiji. Ukupan broj gljiva određen je primenom metode razređenja a AFB_1 je određen primenom imunoadsorpcione enzimske metode (ELISA).

Mikološkim ispitivanjima identifikovano je devet rodova gljiva: *Acremonium, Alternaria, Aspergillus, Cladosporium, Epiccocum, Fusarium, Mucor, Penicillium* i *Rhizopus,* sa ukupnim brojem od 1 x 10^3 to 1 x 10^5 CFU g^{-1}. Najučestalije vrste gljiva su u rodovima *Aspergillus* i *Alternaria*. Među *Aspergillus* vrstama najučestalija je vrsta *A. flavus* sa incidencom od 27,27%. AFB_1 je detektovan u svim uzorcima sa prosečnom koncentracijom od 8,61 μg kg^{-1}.

Dobijeni rezultati ukazuju da pčelarski polen mora biti strogo kontrolisan tokom prikupljanja i njegove dalje prerade. Zbog toga, sprovođenje dobre proizvođačke (pčelarske) prakse podrazumeva definisanje procedura za pčelarske proizvode što bi moglo biti presudno za smanjenje rizika od moguće kontaminacije i dobijanje prirodnih i bezbednih proizvoda bez rizika po zdravlje ljudi.

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THE EFFECT OF NITROGEN FERTILIZER RATES ON GREEN BIOMASS AND DRY MATTER YIELD OF *Sorghum sp.* AT DIFFERENT GROWTH STAGES

J. Ikanović[1], S. Janković[2], V. Popović[3], S. Rakić[1], G. Dražić[4], Lj. Živanović[1], Lj. Kolarić[1]

[1] University of Belgrade, Faculty of Agriculture, Nemanjina Street 6, Zemun, Serbia
[2]Institute for Science Application in Agriculture, Bulevar Despota Stefana St. 68b, Belgrade, Serbia
[3]Institute of Field and Vegetable Crops, Maksima Gorkog Street 30, Novi Sad, Serbia
[4]University of Singidunum, Futura, Belgrade, Serbia
*Corresponding author: bravera@eunet.rs; jela@agrif.bg.ac.rs
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Abstract: The paper investigated the production properties of three sorghum genotypes: NS-Džin (forage sorghum), Zora (Sudan grass) and Siloking (interspecies hybrid) in terms of different nitrogen rates used in side dressing in 2009 and 2010. The subject of study was green biomass and dry matter yield in the stages of intensive growth and tasseling. The results have shown that there have been significant fluctuations in production indicators between the genotypes. In the total average, the lowest yield was recorded for the Sudan grass (85.41 t ha^{-1}). Significantly higher yields were recorded for the interspecies hybrid (90.22 t ha^{-1}) and the forage sorghum (93.51 t ha^{-1}). Although the effect of nitrogen rates depended on weather conditions, i.e. rainfall distribution, the optimal nitrogen rate in both years was 180 kg ha^{-1}.

Key words: nitrogen, sorghum, Sudan grass, interspecies hybrid, yield, green biomass.

Introduction

Sorghum species are becoming popular nowadays, especially as fodder crops, because they regenerate well under favourable weather conditions and give more cuts in a production year, depending on water regime (*Glamočlija et al., 2010).*

Beside water regime, what greatly affects the productivity and quality of green biomass is crop nutrition. Previous studies on this topic have also confirmed the positive effect of side dressing on the quality of forage sorghum. The effect of nitrogen on plant metabolism has been well studied and known (*Avner et al.*,

2006). The optimal nitrogen supply makes plants intensively form nitrogen compounds to synthetize storage proteins (*Glamočlija et al., 2011*).

A general precondition for high yields is to satisfy plant requirements in the best way during the whole growing period. Besides regular food and water supply, this also implies good nutrient absorption, as well as the formation and use of necessary compounds in the optimum ratio and optimum amounts for plant growth (*Ikanović et al., 2010*). Using nitrogen mineral fertilizers in the right way should enable better and more efficient utilisation of the environment (agro-ecological and soil conditions) and crop potential of this plant for higher yields of animal feed per unit area. Yield reflects the potential of a plant to accumulate dry matter and its adaptability to different agro-ecological conditions.

Nitrogen is an element necessary for growth and development of plants in all stages, and symptoms of nitrogen deficiency in actual production are quite intensive and easy to spot (*McLaren, 2003*). Increased nitrogen rates in plants positively affect photosynthesis, as well as the intensity and duration of vegetative organs' activity, thus implying that increased nitrogen accumulation will be a precondition for improving the quality of crop potential of new cultivars.

According to recent research (*Booker, 2007*), nitrogen pollution of ecosystems in global changes has alarmingly increased, so it is assumed that participation rate of Asia in the total nitrogen production from current 35% will have been doubled by 2030. From the aspect of human population, the most unwanted consequences of excess nitrogen use in plant production are those related to accumulating harmful and toxic elements in plant and animal products and jeopardizing food safety (*Erisman et al., 2007*).

Materials and Methods

The study was conducted in 2009 and 2010. A two-factorial field experiment was set up on the experimental field "Radmilovac", in a randomized block system in ten repetitions. The size of basic plots was 10 m^2 (5m by 2m). The study was conducted on samples of three genotypes: *Džin* (forage sorghum bred in 1983), *Zora* (Sudan grass bred in 1983) and *Siloking* (interspecies hybrid bred in 2007) all bred in the Institute of Field and Vegetable Crops in Novi Sad. Different nitrogen rates were used in pre-sowing preparations: 105 kg ha^{-1} (N_2), 150 kg ha^{-1} (N_3), 180 kg ha^{-1} (N_4) and control (N_1) – natural soil fertility, estimated as 60 kg ha^{-1}. Standard cropping practices for forage sorghum were used. Plants were cut in the stage of intensive growth and at the beginning of tasseling (second decade of July and the last decade of August). Mineral fertilizers, such as ammonium-nitrate were applied before sowing. There were two cuts in both years. The productivity of the first and second cuts was determined by measuring fresh biomass from each plot and expressing it in t ha^{-1}.

Obtained data were analysed with STATISTICA 8 for Windows (Stat Soft 2009). Differences between treatments and their significance were determined with the analysis of variance (MANOVA) and LSD- test (0.01% and 0.05%).

Meteorological conditions. Meteorological data were taken from the Meteorological station in Belgrade, Serbia. In the first year, the amount of rainfall in the growing period was about 9.5% higher than a ten-year average. In April and May there was less rainfall than in the summer. Rainfall amounts in the second year were 27% higher than a multi-year average, and about 20% higher than in the first year. The distribution of rainfall in the growing period was even, with maximum rainfall in June (180 litres per square meter).

Table 1. Rainfall (mm) and daily mean temperature ^{0}C for sorghum growing period, Belgrade

Year	Parameter	*Month*						*Average*
		IV	V	VI	VII	VIII	IX	*Sum*
2009	Temperature	16	20	21	24	24	20	21
	Rainfall	6	34	153	79	45	45	362
2010	Temperature	14	18	21	24	24	18	20
	Rainfall	41	85	180	41	54	51	452
Average Sum, ten years	Temperature	15	26	23	25	25	18	21
	Rainfall	15	58	102	53	54	49	331

Monthly heat distribution in the first year has shown that mean temperatures in the summer were lower than a multi-year average for this region. In the second year, lower temperatures were recorded in the spring and the autumn, while in the summer temperatures were at the level of first year (Table 1).

Results and Discussion

The studied production properties of the genotypes have shown great dependence on both treatments (genotypes and applied nitrogen rates) – (Tables 2 and 3).

Green biomass yield. The genotypes had higher production values in 2010 since there was more rainfall in the summer when plants needed more water. This more favourable water regime caused higher productivity of green biomass of these genotypes, when compared to 2009.

Observed by stages, higher green biomass yield was obtained in all genotypes in the stage of growth, while higher dry matter yield was obtained in the stage of tasseling. Dry matter content, however, was also affected by different nitrogen rates. When compared to the total average, the Sudan grass had the lowest yield (85.41 t ha^{-1}), while considerable higher yields were recorded for the interspecies hybrid (90.22 t ha^{-1}) and the forage sorghum (93.51 t ha^{-1}). Nitrogen nutrition affected green biomass yield, so it was considerable higher in all treatments when compared to control. The highest yield, on average for all genotypes, was obtained when 180 kg N ha^{-1} was used. The average green biomass yield obtained in the

second year was about 15% higher, as a result of more favourable water regime, Table 2.

According to *Erić et al.* (2004), Sudan grass yield depends on the time of sowing as well. These authors identified a positive correlation between yield and growing period, i.e. the time of sowing, therefore determining that late sowing make plants have shorter vegetative growth, early-coming generative growth and lower biomass yield.

Table 2. Green biomass yield, t ha^{-1}

Genotypes *N rates*	*2009*	*2010*	*Two-year average*	*LSD* *0.05% and 0.01%*
Genotype (A)				
Siloking	84.52	95.92	90.22	3.3556
NS Džin	85.06	101.95	93.51	4.5661
Zora	80.22	90.60	85.41	
N rates (B)				
N105	84.64	94.06	89.35	
N150	86.31	101.60	90.00	5.851
N180	86.58	102.10	94.36	9.245
Control	79.55	86.96	83.30	
Average	83.84	96.17	89.45	

Table 3. Effect of genotypes and N rates on green biomass and dry matter yield of the investigated sorghum genotypes in 2009-2010

Factors	Green biomass yield		Dry matter yield	
	Stage of growth	Tasseling	Stage of growth	Tasseling
Genotype (A)		***2009***		
Siloking	48.462 b	43.906 a	6.722 b	9.446 a
NS Džin	53.436 a	43.657 a	6.123 c	5.626 c
Zora	45.565 c	36.539 b	8.242 a	7.864 b
N rates (B)				
N105	49.206 c	40.902 c	6.861 b	7.746 c
N150	52.379 b	43.223 b	8.077 a	8.431 b
N180	54.734 a	43.922 a	8.081 a	8.692 a
control	40.299 d	37.421 d	5.099 c	5.712 d
Average ± $S\overline{x}$	**49.154±1.094**	**41.367±0.734**	**7.029±0.260**	**7.645±0.340**
Genotype (A)		***2010***		
Siloking	45.732 a	35.486 a	7.698 b	10.010 a
NS Džin	38.726 b	32.228 c	7.434 c	7.408 c
Zora	36.130 c	33.350 b	8.285 a	9.472 b
N rate (B)				
N105	38.646 b	34.776 c	7.652 c	9.098 b
N150	45.831 a	36.557 b	8.424 b	9.438 a
N180	47.036 a	38.474 a	8.748 a	9.430 a
control	29.272 c	26.278 d	6.399 d	7.889 c
Average ± $S\overline{x}$	**40.196±1.413**	**34.021±0.896**	**7.806±0.167**	**8.964±0.227**

Table 4. Statistical significance of differences between the investigated sorghum properties, by years (F test and LSD test)

	Properties	Test	2009			2010		
			Genotype (A)	N rates (B)	AB	Genotype (A)	N rates (B)	AB
Green biomass yield	Stage of growth	F test	***	***	***	***	***	***
		LSD 5%	0.445	0.514	0.890	1.138	1.315	2.277
		1%	0.605	0.699	1.210	1.548	1.787	3.095
	Tasseling	F test	***	***	***	***	***	***
		LSD 5%	0.490	0.566	0.980	1.033	1.193	2.066
		1%	0.664	0.770	1.332	1.404	1.621	2.808
Dry matter yield	Stage of growth	F test	***	***	***	***	***	***
		LSD 5%	0.032	0.038	0.066	0.043	0.049	0.086
		1%	0.044	0.051	0.089	0.058	0.067	0.117
	Tasseling	F test	***	***	***	***	***	***
		LSD 5%	0.047	0.055	0.094	0.131	0.152	0.263
		1%	0.064	0.074	0.128	0.178	0.206	0.357

^{NS}=P>0.05 *=P<0.05 **=P<0.01 ***=P<0.001

Dry matter yield. Fluctuations in dry matter yield have shown their dependence on the time of cutting and the N rates. Dry matter yield was considerable affected by the N rates, but also the genotypes and the time of cutting.

Higher yield of dry matter was obtained at the stage of tasseling, but it was also affected by the N rates. It should be pointed out that the effect of N rates has shown a great dependence of rainfall distribution during the growing period. It affected both quantity and quality of dry matter in general, resulting in higher dry matter yield in 2010, when the weather conditions were more favourable. The highest dry matter yield was obtained for the interspecies hybrid Siloking, followed by the Sudan grass genotype Zora, whilst the lowest yield was obtained for the forage sorghum genotype Džin. With increasing N rates, the dry matter yield also increased, which was in line with the results of previous research by *Janković et al., 2012; Sikora et. al., 2012; Ikanović et al., 2013; Rakić et al., 2013*, and came as a result of higher yield and better quality of green biomass.

Conclusion

The results of the biennial research indicate that higher nitrogen rates have significant, positive and justifiable effect on production properties of the tested genotypes of sorghum, Sudan grass and interspecies hybrid.

The increased nitrogen nutrition significantly contributed to higher yield of green biomass in the growth stage, while higher dry matter yield was obtained

during the tasseling stage depending on the used nitrogen rates, which was a result of higher yields and better quality of green biomass.

Intense nitrogen nutrition can be justified only when there is favourable water supply, which implies that the effect of using this fertilizer increases only when crops are irrigated and the loss of washing nitrogen into deeper layers of soil decreases.

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Uticaj azota na prinos zelene biomase i suve materije *Sorghum sp.* vrsta po fazama rastenja

J. Ikanović, S. Janković, V. Popović, S. Rakić, G. Dražić, Lj. Živanović, Lj. Kolarić

Rezime

U radu su proučavane produktivne osobine tri genotipa sirka i to: NS-Džin (krmni sirak), Zora (sudanska trava) i Siloking (interspecijes hibrid) u zavisnosti od upotrebljenih količina azota za dopunsku ishranu biljaka tokom 2009. i 2010. godine. Ispitivani su prinos zelene mase u fazama intezivnog porasta i metličenja.
Između ispitivanih genotipova postoje značajna variranja u pokazateljima produktivnosti. U ukupnom proseku najmanji prinos dala je sudanska trava 85,41 t ha^{-1}. Značajno viši prinos bio je kod interspecijes hibrida (90,22 t ha^{-1}) i krmnog sirka (93,51 t ha^{-1}).
Iako je efekat upotrebljenog azota zavisio od vremenskih uslova, odnosno od rasporeda padavina, u obe godine optimalna količina azota bila je 180 kg ha^{-1}.

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Example 2

THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

M. D. Petrović[1], Z. Skalicki[2], V. Bogdanović[2], M. M. Petrović[3]

[1]Faculty of Agronomy, Cara Dušana 34, 32000, Čačak, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[3]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
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Correction – Vol 30 (3), p 407-414 D. V. Anh Khoa, K. Wimmers

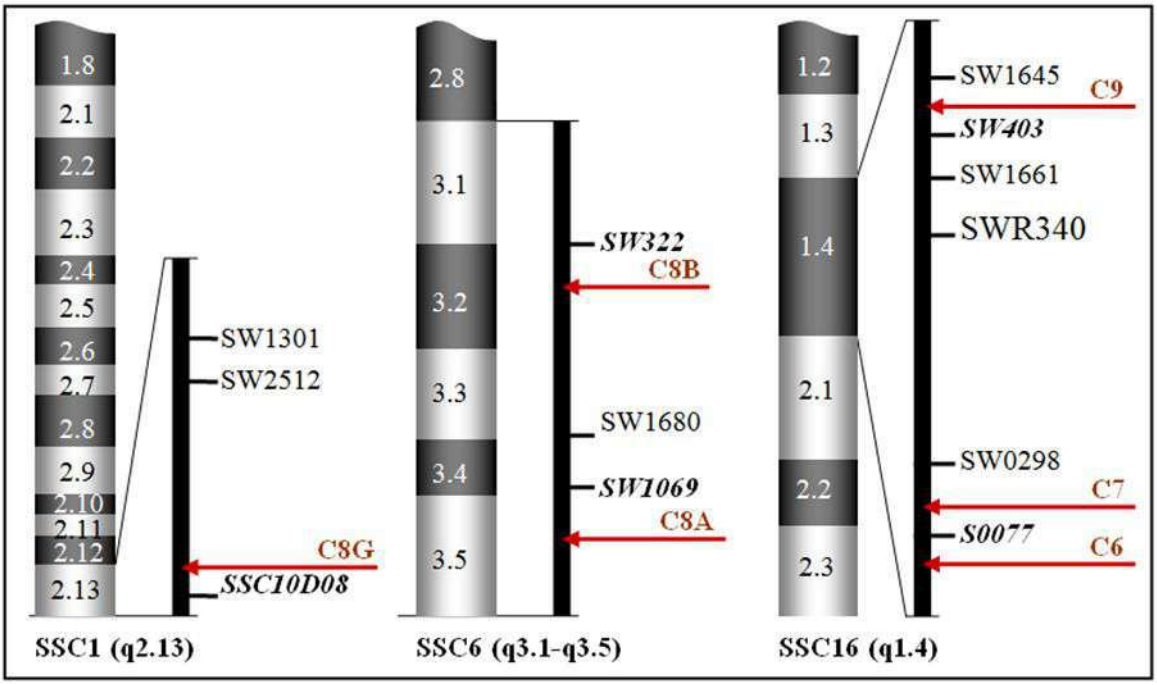

Figure 2. Position of the candidate genes on porcine chromosomes. Linkage is closed to markers in bold and italic