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CHEMICAL COMPOSITION AND BIOLOGICAL PROTEIN VALUE OF MILK FROM LOCAL SREDNOSTAROPLANINSKA SHEEP BREED**

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Abstract: The study was carried out during the grazing period (April-July) with a group of 5 local sheep of the Srednostaroplaninska breed in 3rd lactation. The following system of animal raising was used: grazing on a mountain pasture (April and May), supplementation in April and alpine grazing on high-mountain pastures (June and July).

Samples were taken every month during milking period (April-July). Chemical composition of milk and amino acid composition of protein were studied by ion exchange column chromatography. The objective of the study was to determine the chemical composition and biological protein value of milk from Srednostaroplaninska sheep breed.

The amino acid content in milk protein, namely glutamic acid, proline, lysine, alanine, valine and serine, increased with advance of lactation period, whereas that of the limiting amino acid methionine decreased and subsequently increased till the end of the period. The protein of milk from the Srednostaroplaninska breed had an index of 35.35 % and a biological value of 90.8 %.

The sward type and vegetation stage exerted an influence on the content of essential amino acids, biological value and chemical index of protein of sheep's milk.

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

Introduction

Raising and nutrition of local sheep in mountainous regions as

environmental factors influence the sheep organism and productivity, particularly during the grazing period. Studies on milk productivity of sheep on high-mountain pastures showed a tendency to higher milk yield (*Gerchev* 1998; *Tsochev* 1999). Pasture type and composition of pasture sward during the period does not influence the milk composition significantly (*Gerchev, Mihaylova* 1998; *Gerchev* 1998).

The interest for milk proteins is related to biological and full nutritive value or degree of retention of dietary nitrogen in body (*Gachev*, 1995). Amino acid composition of sheep's milk was studied by *Velev* (1986), *Tanev et al*. (1986), mainly in milk sheep breeds. Comparing the amino acid composition of milk from Karakachan and Tsigai sheep breeds, *Gerchev et al*. (2006) found higher content of proline, valine and isoleucine, as well higher biological value in the first breed.

The objective of the study was to determine chemical composition and biological value of milk from Srednostaroplaninska sheep breed raised in the region of Central Balkan Mountains.

Material and methods

The study was carried out during the grazing period (April-July) with sheep of Srednostaroplaninska breed: in April and May they grazed on a mountain pasture and in the next months of June and July on a high-mountain pasture where the basic grass composition is Nardus stricta. Due to insufficient grazing in April, the animals were supplemented with hay and concentrate.

Milk samples for analysis were taken during the controls from each animal one time monthly from April to July inclusive. Basic chemical composition was determined on Milko-scan 133B. The principle of ion exchange column chromatography was used to determine amino acids of total milk protein. For that purpose the sample was treated by acid hydrolysis with 6 n solution of hydrochloric acid at 110º C for 24 hours. Dissolution of the residue was performed in buffer of pH = 2.2. Sulphur-containing amino acids (methionine and cystine) were determined after sample oxidation with a mixture of carbon peroxide and performic acid. The different amino acids (except for tryptophane) were separated on an Amino analyzer (Amino Acid Analyzer T 339 M. Mikrotehchna – Praha) and their quantity was estimated from their elution volume and standard mixture.

The obtained data was processed by variation statistical methods of Statistika for Windows (Release. 4.3. stat. soft. Inc., 1994).

Results and discussion

The results of average daily milk yield and chemical composition of milk are presented in Table 1. The daily milk yield was highest in April, the differences compared to the other controls varied from 0.24 to 0.46 l and the difference between 1st and 4th controls was significant at $p<0.05$. The results of April were also higher with regard to content of dry matter and milk fat, due to the supplementation of sheep during that period. Besides the milk yield, some of the chemical characteristics also decreased in 2nd control. Dry matter decreased by 1.62 %, but till the end of lactation period it increased by 2.85 % without mathematically significant difference between the controls. Dynamics of fatty substances was similar during that period and the difference of about 3.85 % between 1st and 4th controls had low significance $p<0.05$. Moving the sheep to a high-mountain pasture suggested the low differences between the controls. The changes in dry fat-free residue were minimal and we could not record any tendency. However it was pronounced for the protein, no matter that the dynamics in the increase was very slight and close to that of fat. Similar tendency was recorded in our studies (*Gerchev* 1998; *Gerchev and Mihaylova* 1998) on the effect of pasture type on the milk productivity and composition in Staroplaninski Tsigai sheep breed. No matter that during the different years the lactation curve and milk composition had partial deviations due to environmental conditions.

Table 1. Daily milk yield and chemical composition of milk

Indices	Daily milk		Dry matter		Fat		Dry free residue		Protein	
	L		%		%		%		%	
	x±Sx	C	x±Sx	C	x±Sx	C	x±Sx	C	x±Sx	C
1. control	0.96 ±0.13	13.54	18.42 ±1.61	8.74	7.28 ±2.06	28.30	11.14 ±0.52	4.67	5.22 ±0.31	5.96
2. control	0.78 ±0.13	16.67	16.80 ±0.97	5.77	5.20 ±1.11	21.35	11.61 ±0.33	2.84	5.53 ±0.18	3.25
3. control	0.60 ±0.04	6.67	18.52 ±1.17	6.32	7.19 ±0.4	13.07	11.33 ±0.39	3.44	6.10 ±0.48	7.87
4. control	0.50 ±0.07	14.00	19.65 ±1.14	5.80	8.04 +0.63	7.83	11.61 ±0.54	4.65	6.34 ±0.75	11.83

Data on average values of amino acid composition for the breed by months of the milking period is presented in Table 2. During the period, variation in the amino acid content in milk proteins was different and for

most of them there was an increase from April to June and a subsequent decrease in July. The increase was greatest for glutamic acid ($p<0.05$) and proline ($p<0.05$) with a tendency to increase for arginine, aspartic acid и cysteine. In another amino acid group, an increase of their content in proteins was recorded till the end of lactation (April-July), it was more significant for lysine ($p<0.05$), alanine ($p<0.05$) and valine ($p<0.05$) and the differences for phenylalanine, leucine and isoleucine were mathematically non-significant for the period. For the amino acids methionine and histidine there was a reduction in their content in milk proteins in May and a subsequent increase till July, with significance of the difference between May and July $p<0.01$ for methionine. In the sulphur-containing amino acid serine, the dynamics was contrary to that of methionine, an increase in May and a tendency to decrease till July.

Table 2. Amino acids in milk during the milking period

Amino acids %	Total for the period x+Sx	April x+Sx	May x+Sx	June x+Sx	July x+Sx
Aspartic acid	0.464±0.033	0.424±0.036	0.458±0.040	0.488±0.027	0.484±0.027
Threonine	0.207±0.015	0.205±0.020	0.213±0.019	0.210±0.009	0.201±0.010
Serine	0.176±0.015	0.179±0.021	0.190±0.018	0.183±0.009	0.151±0.010
Glutamic acid	1.362±0.092	1.120±0.100	1.237±0.088	1.558±0.100	1.532±0.081
Proline	0.544±0.041	0.468±0.041	0.491±0.045	0.646±0.045	0.571±0.033
Cysteine	0.038±0.004	0.037±0.003	0.043±0.004	0.043±0.004	0.030±0.005
Glycine	0.102+0.009	0.097±0.009	0.098±0.010	0.112±0.007	0.101±0.009
Alanine	0.219±0.015	0.193±0.015	0.199±0.016	0.240±0.013	0.242±0.015
Valine	0.402±0.029	0.356±0.031	0.372±0.033	0.434±0.026	0.446±0.024
Methionine	0.067±0.009	0.059±0.009	0.042±0.008	0.082±0.012	0.083±0.007
Isoleucine	0.281±0.020	0.260±0.022	0.270±0.025	0.298±0.017	0.294±0.017
Leucine	0.561±0.039	0.515±0.041	0.528±0.046	0.597±0.037	0.604±0.033
Tyrosine	0.189±0.015	0.187±0.018	0.204±0.020	0.182±0.007	0.181±0.014
Phenylalanine	0.264±0.018	0.241±0.023	0.266±0.027	0.262+0.016	0.288±0.007
Histidine	0.172±0.020	0.170±0.038	0.167±0.017	0.175±0.013	0.175±0.011
Lysine	0.477±0.033	0.416±0.038	0.438±0.031	0.514±0.031	0.538±0.032
Arginine	0.170±0.013	0.143±0.016	0.162±0.015	0.192±0.012	0.181±0.009
Total	5.695	5.07	5.38	6.202	6.122

Table 3 presents the distribution of amino acids by groups. The difference between the quantities of non-essential amino acids was about 23 % greater, as compared to that of the essential ones. It is evident that in the months of the period, the essential amino acids were characterized by the

curve of continuous increase, while in the non-essential amino acids, the curve had a peak in June with a subsequent decrease, no matter that the differences were minimal. The content of essential amino acids found by us in the Srednostaroplaninska sheep breed was lower than the average values of *Alekseeva et al*. (1986) for sheep's milk and those of *Stancheva* (2002) for a high-milk sheep population raised in our country.

Table 3 Amino acid groups in the milk

Amino acid group %	Mean for the period x±Sx	April x±Sx	May x±Sx	June x±Sx	July x±Sx
Σ Essential	2.49±0.20	2.27±0.21	2.38±0.21	2.62±0.21	2.67±0.15
Σ Non-essential	3.21±0.24	2.79±0.31	3.00±0.26	3.58±0.26	3.48±0.15
Σ MAMC	2.51±0.02	2.33±0.02	2.45±0.02	2.45±0.02	2.64±0.01
Σ DAMC	065±0.05	0.56±0.05	0.60±0.05	0.71±0.04	0.71±0.04
Σ MADC	1.83±0.13	1.54±0.14	1.70±0.13	2.05±0.13	2.02±0.11
Σ CAA	0.72±0.06	0.64±0.06	0.66±0.06	0.82±0.07	0.75±0.04

MAMC –monoaminomonocarboxylic acids
DAMC –diaminomonocarboxylic
MADC –monoaminodicarboxylic
CAA - cyclic amino acids

At the same time the dynamics of content of monoaminomonocarboxylic and monoaminodicarboxylic acids in milk proteins by months was similar to that of the essential amino acids, while that of diaminodicarboxylic and cyclic amino acids was similar to the non essential ones. According to these characteristics, the values of Tsigai and Karakachan breeds were higher than those of the breed studied by us (*Mihaylova et al.,* 2006).

As a whole, the amino acid content in milk proteins of the Srednostaroplaninska breed was lower, as compared to the Karakachan and Tsigai breed (*Mihaylova et al*., 2006), although they were raised on the same pasture, which we could explain by the year effect and by the fact that their lactation period was covered entirely.

One can judge on the biological value of a given product, comparing the amino acid composition with the so-called "ideal amino acid scales", corresponding to a completely balanced amino acid protein. The method of amino acid index is based on that comparison (*Gachev*, 1995; *Markova*, 1988). Table 4 presents the comparison of the obtained values of the different essential amino acids in sheep's milk of the studied breed by months with the reference values of the "ideal" protein.

The milk of this breed, as a whole, was superior in reference protein, in content of leucine, isoleucine, valine, lysine and in total content of phenylalanine and tyrosine (except for April). Limiting amino acids appeared to be the sum of sulphur-containing methionine and cystine, the index of which varied from 58.0 % in May to 58.3 % till July, which indicated that with advance of sward vegetation, the content of these two amino acids increased.

Table 4. Biological value of sheep's protein

Essential amino acids, g/100 g Total protein	Referene pattern (FAO/ WHO)	Whole egg Protein	April	Index %	May	Index %	June	Index %	July	Index %
Threonine	4,0	4.8	4.03	100.8	3.95	98.5	3.40	85.0	3.80	95.0
Leucine	7,0	8.8	10.2	145.6	9.80	140.0	9.61	137.3	9.88	141.1
Isoleucine	4,0	6.7	5.14	128.5	5.01	125.3	4.80	120.0	4.80	120.0
Valine	5,0	7.2	7.03	140.6	6.90	138.0	7.01	140.2	7.28	145.6
Methionine +	3,5	5.2	1.87	53.4	1.68	48.0	1.79	51.2	2.04	58.3
Cysteine										
Lysine	5,5	6.2	8.20	149.1	8.21	149.3	8.29	138.2	8.79	159.8
Phenylalanine +	6,0	5.7	4.43	73.8	11.20	186.7	7.54	125.7	7.13	119.3
Tyrosine										
Tryptophane	1,0	1.6								
Essential amino acids	36,0	46.3	41.16		45.07		42.42		43.72	
Chemical index	100%		35.48		32.30		34.42		39.23	
Biological value		97%	86.2		96.3		88.9		91.6	

As a whole, the amino acid composition of the milk from this breed increased in May to 45.07 g/100 g total protein, then it decreased in June to 42.42 g/100g during alpine grazing of sheep on a high-mountain pasture and increased again in July. Similar were the changes in the biological protein value of sheep's milk from this breed, as compared to egg protein. According to us, the change of the sward exerted an influence on the content of almost all amino acids in milk, as well as on its biological value. On average for the milking period, it was lower (90.8 %) than the average values of Tsigai and Karakachan breed, 93.6 and 94.7 % respectively (*Mihaylova et al.,* 2006).

Conclusions

Amino acid content in milk protein, namely glutamic acid, proline, lysine, alanine, valine and serine, increased with advance of lactation period, whereas that of limiting amino acid methionine decreased and subsequently increased till the end of the period.

The sward type and vegetation stage exerted an influence on the content of essential amino acids, biological value and chemical index of protein of sheep's milk.

The protein of milk from the Srednostaroplaninska breed had an index of 35.35 % and a biological value of 90.8 %.

HEMIJSKI SASTAV I BIOLOŠKA VREDNOST PROTEINA MLEKA SREDNJE STAROPLANINSKE RASE OVACA

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Rezime

Ispitivanje je izvedeno tokom perioda ispaše (april-jul) sa grupom od 5 lokalnih ovaca srednje staroplaninske rase u trećoj laktaciji. Primenjen je sledeći metod odgoja: ispaša na planinskom pašnjaku (april i maj), dopuna ishrane u apilu I ispaša na visoko-planinskim pašnjacima (jun i juli).

Uzorci su uzimani svakog meseca tokom prioda muže (apil-juli). Hemijski sastav mleka i sastav amino kiselina proteina su ispitivani hromatografijom razmene jona. Cilj ispitivanja je bio određivanje hemijskog sastava i biološke vrednosti proteina mleka od srednjestaroplaninske rase ovaca.

Sadržaj amino kiselina u proteinu mleka, naime glutaminske kiseline, prolina, lizina, alanina, valina I serina, se povećavao sa odmicanjem perioda laktacije, dok se sadržaj limitirajuće amino kiseline metionina smanjio I naknadno povećao do kraja perioda. Protein u mleku ovce srednje staroplaninske ovce je imalo indeks od 35.35 % i biološku vrednost 90.8 %.

Stadijum vegetacije je imao uticaj na sadržaj esencijalnih amino kiselina, biološku vrednost I hemijski indeks proteina u ovčijem mleku.

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