Biotechnology in Animal Husbandry 28 (2), p 241-251 , 2012
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.034/637.04
DOI:10.2298/BAH1202241G

MILK YIELD AND CHEMICAL COMPOSITION OF SHEEP MILK IN SREDNOSTAROPLANINSKA AND TETEVENSKA BREEDS

G. Gerchev[1], G. Mihaylova[2]

[1]Research Institute of Mountain Stockbreeding and Agriculture, 5600, Troyan, Bulgaria
[2]Faculty of Agriculture, Trakia University, 6000, Stara Zagora, Bulgaria
Corresponding author: gercho_g@abv.bg
Original scientific paper

Abstract: The study was conducted from April to July on pastures located at different altitudes and of different sward composition during the milking period of Srednostaroplaninska and Tetevenska sheep. Morning bulk milk was sampled for analysis monthly between April and end of July. The purpose of the study was to establish the milk yield and to investigate the chemical composition of sheep milk obtained from Srednostaroplaninska and Tetevenska breeds reared on pastures at a different altitude and with different sward composition. The Tetevenska breed had a higher daily milk yield and exhibited a high percentage of casein in milk, while the milk from Srednostaroplaninska sheep had a higher content of macro elements - calcium, potassium, magnesium and some trace elements (iron and manganese). The milk fat content, including the percentages of saturated and unsaturated fatty acids in milk obtained from studied breeds, was similar. There was a tendency towards higher percentage of polyunsaturated and long-chain fatty acids in the milk of Srednostaroplaninska sheep, reared on mat grass pastures. Milk casein content was higher in the milk of Tetevenska sheep, as well as the content of non-essential amino acids as compared to Srednostaroplaninska sheep.

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

Introduction

Rearing local sheep on mountainous and highland pastures during the milking period allows for production of nutritionally complete dairy products. The interest to milk proteins is raised by their biological and nutritional value or whole body nitrogen retention. The comparison of amino acid content of Karakachan and Tsigay sheep during the grazing period established higher content of proline, valine and isoleucine and higher biological value of proteins in the former sheep breed (*Gerchev et al., 2006*).

Fat contains a number of specific functional substances with therapeutic properties: polyunsaturated fatty acids, including conjugated linoleic acid (CLA) (*Jarheis,2000*). Investigations on milk fat composition in Churra sheep demonstrated a low heritability for saturated and monounsaturated fatty acids and a potential for genetic improvement in polyunsaturated fatty acids (*Sanches et al., 2010*). In Italian sheep breeds, *Signorelli et al. (2008)* established differences in the milk content of CLA and PUFA, but not of monounsaturated fatty acids.

According to *Antunovic et al. (2001)*, *Celik and Ozdemir (2003)* and *Khan et al. (2006)* food is the main source of minerals for the animal organism. Also, it is responsible for the mineral content of milk during the lactation period.

The purpose of the study was to establish the milk yield and to investigate the chemical composition of bulk sheep milk in Srednostaroplaninska and Tetevenska breeds reared on pastures at a different altitude and different sward composition.

Material and Methods

The study was performed during the grazing period (April-July) with two herds of 12 sheep from Srednostaroplaninska and Tetevenska breeds. They were reared using the traditional system of Central Balkan Mountains – gradually moving sheep from mountainous (400-500 m altitude) to highland pastures (1400 m altitude for the Srednostaroplaninska sheep breed and 800-1000 m altitude for the Tetevenska sheep breed) according to the change in swards and sheep' productivity. During the grazing period, additional feed was not offered. Milk samples for analysis were collected during the bulk milk control one monthly between April and end of July.

The main chemical composition was determined on a Milko-skan 133B analyzer. Amino acids of total milk protein were assayed using the ion exchange chromatography technique. The sample was submitted to acid hydrolysis with 6 N hydrochloric acid at 110º C for 24 h and buffer with pH 2.2. Sulfur-containing amino acids (methionine and cysteine) were assayed after oxidation of samples with a mixture of hydrogen peroxide and performic acid. The separation of amino acids was performed on Amino Acid Analyzer T 339M, (Mikrotehchna – Praha), and their quantity – calculated according to eluant volumes in a standard mixture.

Milk fat extraction was done by the method of Rose-Gottlieb. Methyl esters of fatty acids were separated on a gas chromatograph “Pay-Unicam 304” with flame ionization detector and EC^{TM}-WAX column, 30 m, ID 0.25 mm, film:0.25 μm.

Macro- and trace element were determined after dry ashing of samples in a muffle furnace at 550°C. The ashed material was dissolved in hydrochloric acid. The content of K and Na was assayed on a flame photometer (Flamom-B), the

content of phosphorus – spectrophotometrically by the molybdate/vanadate technique. The other elements were assayed by atomic absorption spectrophotometry (Perkin-Elmer 380).

Data were statistically processed by Statistica for Windows (Release, 4.3, Stat. Soft. Inc., 1994).

Results and Discussion

The daily milk yield and milk dry matter in both studied sheep breeds are relatively high, accounting for highland conditions of rearing and milk production (Table 1). Higher milk yield ($p<0.05$) and tendency for insignificantly high content of dry matter and milk fat were established in Tetevenska breed. The difference in non-fat milk solids was very low, whereas the differences in protein and casein content were bigger, with insignificantly higher values in Tetevenska sheep milk. Our data about the chemical composition of milk in both studied breeds corresponded to those of *Genkovski (2002)*, but were substantially higher with respect to average daily milk yields.

Table. 1 Daily milk yield and chemical composition of milk during the milking period

Quatitative indices	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
Daily milk, l	0.96±0.12	1.15±0.28
Dry matter, %	18.54±1.25	18.69±1.56
Fat, %	7.13±1.42	7.25±1.38
Dry free residue, %	11.41±0.42	11.44±0.27
Protein, %	5.71±0.43	6.08±0.54
Casein	4.08±0.35	4.41±0.41
Non casein protein %	1.63±0.10	1.63±0.13
Lactose, %	5.10±0.42	4.76±0.30
Solids, %	0.93±0.07	0.99±0.03

Table 2. Mineral elements of milk during milking period

Mineral elements	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
Macro elements		
Ca, mg/100g	223±2**	208±4
P, mg/100g	145±1	145±4
K, mg/100g	131±1***	110±3
Na, mg/100g	49±1	50±1
Mg, mg/100g	1807±53***	1657±16
Trace elemends		
Fe, mg/100g	0.181±0.015**	0,120±0.011
Cu, mg/100g	0.043±0.02	0,040±0.003
Zn, mg/100g	0.709±0.032	0,618±0.076
Mn, mg/100g	0.032±0.002***	0,016±0.001

**P<0.01
***P<0.001

Pasture vegetation is the source of macro- and trace elements in milk during the studied lactation period (Table 2). Macroelement content was higher in the milk of the Srednostaroplaninska breed, reared on a mat grass pasture, mainly with respect to calcium ($p<0.01$), potassium ($p<0.001$) and magnesium ($p<0.001$). The concentrations of phosphorus and sodium were similar. Our results were in agreement with those of *Todorova et al. (2003)*, who reported higher levels of Ca, P, Na and Mg in the biomass of mat grass pastures at an altitude of 420 m, of K – at 990 m, compared to the biomass collected at altitudes of 600 and 1500 m, thus confirming that the higher macroelement content of milk from Srednostaroplaninska sheep was consequent to their higher content in grass vegetation.

A similar trend was established for trace elements in milk, with significant interbreed differences for iron ($p<0.01$) and manganese ($p<0.001$) concentrations. In previous studies of ours *(Mihaylova et al., 2005; 2006)* on milk mineral composition in Karakachan and Tsigay sheep, iron and zinc concentrations were lower in milk produced at a higher altitude. *Angelov et al. (1998)* reported iron contents of 71.0 to 145.7 mg/kg and manganese content 77.3–280.9 mg/kg at highland pastures of Central Balkan Mountains. The high concentration of iron and zinc was due to powder layering in pastures from surface mine.

Saturated fatty acids in milk fat were high in both breeds (Table 3). The percentage of short-chain fatty acids (butyric, caproic and caprylic) was higher in the milk fat of Tetevenska sheep whereas the milk of Srednostaroplaninska sheep

was richer in capric acid. In both studied breeds, the concentrations were higher as compared to Karakachan sheep, reared in the Central Balkan Mountains region *(Mihaylova et al., 2008)*.

Table 3. Saturated fatty acids

Fatty acids%	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
C 4:0	3.14±0.23	3.59±0.284
C 6:0	2.84±0.31	2.96±0.231
C 8:0	2.71±0.41	2.92±0.335
C 10:0	6.79±0.54	5.88±0.624
C 12:0	3.38±0.24	3.79±0.257
C 14:0	10.59±0.61	11.71±0.370
C 15:0	0.96±0.22	0.67±0.109
C 16:0	25.12±0.825	25.80±0.711
C 17:0	2.25±0.34	1.09±0.242
C 18:0	13.02±0.682	12.52±0.461

The trend of lauric acid (C12:0) content was similar to that of short-chain fatty acids. Myristic acid (C14:0), which determines the specific odour of sheep milk, had a relatively high content in both breeds, higher in Tetevenska sheep milk. The values were significantly higher than those reported in Karakachan and Tsigay sheep breeds *(Mihaylova et al., 2008; Gerchev end Mihaylova, 2009)*.

The share of palmitic (C16:0) and stearic (C18:0) acid in the saturated fatty acids group was significant, but their concentrations in studied milk varied. From fatty acids with odd number of carbon – C15:0 and C17:0, margaric acid tended to be at higher amounts in the milk fat of Srednostaroplaninska sheep breed. These data do not correspond to reported concentrations of C16:0, C17:0 and C18:0 in Karakachan sheep milk, but instead are similar to the milk fat composition of Tsigay sheep, which are reared in the same conditions *(Mihaylova et al., 2008; Gerchev and Mihaylova, 2009)*.

The content of unsaturated fatty acids – C10:1, C12:1, C14:1 и C16:1 in milk fat (Table 4) was low in general, without statistically significant differences between both breeds. Oleic acid content (C18:1) in milk was slightly higher in Srednostaroplaninska sheep. In the view of *Mihaylova et al. (2008)* the percentage of C18:1 (especially of some of its isomers) was the highest at the beginning of the grazing period. In many other reports, a positive correlation between C18:1 and CLA, as oleic acid is a substrate in CLA synthesis.

Table 4. Unsaturated fatty acids

Fatty acids%	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
C10:1	0.151±0.043	0.185±0.030
C12:1	0.156±0.096	0.036±0.013
C14:1	0.298±0.220	0.208±0.052
C16:1	0.256±0.176	0.512±0.148
C18:1	24.426±0.994	24.171±0.404
C18:2	2.809±0.248	2.598±0.192
C18:3	1.512±0.174	1.336±0.104

From polyunsaturated fatty acids, linoleic acid (C18:2) content was higher than that of linolenic acid (C18:3), but without any significant interbreed differences. The levels of these fatty acids in studied sheep breeds were higher as compared to those in Karakachan and Tsigay sheep, reared in Rhodopes and Stara Planina mountains *(Mihaylova et al., 2006a, Gerchev and Mihaylova, 2009),*

The total amount of saturated fatty acids (SFA) in milk fat (Table 5) was similar in both breeds. High SFA concentrations were related to lower content of both monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) with e trend towards higher concentrations in the milk of the Srednostaroplaninska sheep breed. The results corresponded to data obtained for Karakachan and Tsigay sheep milk, reared in the same region of Central Balkan Mountains *(Mihaylova et al., 2008; Gerchev end Mihaylova, 2009)*. Similar conclusions were also made by *Alexiev (2010)* in his research on milk from Pleven Blackhead sheep.

Table 5. Groups of fatty acids in milk from sheep

Fatty acids%	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
Σ SFA	70.84±5.22	71.00±3.56
Σ MUFA	25.08±1.40	25.01±0.62
Σ PUFA	4.52±0.42	3.93±0.30
Σ C4:0-C11:0	15.48±1.49	15.27±1.48
Σ C12:0-C16:1	40.66±2.31	42.48±1.66
ΣC17.0-C25:0	44.03±2.44	41.71±1.35

The variations in amino acid content of milk protein during the period were miscellaneous (Table 6). Tetevenska sheep milk exhibited higher concentrations of

non-essential amino acids, which is in agreement with the higher casein content of protein. The proportion of threonine, serine and arginine in Tetevenska sheep milk was substantially higher than those in the Srednostaroplaninska breed ($p<0.01$). Similar tendencies were observed with regard to aspartic acid, alanine, glutamic acid and tyrosine ($p<0.05$), while the difference in phenylalanine, histidine, lysine and valine were not significant ($p>0.05$). The dynamics off cysteine in both breeds was similar, unlike methionine content that was higher in Tetevenska sheep milk.

Table 6. Amino acids in milk during the milking period

Amino acids, %	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
Aspartic acid	0.368±0.033	0.464±0.025**
Threonine	0.136±0.015	0.207±0.011* **
Serine	0.106±0.015	0.176±0.011* * *
Glutamic acid	1.110±0.092	1.362±0.079* *
Proline	0.484±0.041	0.544±0.031
Cysteine	0.037±0.004	0.038±0.004
Glycine	0.078±0.009	0.102±0.006**
Alanine	0.179±0.015	0.219±0.011* *
Valine	0.333±0.029	0.402±0.023
Methionine	0.046±0.009	0.067±0.009
Isoleucine	0.237±0.020	0.281±0.017
Leucine	0.485±0.039	0.561±0.033
Tyrosine	0.143±0.015	0.189±0.011**
Phenylalanine	0.218±0.018	0.264±0.014
Histidine	0.149±0.020	0.172±0.011
Lysine	0.388±0.033	0.477±0.031
Arginine	0.112±0.013	0.170±0.009***

** $p<0.01$
***$p<0.001$

The studied amino acids, divided into groups depending on whether they were essential or non-essential, and on the number of amine and carboxylic acid groups, are shown in Table 7. The amounts of essential amino acids in the milk of the two breeds were comparable whereas the content of non-essential amino acids was considerably higher ($p<0.001$) in Tetevenska sheep milk. The total amount of essential amino acids in Srednostaroplaninska and Tetevenska sheep milk was

lower than values reported by *Alexeeva et al. (1986)*, and values in a high-production Bulgarian sheep population *(Stancheva, 2002)*.

Table 7. Amino acid groups in the milk

Amino acid group %	Srednostaroplaninska Sheep	Tetevenska Sheep
	x±Sx	x±Sx
Σ Essential	2.26±0.17	2.49±0.20
Σ Non-essential	2.32±0.18	3.21±0.25***
Σ MAMC	1.75±0.13	2.47±0.02***
Σ DAMC	0.50±0.04	0.65±0.05*
Σ MADC	1.48±0.06	1.83±0.13*
Σ RAA	0.73±0.07	0.72±0.06

MAMC – monoaminomonocarboxylic amino acids
DAMC – diaminomonocarboxylic amino acids
MADC – monoaminodicarboxylic amino acids
RAA - ring-containing amino acids
*P<0.05 ***P<0.001

At the same time, Tetevenska sheep milk had a higher content of monoamino monocarboxylic amino acids ($p<0.001$). The same was valid for diamino monocarboxylic and monoamino dicarboxylic amino acids, although at a lower statistical significance. The contents of ring-containing amino acids were similar. In general, the content of amino acids in milk protein of Srednostaroplaninska and Tetevenska sheep was lower as compared to Karakachan and Tsigay sheep *(Mihaylova et al., 2006b)*, although Srednostaroplaninska sheep grazed on the same pastures, which could be attributed to the effect of breed on chemical composition of milk.

Conclusion

The Tetevenska breed had a higher daily milk yield and exhibited a high percentage of casein in milk, while the milk from Srednostaroplaninska sheep had a higher content of macroelements calcium, potassium, magnesium and some trace elements (iron and manganese).

The milk fat content, including the percentages of saturated and unsaturated fatty acids in studied breeds, was similar. There was a tendency towards higher percentage of polyunsaturated and long-chain fatty acids in the milk of Srednostaroplaninska sheep.

Milk casein and total protein contents were higher in the milk of Tetevenska sheep, as well as the content of non-essential amino acids as compared to Srednostaroplaninska sheep.

Prinos i hemijski sastav mleka ovaca srednje- staroplaninske i tetevenske rase

G. Gerchev, G. Mihaylova

Rezime

Istraživanje je izvedeno u periodu od aprila do jula, na pašnjacima koji se nalaze na različitim nadmorskim visinama i različitog travnog sastava, tokom perioda laktacije ovaca srednje-staroplaninske i tetevenske rase. Zbirni uzorak mleka sa jutarnje muže je uziman mesečno u periodu od aprila do kraja jula.

Cilj istraživanja je bio da se utvrdi prinos mleka i ispita hemijski sastav mleka dobijenog od ovaca srednje-staroplaninske i tetevenske rase koje su gajene na pašnjacima na različitim nadmorskim visinama i različitog travnog sastava.

Tetevenska rasa ovaca je imala viši denvni prinos mleka i utvrđen je viši sadržaj kazeina u mleku, dok je mleko ovaca srednje-staroplaninske rase imalo veći sadržaj makro elemenata – kalcijuma, kalijuma, magnezijuma i nekih mikro elemenata (gvožđe i magnezijum).

Sadržaj mlečne masti, uključujući sadržaj zasićenih i nezasićenih masnih kiselina u mleku dobijenom od ispitivanih rasa ovaca, je bio sličan. Utvrđena je tendencija ka većem sadržaju poli-nezasićenih masnih kiselina i masnih kiselina dugog lanca u mleku ovaca srednje-staroplaninske rase koje su gajene na pašnjacima gde raste trava *Axonopus fissifolius*.

Sadržaj kazeina u mleku je bio veći u mleku tetevenske ovce, kao i sadržaj ne-esencijalnih amino kiselina, u poređenju sa srednje-staroplaninskom rasom ovaca.

References

ALEXIEV Y. (2010): Effect of shearing on some productive and physiological traits in different breeds and categories of sheep. PhD thesis, Institute of Mountain Stockbreeding and Agriculture, p.350.

ALEXEEVA N.Y., ARISTOVA B.P., PATRATIY A.P.,. POTAPOV A.S,. SEREBRYANNIKOVA V.A., TOSHEV Y.V., FETISOV E.A, SHIDLOVSKAYA

V. P. (1986): Composition and properties of milk as a raw product for the dairy industry. Moskva, Agropromizdat.
ANGELOV L., KAFEDZHIEV V., ODZHAKOVA T.S., HRISTOV M. (1998): Effects of environmental geochemical factors and sward vegetation on the transfer of some essential trace elements I. Provision of sheep with manganese, iron and selenium. Animal Sciences, 5, 71-76.
ANTUNOVIC Z., STEINER Z., SENCIC D., MANDIC M., KLAREC T. (2001): Changes in ewe milk composition depending on lactation stage and feeding season. Czech Journal of Animal Science, 46, 2, 75-82.
CELIK S., OZDEMIR S. (2003): Chemical composition and major minerals of Avassi sheep milk during lactation. Milcwissenschaft-Milk Science International, 58, 7-8, 373-375.
GENKOVSKI D. (2002): Comparative characteristics of sheep of different breeds and strains, reared in the Central Balkan Mountains. PhD. thesis, p. 138.
GERCHEV G., MIHAYLOVA G., SLAVOV R. (2006): Chemical and mineral composition of sheep milk from the region of Central Balkan Mountains. Food Industry, 7,25-29.
GERCHEV G., MIHAYLOVA G. (2009): Variation in fatty acid composition of milk from Tsigai sheep in the region of Central Balкan Mountains. Biotehnology in animal husbandry, Belgrade, Book 2, 25, 5-6, 945-952.
JAHREIS G. (2000): Functional foods. Fettsäuren und fettähnliche inhaltsstoffe der milch mit functional - food-charakter. Deutche Molkerei Zeitung, 7, 320-324.
HAENLEIN, MOIOLI B. (2008): Breed differences in sheep milk fatty acid profiles: Opportunities for sustainable use of animal genetic resources. Small Ruminant Research, 78, 1, 24-31.
KHAN Z., ASHRAF M., HUSSAIN A., MCDOWELL L., ASHRAF M. Y. (2006): Concentracions of minerals in milk of sheep and goads grasing similar pastures in a semiarid region of Pakistan. Small Ruminant Research, 65, 274-278.
MIHAYLOVA G., GERCHEV G., PETKOV G., GROZEVA M. (2005): Mineral concentration dynamics in milk during the lactation period of Tsigay sheep. Ecology and Future, 4, 2, 40-43.
MIHAYLOVA G., GERCHEV G., GENKOVSKI D. (2006): Dynamics of trace elements in Karakachan sheep milk during the lactation period. Ecology and Future, 1, 42-47.
MIHAYLOVA G., KAFEDJIEV V., ODJAKOVA TS. (2006a): Fatty acid content of milk from Karakachan sheep reared in the Rhodopes Mountain. Ecology and Future, 1, 22-25.
MIHAYLOVA G., GERCHEV G., BEEV G. (2006b): Biological value of milk protein in Tsigay and Karakachan sheep breeds. Animal Sciences, 3, 34-37.
MIHAYLOVA G., GERCHEV G., NAYDENOVA N. (2008): Changes in milk fat composition in Karakachan sheep reared in Central Balkan Mountains.

Anniversary Scientific Conference "80 years Agrarian Science in the Rhodopes", Smolyan, Proceedings, 110-113.
SANCHES J.P., SAN PRIMITIVO F., BARBOSA E., VARONA L., DELA FUENTE F.L.(2010): Genetic determination of fatty acid composition in Spanish Churra sheep milk. J. Dairy Sci., 93, 1, 330-339.
STANCHEVA N. (2002): Phenotype and genotype parameters of selection traits in the new high-productivity dairy sheep population in Bulgaria. PhD. Thesis, Sofia.
SIGNORELLI, F., CONTARINI G., ANNICHIARICO G., NAPOLITANO F., ORRU L., CATILLO G., SANCHES G. J. P., SAN PRIMITIVO F., BARBOSA E., VARONA L., DELA FUENTE L. F. (2010): Genetic determination of fatty acid composition in Spanish Churra sheep milk.J. Dairy Sci., 93, 1, 330-339.
TODOROVA P., PAVLOV D., TODOROV M. (2003): Composition and feeding value of permanent grassland in the Central Balkan Mountains. Optimal forage systems for animal production and the environment, 8, 215-218.

Received 28 March 2012; accepted for publication 14 May 2012