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Monitoring of blood metabolic profile and milk quality of ewes during lactation in organic farming

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Abstract

The aim of this research was to monitor the metabolic profile of blood and the quality of ewes' milk during lactation in organic farming. Biological investigations were carried out on 32 clinically healthy Merinolandschaf ewes during the 3th lactation on the 20th, 60th and 100th day of lactation. Ewes' milk was analyzed for the non fat dry matter, milk fat, protein, lactose, urea, the somatic cells count (SCC) and te total viable cell number (CFU), as well as for the concentration of fatty acids, atherogenic (AI), thrombogenic (TI) and Δ9-desaturase activity index. Concentrations of minerals (Ca-calcium, P-phosphorus-inorganic, Mg-magnesium, and Fe-iron), biochemical parameters (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total protein, albumin, globulin, NEFA-non-esterified fatty acids, BHBA-beta-hydroxybutyrate) and enzyme activity (ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase, CK-creatine kinase and GGT-γ-glutamyl transferase) were analyzed in blood serum. Chemical composition of milk differed among different stages of lactation, which was marked by the increased content of milk fat, the decreased urea concentration, as well as by numeorus changes of fatty acid concentration observed along with lactation progression. Number of SCC and CFU in milk increased during lactation. AT and TI were appropriate in all stages of lactation, which resulted in satisfactory quality of ewes' milk from organic farming. Determined concentrations of certain biochemical parameters (NEFA, triglycerides, VLDL-cholesterol, Ca and Fe) in blood of ewes originating from organic farming indicated lower deficit of energy during the 20th day of lactation, as well as a lack of Ca and Fe concentrations in blood, which most likely occurred due to higher loss through milk. Accordingly, the blood metabolic profile can be considered as an indicator for feeding and health status of ewes during lactation in organic farming.

Key words: ewes' milk, lactation stage, biochemical parameters, fatty acids, organic farming

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Introduction

Organic farming of ewes becomes increasingly important why a good quality control and monitoring of production are necessary. Ewes' health and feeding status also need to be monitored. The blood metabolic profile is an important laboratory diagnostic technique that can be used to assess the nutritional status and animal health (Herdth et al., 2000). Therefore, monitoring the metabolic profile of ewes in organic farming imposes as good quality measure of surveilance. Lactation is a very demanding period for an animal as its nutritional needs are increased. In conventional production during this period, especially in the first half of lactation, it is difficult to satisfy the nutritional requirements of animals because of high milk production. In early lactation, energy intake is lower if compared to animals' needs, indicating negative energy balance, which mobilizes body reserves. Consequently, significant changes may occur in the ewes in early lactation period, which can lead to metabolic disorders. Arfuso et al. (2016a) reported that cow adjusted to the resulting negative energy balance with the mobilization of lipids adipose tissue that, if excessive, could lead to many transition disorders compromising the offspring's growth and well-being. Mobilization of body reserves firstly activated body fat reserves and changes of NEFA and BHBA concentrations in blood serum of animals (Kokkonen et al., 2005; van Knegsel et al., 2007), as well as some other blood metabolites (insulin and glucose). However, in comparison to conventional system, ewes in organic farming have slightly worse production traits (Klir et al., 2013; Melissiova et al 2015), and lower risk of the above mentioned occurrence.

Organic farms aim for an ecological approximation of livestock production with minimal environment impact, rather than the maximization of production yields of animals (Angeles-Hernandez et al., 2014). Monitoring the metabolic profile by determining the concentration of biochemical parameters in the blood of small ruminants gives us a clearer picture of their nutritional and health status even before the changes are visible on an animal (Antunović et al., 2011; Šoch et al., 2011). It is almost indispensable in organic breeding, where permitted veterinary interventions are strictly regulated and limited (Arfuso et al., 2016b).

Milk quality is estimated according to the numerous physical, technological and nutritional factors. Basic chemical composition (fat, protein and lactose), physico-chemical properties and micro-ingredients (minerals, vitamins, fatty acid, cholesterol, terpenes, etc.) of milk are usually used for assessment of milk quality. Milk from ruminants is a well known source of fatty acids important for human health (Tsiplakou et al., 2010). The content of those is however significantly dependant on the nutrition (De la Fuente al., 2009). Ewes' milk composition is well documented, although their blood metabolic profile during lactation in organic breeding still needs to be researched. The aim of this study was to monitor blood metabolic profile and quality of ewes' milk during lactation in organic farming.

Material and methods

Animals, locations of investigation, diets and analyses

Biological investigations were carried out on 32 clinically healthy Merinolandschaf ewes during lactation. Ewes were kept on farm during winter season. Rearing and feeding of ewes was organized according to the Council Regulation (EC) 834/2007 on organic production. Ewes' selection was carried out according to the register of flock containing 200 ewes of Merinolandschaf breed. In addition to the uniform physical development, good health and good physical fitness, the criteria for selection of ewes were the age, number of lactations, ewes origin and litter size. Ewes were of an average age of 4 years in the 3rd lactation with one lamb in litter. This research was performed during the year 2016 at the Ursic family farm (in Croatia, located 35 km south-east of Osijek, 42.150^0 N 52.647^0 E), with mean annual temperature of 11.4 °C, mean annual humidity 87 % and sum of annual rainfall 704.6 L. From February to May 2016, monthly mean temperature for this area was 10.5 °C (range from -2.9 to 30.1 °C), mean monthly value for relative humidity was 87 % and the sum of rainfall was 252.1 L. For the mentioned period of time and the area, the calculated THI was 51.4. This area is located in the Baranja region at an altitude of approximately 91 meters. Ewes were tested in three periods according to the stage of lactation: 20th, 60th and 100th day of lactation (±5 days). Ewes were fed a feeding mixture (0.5 kg/day: 71 % corn, 10.5 % barley, 16.5 %

soybean meal, 2 % mineral-vitamin premix) and meadow hay (*Lolium perenne, Lolium italicum, Phleum phleoides, Trifolium repens, Dactylis glomerata*) from organic farming *ad libitum*. Water was provided *ad libitum* to all ewes during the whole period of research. During the research lambs were kept with the ewes and were allowed to suckle *ad libitum*. One day prior to milk sampling, lambs were separated from their mothers.

Sampling of milk and blood was carried out by hand milking on the 20th, 60th and 100th day of lactation. Milk samples were collected in plastic bottles (200 mL), which were placed into mobile coolers, chilled to 4 °C and transferred in that manner to ensure laboratory analysis within 24 hours. Ewes' milk was analyzed for the non fat dry matter, milk fat, protein, lactose, urea, somatic cell count (SCC) and the total viable cell counts (CFU). The non fat dry matter was determined by subtracting the milk fat from the dry matter which is determined by drying. Analysis of fat, protein, lactose and urea concentrations in milk was carried out by infrared spectrometry (HRN EN ISO 9622: 2001) on the MilkoScan FT 6000 analyzer within the Comby system. Somatic cells count was determined by a fluoro-opto-electronic method (HRN EN ISO 13366-2/ Correction. 1. 2007) using a Fossomatic 5000 analyzer, and the number of CFU with epifluorescent method of flow cytometry (IDF 161A: 1995). In order to the obtain normal distribution of SCC and CFU, values were expressed as logarithms (log 10).

Milk fat separation was ensured by mixing a specific volume of milk with selected organic solvents (ammonia, ethanol, ether, petroleum ether) to allow the solution to stand in order to separate the layers. An aliquot of supernatant was evaporated and dried in oven until reaching a constant weight (Trajković et al., 1983).

The fatty acid methyl esters were prepared by a gas chromatography according to the HRN EN ISO 12966-2:2011 standard. Conditions and method applied corresponded to the HRN EN ISO 12966-1:2015 standard. The samples were analyzed by a gas chromatograph 7890B (Agilent Technologies, USA), using a 100 m working capillary column Rtx®-2560 (biscyanopropyl polysiloxane) with a diameter of 0.25 mm and the thickness of the stationary phase 0.20 microns (Restek, USA), a split-splitless injector (temperature 225 °C and pressure of 35.8 psi) with flame-ionization detector and temperature of 250 °C. The used 1 μL of sample volume was injected into the system. In the beginning, oven temperature was 100 °C with holding time for 4 minutes, which was increasing with a rate of 3 °C/min to 240 °C/min and holding for 11 minutes. Run time was 61.67 minutes. Carrier gas was nitrogen (99.9999 %) at a constant flow rate of 1.2 mL/min. The hydrogen flow was 30 mL/min, air flow was 250 mL/min, and the makeup gas flow (nitrogen) was 45 mL/min.

Table 1. Chemical and fatty acid composition of feed mixture and hay

Indicators	Feed mixture	Hay
Chemical composition (%)		
Dry matter	89.77	93.81
Crude proteins	14.73	9.97
Ether extract	2.95	0.97
Crude fiber	3.30	32.51
NDF	9.13	66.41
Ash	3.85	5.55
NEL, MJ/kg	7.26	4.71
Fatty acids (%)		
C4:0	ND	0.43
C12:0	ND	0.46
C14:0	0.08	0.99
C14:1	ND	0.22
C15:0	ND	0.72
C16:0	16.15	38.70
C16:1	0.13	0.51
C17:0	0.10	0.57
C18:0	2.52	3.68
C18:1 c9	26.32	6.82
C18:2 t9,12	ND	0.64
C18:2 c9,12	51.19	18.06
C20:0	0.43	1.15
C20:1 c11	0.31	0.54
C18:3 c9c12 c15	2.14	21.80
C21:0	ND	0.55
C22:0	0.25	1.41
C22:1	ND	0.55
C20:4	0.07	0.84
C24:0	0.31	1.36
SFA	19.83	50.02
UFA	80.17	49.98
MUFA	26.76	8.64
PUFA	53.40	41.34

NDF-neutral detergent fiber, NEL-net energy for lactation, SFA-saturated fatty acids, UFA-unsaturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids, ND-not determined.

Fatty acid methyl esters were identified by comparing retention times of 37 fatty acid methyl ester standards and samples analyzed under the same conditions. Prior to analyzing the samples and standards, a certified reference material (CRM) was prepared and analyzed under the same conditions. The result was expressed as percentage (%) of individual fatty acids in the total fatty acids. The detection limit was 0.01 %.

Atherogenic (AI) and thrombogenic (TI) indices of milk were calculated according to the Ulbricht and Southgate (1991) equations:

$$AI=[(12:0+4(14:0)+16:0]/[(n6+n3)PUFA+18:1+\Sigma MUFA]$$

$$TI=(14:0+16:0+18:0)/[(0.5\times 18:1)+0.5(\Sigma MUFA)+0.5(n6PUFA)+3(n3PUFA)+(n3PUFA/n6PUFA)]$$

Index of Δ^9-desaturase activity was determined from the following ratios: C14:1/C14:0, C16:1/C16:0 and C18:1/C18:0.

After morning feeding blood samples were collected from the jugular vein (10 mL) into serum Vacutainer tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) with addition of the ethylenediamine tetra-acetic acid (EDTA). The EDTA tubes were inverted several times to ensure adequate mixing of the blood with anticoagulant. Blood serum was separated by centrifugation (10 minutes) at a speed of 3000/minute. Concentrations of minerals (Ca-calcium, P-phosphorus-inorganic, Mg-magnesium, and Fe-iron), biochemical parameters (urea, glucose, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total protein, albumin, globulin, NEFA-non-esterified fatty acids, BHBA-beta-hydroxybutyrate) and enzyme activity (ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase, CK-creatine kinase and GGT γ-glutamyl transferase) were analyzed on the Olympus AU400 device (Beckman Coulter, Tokyo, Japan) at 37 °C. Olympus System Reagents (OSR) were manufactured and distributed by Olympus Diagnostic GmbH (Irish Branch), Lismeehan, Ireland. The concentration of NEFA was measured with the enzymatic colorimetric method (Randox Laboratories Ltd., Crumlin, UK) and the concentration of BHBA was measured with the colorimetric method (RANBUT, Laboratories Ltd., Crumlin, UK). Concentration of VLDL (very low density lipoproteins) was calculated with triglycerides/5.

Statistical analysis

Data were analyzed with the statistical software SAS 9.3®. The results of milk and blood were presented as arithmetic mean and standard error of mean estimated with MEANS procedure. Data were analyzed with one way repeated measures ANOVA. Means were compared by applying the Tukey test and the differences between lactation stages were declared significant at $P<0.05$ level.

Results and discussion

Table 2 presents chemical composition, as well as number of somatic cells and CFU of ewes' milk from organic farming through different lactation stages. Most of the analyzed parameters of milk chemical composition from organic farming significantly differed among different lactation stages. The milk fat content, the somatic cells count and CFU significantly increased from 20th to 100th day of lactation, while the concentration of urea significantly decreased on the 100th day, compared to the 20th day of lactation. The composition of milk changes during lactation, and is characterized by increased fat content and somatic cells count (Pulina and Nudda, 2004). Melissiova et al. (2015) obtained similar content of lactose, dry matter and protein, as well as slightly higher content of fat in ewes' milk from organic farming in Greece. Opposite results were determined by Kraličkova et al. (2012), who reported significant effect of lactation stage on content of dry matter, proteins and lactose in organic milk of East Frisian sheep in Czech Republic. These differences were determined by monitoring the sheep from the 75th to 190th day of lactation, which was probably the reason for the obsereved changes.

Novotna et al. (2009) examined milk samples from the organic production of crossbred sheep (50 % Lacaune, 37.5 % East Friesian and 12.5 % Improved Wallachian) and reported a significant increase in most of the basic milk ingredients (dry matter, protein, fat) within the lactation period, except for the lactose content, which remained constant and decreased at the end of lactation. In the milk of Istrian Pramenka sheep kept in organic farming in Slovenia, Klir et al. (2013) determined higher contents of fat (7.48 %) and protein (6.13 %), but lower lactose content (3.93 %). Such data might

be associated with longer lactation (197 days), if compared to the current research. In comparison to data relating fat content of milk exaimend by Klir et al. (2013), lower content in milk of sheep from organic farming was associated with duration of suckling period of lambs, when sheep withhold milk and keep it for lamb, and when known that the last jets are the richest in milk fat.

Table 3 presents concentration of fatty acids in organic ewes' milk, atherogenic (AI) and thrombogenic (TI) indices and activity of Δ^9-desaturase, during different lactation stages. Most of the short-, medium- and long-chain fatty acids significantly differed among lactation stages ($P \leq 0.026$). In the present research, concentration of oleic acid significantly ($P<0.05$) decreased on the 60th and 100th day (23.01 and 22.17 g/100 g, respectively), if compared to the 20th day of lactation (26.39 g/100 g). Similarly, significant decrease in linolenic acid was determined on the 60th (0.78 g/100 g) and 100th day (0.76 g/100 g), if compared to the 20th day (1.04 g/100 g). The results proved a significant ($P<0.05$) increase in the total saturated fatty acids (SFA), as well as a decrease ($P<0.05$) in the total unsaturated fatty acids (UFA) and monounsaturated fatty acids (MUFA) as lactation progressed. Chilliard et al. (2003) noted that, when energy balance was negative, animal mobilized lipids stored in adipose tissue, which explained that 59 % of milk variability C18:0 and C18:1 was linked to changes in energy balance. After the lactation peak, greater part of exogenous fatty acids was taken up by the adipose tissue (Chilliard, 1993). In milk of Karagouniko and Chios ewes in Greece, Sinannoglou et al. (2015) determined significant decrease of C4:0 and total polyunsaturated fatty acids (PUFA), as well as significant increase of C14:0, C15:0, C16:0 and C20:0 concentrations. De la Fluente et al. (2009) examined milk of Churra sheep and found asignificant effect of lactation stage on the most of fatty acids´ concentrations. The ALA concentration varied between 0.76 and 1.04 g/100 g in ewes' milk, which was generally adequate in all stages, and was influenced by feeding mixtures and hay fatty acid profile (Table 1).

In the present research, the values of AI and TI significantly increased during the lactation and were adequate (<3). Similar AI and TI results in milk of ewes in Greece were observed by Sinannoglou et al. (2015). Atherogenic and thrombogenic indices showed that C12:0, C14:0 and C16:0 fatty acids were atherogenic, while C14:0, C16:0 and C18:0 were thrombogenic (Ulbricht and Southgate, 1991). In the present research, significant activity of index Δ^9-desaturase was determined only for the ratio of C14:1/C14:0, which was increasing, while the other two ratios were not significantly different with respect to the stage of lactation. The above changes showed that the ratio of C14:1/C14: 0 was the best indicator of Δ^9-desaturase because the concentration of C14:0 in the milk fat was product of *de novo* synthesis in the mammary gland, while the fatty acid C16:0 and C18:0 may be absorbed in the intestine (Cabiddu et al., 2005). Results of the present research indicate a satisfactory quality of ewes' milk produced in organic farming.

Table 2. Chemical composition, somatic cell counts (SCC) and total viable cell count (CFU) in ewes' milk from organic farming

Parameters	Lactation stage, days (mean)			SEM	P-values
	20th	60th	100th		
Dry matter without fat (%)	11.25	11.06	11.03	0.06	0.179
Milk fat (%)	4.31^{a}	4.97^{ab}	5.45^{b}	0.13	0.004
Protein (%)	5.26	5.19	5.10	0.06	0.616
Lactose (%)	4.87	4.75	4.82	0.03	0.108
Urea (mg/dL)	40.50^{a}	39.20^{ab}	33.35^{b}	0.69	0.046
$\log_{10}$ (SCC), mL	5.22^{a}	5.24^{ab}	5.60^{b}	0.07	0.049
$\log_{10}$ (CFU), mL	3.50^{a}	3.98^{ab}	4.38^{b}	0.12	0.031

sd-standard deviation; SEM-standard error of mean
^{a,b}data within the same row with different superscripts differ significantly ($P < 0.05$)

Table 3. Concentration of fatty acids (g/100 g fatty acids), atherogenic (AI), thrombogenic (TI) and Δ^9-desaturase indices in ewes' milk during different lactation stages

Fatty acids	Lactation stage, days (mean)			SEM	P-values
	20th	60th	100th		
C4:0	1.23[a]	1.08[b]	1.09[b]	0.02	<0.001
C6:0	1.44	1.51	1.48	0.02	0.454
C8:0	1.65[b]	1.87[a]	1.77[ab]	0.03	0.026
C10:0	5.67[b]	7.09[a]	6.75[a]	0.15	<0.001
C11:0	0.06[b]	0.10[a]	0.11[a]	0.005	<0.001
C12:0	3.77[b]	4.96[a]	4.83[a]	0.12	<0.001
C13:0	0.11[b]	0.14[a]	0.12[ab]	0.004	0.012
C14:0	10.01[b]	11.69[a]	12.22[a]	0.17	<0.001
C14:1	0.09[b]	0.16[a]	0.17[a]	0.01	<0.001
C15:0	1.26[b]	1.53[a]	1.55[a]	0.03	<0.001
C16:0	24.48[b]	25.88[a]	26.57[a]	0.23	<0.001
C16:1	0.65[b]	0.72[a]	0.75[a]	0.01	0.001
C17:0	1.23	1.16	1.16	0.02	0.206
C17:1 c10	0.40[a]	0.24[b]	0.39[a]	0.02	<0.001
C18:0	13.61[a]	12.24[b]	11.90[b]	0.19	<0.001
C18:1 t9	2.05[a]	1.54[b]	1.41[b]	0.04	<0.001
C18:1 (OA)	26.39[a]	23.01[b]	22.17[b]	0.35	<0.001
C18:2 t9 t12	0.20[a]	0.17[ab]	0.13[b]	0.007	<0.001
C18:2 (LA)	2.59	2.19	2.36	0.07	0.088
C20:0	0.41[c]	0.50[b]	0.56[a]	0.01	<0.001
C18:3 c6 c9 c12	0.02[b]	0.06[a]	0.05[a]	0.005	0.002
C20:1	0.08[b]	0.12[a]	0.10[ab]	0.01	0.011
C18:3 (ALA)	1.04[a]	0.78[b]	0.76[b]	0.03	<0.001
C21:0	0.87[a]	0.46[b]	0.60[b]	0.03	<0.001
C20:2	0.04	0.02	0.02	0.005	0.056
C22:0	0.14[b]	0.14[b]	0.25[a]	0.008	<0.001
C20:3	0.003	0.01	0.006	0.001	0.734
C20:4	0.06[c]	0.19[a]	0.13[b]	0.008	<0.001
C23:0	0.17[b]	0.06[c]	0.22[a]	0.01	<0.001
C22:2	0.06[ab]	0.05[b]	0.08[a]	0.006	0.050
C24:0	0.05[b]	0.07[b]	0.13[a]	0.006	<0.001
C20:5 (EPA)	0.09[a]	0.09[a]	0.06[b]	0.007	0.022
C24:1	0.02[b]	0.05[a]	0.07[a]	0.005	<0.001
C22:6 (DHA)	0.10	0.10	0.09	0.005	0.967
SFA	66.16[b]	70.47[a]	71.30[a]	0.42	<0.001
UFA	33.84[a]	29.53[b]	28.70[b]	0.42	<0.001
MUFA	29.68[a]	25.85[b]	25.06[b]	0.38	<0.001
PUFA	4.16	3.68	3.64	0.09	0.058
AI	2.08[b]	2.67[a]	2.84[a]	0.06	<0.001
TI	1.47[b]	1.73[a]	1.84[a]	0.03	<0.001
	Δ^9-desaturase index				
C14:1/C14:0	0.009[b]	0.014[a]	0.014[a]	0.0004	<0.001
C16:1/C16:0	0.027	0.028	0.028	0.0004	0.188
C18:1/C18:0	1.947	1.903	1.890	0.025	0.628

Sd-standard deviation; SEM-standard error of mean; OA-oleic acid; LA-linoleic acid; ALA-alpha-linolenic acid; EPA-eicosapentaenoic acid; DHA-docosahexaenoic acid; SFA-saturated fatty acids; UFA-unsaturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; AI-Atherogenic index; TI-thrombogenic index.
[a,b]data within the same row with different superscripts differ significantly (P<0.05)

In this research, concentration of urea, tryglicerides, Fe and VLDL in ewes' blood significantly increased on the 100th day if compared to the 20th day. Opposite effect was determined for the concentration of albumin, phosphorus and NEFA (Table 4). In comparison to the mentioned reference values, lower concentrations of Ca, Fe and cholesterol were determined, while the total protein contents were on the lower limit of reference values.

Assessment of ewes' energy status was performed by determining cholesterol, glucose, tryglicerides concentrations, as well as NEFA and BHBA in blood, while concentration of urea, total protein and albumin, as well as activity of CK enzyme were used as an indicator of ewes' protein supply through feed (Antunović et al., 2009). Upon analyzing the concentrations of the above mentioned biochemical parameters in lactating ewes, there were no significant deviations in comparison to the reference values. Such findings indicated satisfactory supply of energy through diets, except on the 20th day of lactation. Simultaneously, by analyzing the concentration of urea, total proteins and albumins, as well as CK activity, it was proven that protein supply from diets was satisfied. However, lower concentrations of Fe in blood could be related to its loss through milk, as well as to its deficit in the diets.

Liesgang et al. (2007) determined lower concentration of Ca in ewes' blood after lambing and in early lactation, which might be associated to the increased secretion of Ca through milk and its rearrangement in bones. Antunović et al. (2011) determined a drop down of cholesterol and increased tryglicerides in the blood serum during the lactation, which was explained by the high cholesterol requirements by dams for milk synthesis.

Table 4. Concentration of selected biochemical parameters and enzyme activity in the blood of ewes in organic farming during different lactation stages

Parameter	Lactation stage, days (mean)			SEM	P-values	Reference values[1]
	20th	60th	100th			
Glucose, mmol/L	4.06	3.84	3.71	0.058	0.127	2.78-4.44
Urea, mmol/L	3.79[b]	4.23[ab]	4.61[a]	0.119	0.017	2.86-7.14
Total proteins, g/L	59.68	60.55	59.48	0.706	0.816	60-79
Albumins, g/L	26.07[a]	25.23[ab]	24.24[b]	0.315	0.026	24-30
Globulins, g/L	33.61	35.32	35.24	0.624	0.517	35-57
Cholesterol, mmol/L	1.31	1.32	1.28	0.024	0.759	1.35-1.97
Triglyceride, mmol/L	0.19[b]	0.21[ab]	0.23[a]	0.005	0.003	0.19-0.25[2]
HDL, mmol/L	0.89	0.85	0.83	0.015	0.304	1.09-1.18[2]
LDL, mmol/L	0.33	0.39	0.34	0.012	0.215	0.36-0.40[2]
VLDL, mmol/L	0.038[a]	0.043[ab]	0.047[b]	0.001	0.004	0.03-0.04[3]
Phosphorus-inorganic, mmol/L	1.86[a]	1.79[ab]	1.56[b]	0.043	0.020	1.62-2.36
Ca, mmol/L	2.14	2.23	2.21	0.020	0.232	2.88-3.20
Mg, mmol/L	0.88	0.93	0.92	0.009	0.052	0.90-1.26[3]
Fe, μmol/L	17.01[b]	18.34[ab]	21.00[a]	0.744	0.027	29.7-39.7
NEFA, mmol/L	0.35[a]	0.11[b]	0.13[b]	0.019	<0.001	>0.345[4]
BHBA, mmol/L	0.40	0.36	0.39	0.010	0.238	<0.849[4]
AST, U/L	112.56[ab]	120.35[a]	107.04[b]	2.388	0.010	60-280
ALT, U/L	23.29	23.08	21.88	0.518	0.477	11-40
ALP, U/L	99.45[a]	126.21[b]	129.70[b]	4.369	0.006	68-387
GGT, U/L	56.42	59.09	54.62	1.392	0.325	15-60
CK, U/L	97.82	129.87	98.23	6.031	0.052	100-584

Mean=mean value; SEM=mean standard error
[1]Kaneko et al. (2008); [2]Antunović et al. (2011); [3]Nazifi et al. (2002); [4]Karagiannis et al. (2014);
HDL-high density lipoprotein, LDL-low density lipoprotein, VLDL- very low density lipoproteins, NEFA-non-esterified fatty acids, BHBA-beta-hydroxybutyrate, AST-aspartate aminotransferase, ALT-alanine aminotransferase, ALP-alkaline phosphatase, GGT- γ-glutamyl transferase, CK-creatine kinase
[a,b]data within the same row with different superscripts differ significantly (P<0.05)

Elevated conectrations of triglycerides in the blood of lactating ewes might be associated with negative energy balance that accompanied the increased mobilization of fat in adipose tissue (Sobiech et al. 2008). As a result of the limited liver capacity to export triglycerides as VLDL, the liver storage of triglyceride in the interior of the hepatocytes ends up decreasing triglyceride concentration in the serum (Garcia et al., 2011). As a result, body fat reserves are mobilized into blood in the form of non-esterified fatty acids (NEFAs) under conditions of energy deficit and stress (Sato and Inoue, 2006).

Significantly higher NEFA concentration in ewes' blood suggested a negative energy balance which were characterized by increased mobilization of NEFA from body reserves in ewes during the 20th day of lactation. The increase in the NEFA and BHBA concentrations in blood of ewes on the 20th day with decreased glucose in blood could also reflect a low energy intake. The obsereved changes could be related to using glucose for milk lactose synthesis with insufficient dietary supply to maintain blood glucose homeostasis (Roubies et al., 2006). Abdelatif et al. (2009) determined gradual increase of urea concentration in lactating ewes along the lactation, which was presumably related to the increase in feed intake associated with higher nutrient demands. Greater concentration of urea in serum of ewes could also be a result of catabolizing muscle protein when large amounts of body reserves were mobilized (Whitney et al., 2009).

AST enzyme activity was significantly higher on the 60th day of lactation, if compared to the 100th day, while the ALP activity significantly increased on the 60th and 100th day, if compared to the 20th day of lactation. Such results could be caused by more intense liver function of lactating ewes, to meet the energy and protein requirements for maintenance and milk production (Roubies et al., 2006). Whitaker (1997) reported AST enzymes to be responsible for the protein balance, which was especially important in the period of intensive metabolism during the lactation peak. The activity of other enzymes in ewes´ blood was not significantly different considering different stages of lactation in organic farming. In comparison to the mentioned reference values (Kaneko et al., 2008), the presented research results did not show any deviations in enzymatic activities.

Conclusion

Chemical composition of milk differed among different stages of lactation, thus resulting in the increased content of milk fat, SCC and CFU, as well as in the decreased urea concentration. Furthermore, many changes in milk fatty acid concentration were observed along with the lactation progression. AT and TI were adequate in all stages of lactation, thus resulting in satisfied quality of ewes´ milk produced in organic farming. Concentrations of certain biochemical parameters (NEFA, triglycerides, VLDL cholesterol, as well as Ca and Fe) determined in blood of ewes from organic farming indicated lower deficit of energy on the 20th day of lactation, as well as a deficit of calcium and iron, which probably occurred due to higher excretion through milk. Determination of the blood metabolic profile can be used to monitor feeding and health status of ewes in organic farming.

Praćenje metaboličkog profila i kvalitete ovčjeg mlijeka tijekom laktacije u ekološkoj proizvodnji

Sažetak

Cilj istraživanja bio je utvrditi metabolički profil ovaca i kvalitetu ovčjeg mlijeka tijekom laktacije u ekološkoj proizvodnji. Istraživanje je provedeno na 32 ovce Merinolandschaf pasmine u trećoj laktaciji 20., 60. i 100. dana laktacije. U mlijeku je utvrđen udjel suhe tvari bez masti, sadržaj mliječne masti, bjelančevina, laktoze, koncentracija ureje, broj somatskih stanica (SCC) i ukupan broj mikroorganizama (CFU), kao i koncentracija masnih kiselina, aterogeni (AI) i trombogeni (TI) indeksi te indeksi aktivnosti enzima Δ^9-desaturaze. U serumu ovaca utvrđene su koncentracije minerala (Ca-kalcij, P-anorganski fosfor, Mg-magnezij i Fe-željezo), biokemijskih pokazatelja (urea, glukoza, kolesterol, HDL-kolesterol, LDL-kolesterol, trigliceridi, ukupne bjelančevine, albumin, globulin, NEFA-neesterificirane masne kiseline, BHBA-beta-hidroksibutirat) i aktivnosti enzima (ALT-alanin aminotransferaze, AST-aspartat aminotransferaze, ALP-alkalne fosfataze, CK-kreatin kinaze i GGT-γ-glutamil transferaze).

Utvrđene su značajne razlike u kemijskom sastavu mlijeka, odnosno povećanje udjela mliječne masti i smanjenje koncentracije ureje, kao i promjene masnokiselinskog sastava mlijeka s napredovanjem laktacije. Broj somatskih stanica i ukupan broj mikroorganizama u mlijeku su rasli s napredovanjem laktacije. Osim toga, vrijednosti AI i TI bile su odgovarajuće u svim stadijima laktacije, što je rezultiralo povoljnoj kvaliteti ovčjeg mlijeka iz ekološke proizvodnje. Utvrđene koncentracije pojedinih biokemijskih pokazatelja (NEFA, triglicerida, VLDL kolesterola, Ca i Fe) u krvi ovaca u ekološkoj proizvodnji ukazuju na slabiju opskrbljenost energijom 20. dana laktacije, kao i smanjenje koncentracije Ca i Fe u krvi, zbog njihovog izlučivanja mlijekom. Prema navedenome, metabolički profil se može koristiti kao pokazatelj hranidbenog i zdravstvenog statusa ovaca u laktaciji u ekološkoj proizvodnji.

Ključne riječi: ovčje mlijeko, stadij laktacije, biokemijski pokazatelji, masne kiseline, ekološka proizvodnja

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