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AMINO ACID COMPOSITION OF MILK FROM TSIGAI AND KARAKACHANSKA SHEEP BREEDS REARED IN THE CENTRAL BALKAN MOUNTAINS REGION[1]

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Abstract: The study focused on the contents of amino acids in mixed sheep milk samples, obtained from thoroughbred Tsigai and Karakachanska ewes, reared in the Central Balkan Mountains area. The ewes, allocated in two groups, were equalized in age, lambing time and put under similar conditions of rearing. The period of study covered only the ewes' milking period during which four milk controls were conducted, in accordance with the active Instructions on Milkiness Control. The samples for analysis were collected on monthly basis from mixed milk of the ewes in each group, subsequent to combining the milk amounts from the evening and morning milkings, observing the rules for milk sample collecting. Until the beginning of analysis the milk samples were stored at temperature values below zero. The amino acids were determined by acid hydrolysis of the sample and separating each amino acid on Amino Acid Analyzer T 339 M (Mikrotechna - Praha).

Evidence showed that except for cystine the milk of Tsigai ewes contained greater amounts of each amino acid than the samples of Karakachanska breed ewes. In the sheep milk examined, the monoaminocarbon acids were present in the highest amounts (38-39%), followed by monoaminodicarbon acids (30%), and last came diaminocarbon acids (10.6%). Compared with egg protein, the biological value of sheep milk was 92.2 and 93.4% for Tsigai and Karakachanska breeds accordingly. The highest reference protein pools in the sheep milk (FAO/WHO) were found for lysine – 140 and 142 for Groups 1 and 2 correspondingly.

Key words: sheep milk, amino acids, biological value

Introduction

Sheep of Karakachanska and Tsigai breeds are valuable genetic resources for mountain and fore mountain regions of our country for the production of milk and meat. The composition and processing characteristics of the milk produced from these breeds reared in various regions across the country have been subject of continuous researches (*Raichev et al., 1987; Slavov et al., 1990; Gerchev, 1998; petrova et al., 1998; Odjakova et al., 2002; 2002a; Genkovski, 2002; Kafedjiev et al., 1998; Gerchev et al., 1998, etc.*). Despite the numerous studies, there are no data on the amino acid composition of protein in milk from Tsigai and Karakachanska sheep breeds.

Some Bulgarian researchers who have conducted studies on the amino acid composition of sheep milk are *Shalichev and Tanev (1973), Tanev (1975), Tanev and Yovcheva (1976), Tanev et al. (1986), Velev (1986), Stancheva (2002*).

Tanev et al. (1986) found that in milk from Avassi breed there is a third limiting amino acid – threonine, unlike the other two breeds investigated, while the casein in milk of Pleven Blackhead sheep breed was determined to be of the highest biological full value.

Velev (1986) examined the amino acid composition of milk from Local Starozagorski sheep breed. The author found that sheep milk is slightly different from cow milk in amino acid composition, and also that there is no great difference between milk from Starozagorski sheep breed and that of other breeds.

Stancheva (2002) recorded a value of 46.06 g/l for the total amino acid content in sheep milk from the newly bred population of high milking capacity. The amount of essential amino acids was 19.03 g/l (41.32%), and that of inessential ones – 27.03 g/l (58.68%). Among the essential amino acids, the highest percentages were determined for leucine (10.09%), followed by lysine (8.40%) and valine (6.73%). The lowest content was determined for the sulphur containing amino acid methionine (5.99 g/l). Among the inessential amino acids glutamic acid was present in the highest percentage (19,08%), followed by proline

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(10,63%) and aspartic acid (7,27%). The amino acid index of this milk was 67.0, with methionine as limiting amino acid.

Sawaya et al. (1984) reported differences in the contents of glutamic acid, lysine and aspartic acid in the milk form two Arab sheep breeds – Najdi and Nuaimi. The research included comparative assessment of sheep and cow milk, and the authors found higher levels of sulphur containing amino acids in the sheep milk.

The aim of our study was to determine the amino acid contents in the protein of milk from Tsigai and Karakachanska sheep breeds, reared under the conditions of the Balkan Mountains.

Material and methods

The study was conducted in the region of the Balkan Mountains during the grazing season (April – June) of two groups of 50 ewes at their 1st to 5th lactation: Group 1 was comprised of thoroughbred Tsigai ewes, and Group 2 – of Karakachanska breed ewes. The ewes in both groups were equal in age and lambing time, and they were put under the same conditions of rearing. Until March the animals were kept indoors, fed concentrate forage and hay, in April they were grazed but also received some concentrate, while during the rest of the study period they were only grazed.

The study covers only the milking period of ewes, through which four milk controls were made, pursuant to the Instruction for Milking Capacity Control. Samples for analysis were collected on monthly basis from mixed milk of the morning and evening milking of the animals in each group, in compliance with the rules for milk sampling. The samples were stored at temperature below zero until the analyses were performed.

The amino acid composition was determined by acid hydrolysis of the sample with 6 N solution of hydrochloric acid at 110°C for 24 h, and dissolving the residue in a buffer of pH=2.2. The amino acids (except for tryptophane) were separated on Amino Acid Analyzer T339 M (Mikrotechna – Praha), while their amounts were calculated by their eluant volume in a standard mixture.

The data obtained were processed by Statistica for Windows (release 4.3, Stat. Soft. Inc., 1994).

Results and discussion

The average values for the amino acid contents in sheep milk of the breeds examined are listed in table 1. The milk of Tsigai sheep had higher values than Karakachanska breed for the contents of each amino acid, with the exception of cystine. The greatest difference was registered for proline ($p < 0.05$) and histidine, whose amounts were by 16% higher for Tsigai ewes than for Karakachanska breed ewes. Next came glycine and arginine where the difference was 13% in favour of Tsigai breed.

Table 1. Amino acids in the bulk milk during the milking period

(n=4)

Amino acids, %	Group 1		Group 2	
	x	S	x	S
Aspartic acid	0,431	0,068	0,387	0,018
Threonine	0,218	0,033	0,203	0,009
Serine	0,225	0,040	0,199	0,019
Glutamic acid	1,126	0,181	0,998	0,046
Proline	0,532	0,053	0,444	0,019
Cysteine	0,040	0,006	0,040	0,004
Glycine	0,094	0,014	0,082	0,004
Alanine	0,191	0,032	0,171	0,003
Valine	0,322	0,052	0,294	0,010
Methionine	0,138	0,020	0,123	0,008
Isoleucine	0,238	0,039	0,217	0,009
Leucine	0,501	0,078	0,451	0,018
Tyrosine	0,195	0,039	0,170	0,012
Phenylalanine	0,223	0,036	0,192	0,009
Histidine	0,161	0,025	0,135	0,010
Lysine	0,401	0,063	0,358	0,014
Arginine	0,145	0,025	0,126	0,009
Total	5,182	0,784	4,565	0,142

The results we obtained for separate essential amino acids, except for methionine, measured for milk of Tsigai sheep were lower than the average values reported by *Alexeeva et al. (1986*) for sheep milk.

Table 2. Amino acid groups in the milk examined

(n=4)

Amino acid groups, %	Group 1		Group 2	
	x	S	x	S
Σ Essentials	2,277	0,360	2,046	0,077
Σ Non-essentials	2,905	0,425	2,542	0,095
Σ MAMC*	1,968	0,307	1,779	0,070
Σ DAMC*	0,547	0,087	0,484	0,022
Σ MADC*	1,557	0,247	1,385	0,064
Σ CAA*	1,110	0,145	0,940	0,030

*MAMC – monoaminomonocarbonic amino acids
DAMC - diaminomonocarbonic
MADC - monoamonodicarbonic
CAA – cyclic amino acids

Table 2 shows the amino acid groups in sheep milk from the breeds studied. The amount of inessential amino acids exceeded by 21.6% (Tsigai) and 19.5% (Karakachanska breed) that of the essential ones. The essential amino acid amounts estimated in the milk of Tsigai ewes were by 10% higher than in the milk of Karakachanska breed. The difference in the concentration of inessential amino acids was 12.5% in favour of Tsigai breed ewes.

In the milk samples studied the highest percentage was found for the monoaminomonocarbonic amino acids – 38-39%, followed by monoaminodicarbonic ones with 30% and last came the diaminomonocarbonic amino acids – 10.6%. Nearly similar were the values estimated for total amount of essential amino acids in milk samples of both Tsigai and Karakachanska sheep breeds – about 44-45%; this value is a little less than the one reported for Starozagorsha sheep breed by *Velev (1986*).

The biological value of a product is evaluated by comparing the data for its amino acid composition with the so-called "ideal amino acid scales" that correspond to a protein with totally balanced amino acid index. Based on this comparative evaluation is the method of amino acid index (amino acid score) that illustrates the contents of essential amino acids versus the "ideal" protein (*Markova, 1988; Gachev, 1995*).

The biological full value of protein in sheep milk obtained during the milking period from the animals examined is presented in table 3. The limiting amino acid for milk compared to full egg protein is methionine+cystine, with chemical index of 66% (Group 1) and 67.9% (Group 2). Concerning the biological value of the milk studied and compared with egg protein (where it is considered to be 97%) the results were 92.2% (Tsigai ewes) and 93.4% (Karakachanska breed).

Table 3. Biological value of sheep's milk protein

Essential amino acids, g/100 g total protein	Full egg protein	Reference pattern (FAO/WHO)	Group 1		Group 2	
			Amino acids	Score, %	Amino acids	Score, %
Threonine	4,8	4,0	4,2	105	4,4	110
Leucine	8,8	7,0	9,7	138	9,9	141
Isoleucine	6,7	4,0	4,6	115	4,6	115
Valine	7,2	5,0	6,2	124	6,4	128
Methionine	5,3	3,5	2,7	100	2,7	103
Cysteine			0,8		0,9	
Lysine	6,2	5,5	7,7	140	7,8	142
Phenylalanine	5,7	6,0	4,3	135	4,2	132
Tyrosine			3,8		3,7	
Tryptophane	1,6	1,0	-	-	-	-
Total essential amino acids	46,3	36,0	44,0	-	44,6	-

The same table contains also the data for contents of essential amino acids in sheep milk of the breeds examined and the corresponding amino acid index, calculated on the basis of FAO/WHO (1973) databases for reference protein. Lysine turned out to contain the highest pools with regard to reference protein, with amino acid indices 140 and 142 for Groups 1 and 2, accordingly, followed by leucine (138 – Group 1 and 141 – Group 2). The aromatic amino acids in the milk of the sheep breeds studied also contained greater pools regarding the reference protein – their amino acid index was 1.3 times higher than the reference protein. The sheep milk quality can be assessed also on the grounds of amino acid indices of the sulphur-containing amino acids (methionine + cystine) – 100 and 103 for groups 1 and 2, respectively; their amounts were the same as those of reference protein. The total quantities of essential amino acids in the milk of both sheep breeds was 44.0 g/100 g (Group 1) protein and 44.6 g/100 g protein (Group 2) and exceeded by 18% that of reference protein (36%).

The ratio lysine / arginine is used by some authors to describe the atherogenic properties of protein (Tsanev et al., 1995, after Rajamohan et al., 1986), and the lower values indicate anti-atherogenic effect. The values we obtained for this ratio in sheep milk were 2.77 (Tsigai breed) and 2.84 (Karakachanska breed) and these were rather higher than the data found by *Tsanev et al. (1995*) for carp (1.65) and shark (1.67).

Conclusions

The milk from Tsigai breed ewes contains higher contents of the separate amino acids, except for cystine, than that of Karakachanska sheep breed.

The In the milk samples of the breeds studied the highest percentage was found for the monoaminomonocarbonic amino acids – 38-39%, followed by monoaminodicarbonic ones with 30% and last came the diaminomonocarbonic amino acids – 10.6%.

The biological value of sheep milk compared to egg protein was 92.2 and 93.4% for Tsigai and Karakachanska breeds, respectively.

As regards the reference protein (FAO/WHO), lysine had the highest pools and amino acid indices 140 and 142 for the studied Group 1 and 2.

SASTAV AMINO KISELINA U MLEKU OVACA RASA CIGAJA I KARAKAČANSKA KOJE SU GAJENE U CENTRALNOM BALKANSKOM PLANINSKOM REGIONU

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Fokus istraživanja je bio u sadržaju amino kiselina kod mešanih uzoraka ovčijeg mleka dobijenih od čistokrvnih ovaca rase cigaja I karakačanska koje su gajene u centralnom planinskom regionu Balkana. Ovce podeljene u dve grupe su bile jednake po uzrastu vremenu jagnjenja i dražne u istim uslovima odgoja. Period istraživanja je pokrivao samo periode muže ovaca kada su urađene četiri kontrole mlečnosti prema važećim Instrukcijama kontrole mlečnosti. Uzorci za analizu su uzimani mesečno od mešanog mleka ovaca u svakoj grupi, a zatim su kombinovane količine mlkea iz jutarnj i večernje muže poštujući pravila za uzorkovanje mlkea. Do početka analize uzorci mleka su bili skladišteni na temperaturi ispod nule. Amino kiseline su određivane kiselom hidrolizom uzorka i odvajanjem svake amino kiseline na aparatu Amino Acid Analyzer T 339 M (Mikrotechna - Praha).

Rezultati su pokazali da osim cistina mleko cigaja ovaca je sadržavalo veće količine svake amino kiseline nego uzorci mleka karakačanske ovce. U ispitanom mleku, monoaminokarbonske kiseline su bile prisutne u najvećim količinama (38-39%), zatim monoaminodikarbonske kiseline (30%), i na kraju diaminokarbonske kiseline (10.6%). U poređenju sa proteinom jaja, biološka vrednost ovčijeg mleka je bila 92.2 i 93.4% cigaju i karakačansku rasu. Najveći referentni depoi proteina u ovčijem mleku (FAO/WHO) su utvrđeni za lizin – 140 i 142 za grupe 1 i 2.

Ključne reči: ovčije mleko, amino kiseline, biološka vrednost

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