Biotechnology in Animal Husbandry 31 (1), p 45-53 , 2015
Publisher: Institute for Animal Husbandry, Belgrade-Zemun

ISSN 1450-9156
UDC 637.043:637.12'632
DOI: 10.2298/ BAH1501045G

FATTY ACID COMPOSITION OF MILK FAT IN MILK OF TZIGAY SHEEP AND THEIR F2 CROSS-BREEDS WITH CHIOS

G. Gerchev[1], N. Naydenova[2], S. Slavkova[1], G. Mihaylova[2]

[1]Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., 5600, Troyan, Bulgaria
[2]Agrarian Faculty of Thracian University, Students' campus, 6000, Stara Zagora, Bulgaria
Corresponding author: gercho_g@abv.bg
Original scientific paper

Abstract: The study was conducted on aggregate milk samples, which were taken every month during the milking period from Tzigay sheep and their F2 cross-breeds of Chios, raised in the conditions of the Central Balkan Mountain. The fat extraction of milk samples was done by the Rose-Gottlieb method. Fatty acid composition was determined on a gas chromatograph with flame ionization detector and capillary column. The aim of the study was to follow the changes in the composition of fatty acids in the milk fat of milk of Tzigay sheep and their F2 cross-breeds. The saturated fatty acids in milk of the two groups had high values during both consecutive years, as they varied from 67.05% in milk of Tzigay sheep in the second lactation up to 70.87% at their F2 cross-breeds. The content of myristic acid was correspondingly 8.22-8.88% at Tzigay sheep and 8.45-8.74% at their F2 cross-breeds. The total amount of polyunsaturated fatty acids in the examined milk for the two types of sheep was comparatively low with near concentrations (4.39-5.20%) in the period of the two years. The milk of the two groups had high values of the correlation SFA/PUSFA (15.71 and 13.17) and low values of PUSFA/SFA (0.06-0.08). Monounsaturated fatty acids, represented mainly by the oleic acid (C18:1) varied during both periods from 21.92% to 25.32% and appeared as a substratum in the synthesis of CLA. The short-chain fatty acids (C4:0-C11:0) had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids (C17iso-C25:0) maintained close concentration in the milk of Tzigay breed, while their content in the milk of F2 cross-breeds was increased.

Key words: sheep milk, milk fat, fatty acids

Introduction

For many years fatty acids in milk and milk products were associated with raised serum cholesterol, obesity and illness *(Talpur, 2007)*. Extensive researches on the effects of various fatty acids on human health indicate that only a few fatty acids are responsible for the negative effects on the health of consumers *(Simopoulos, 2002)*. Some saturated fatty acids as lauric, miristic and palmitic acids may have effect on the total cholesterol but only C16:0 has a proven impact of raising coronary heart diseases *(Williams, 2000).* Research however is not focused only on the negatives of the fatty acid profile of milk. It contains unsaturated fatty acids including conjugated linoleic acid (CLA), which can reduce total cholesterol, exert anticarcenigenic and antidiabetic activity and has immunomodulating effect ***(****Mills et al., 2011)*. It is important to compare quantitative contents of saturated and unsaturated fatty acids, by calculating the atherogenic index recommended by *Ulbright and Southgate (1991)*. Another way for estimating fatty acid composition is the ratio omega 6/omega 3, which has antagonistic physiological functions necessary for the human body *(Simopoulos, 2002).*

Many studies have focused on the various factors affecting the content of the different fatty acids in milk – breed, nutrition, stage of lactation and others. *Signorelli et al. (2008)*, found that there were no differences in the content of conjugated linoleic acid (CLA) and polysaturated fatty acids in local Italians breeds, while differences were reported in monounsaturated ones. In studying the composition of milk fat in sheep of Churra breed, *Sanchez et al. (2010)* found a low heritability in saturated and monounsaturated fatty acids and a potential for genetic variations in polyunsaturated fatty acid. Studying the fatty acid profile of four sheep breeds (Avasi, Lakaune, Friesland and Chios), *Tsiplakou et al. (2008)* proved that the breed had not influenced the fatty acid region of the Central Balkan Mountains profile. The same author found that when using pastures that led to lower correlations of saturated and higher shares of unsaturated fatty acids.

The aim of the study was to follow out the changes in the milk fatty acid profile during the two consecutive lactating periods of Tzigay sheep and their F2 cross-breeds of Chios breed, raised in the conditions of the Central Balkan Mountain, and to determine the value of the atherogenic index and ratio omega 6/omega 3 as indicators for assessment of the functional qualities of the milk.

Materials and Methods

The study was conducted on aggregate milk samples, which were taken from Tzigay sheep and their F2 cross-breeds of Chios breed, raised in the conditions of the Central Balkan Mountain. The milk samples were taken once a month during the milking period at the time of the three milk controls. The milk was analysed during the May-July period, which covered the grazing raising of sheep on mountain pastures, and after June on high-mountain ones. The samples for analysis were taken from the total milk quantity obtained from 12 sheep in groups at second lactation.

The extraction of fat from the milk samples was performed in the laboratory of Dairying Department at the Agrarian Faculty of the Thracian

University, city of Stara Zagora by the method of Röse-Gottlieb. The methyl esters of the fatty acids were separated by a gas chromatograph. Fatty acid composition was determined on a gas chromatograph 'Pay-Unicam 304' with flame ionization detector and capillary column ECTM-WAX, 30 m, ID 0.25 mm, Film: 0.25µm.

Atherogenicity index was calculated as the content ratio of SFA/unsaturated FA using the following formula proposed by *(Ulbricht and Southgate, 1991)*:

$$IA = \frac{4xC14:0 + C16:0 + C18:0}{\sum MUFA + \sum PUFA}$$

The data was processed in a variance statistical way through Statistica for Windows (Release. 4.3. stat. soft. Inc., 1994), and the average values were compared according to the tables of t-test of Student-Fisher.

Results and Discussion

Table 1 shows the results for the content of saturated fatty acids in milk of Tzigay sheep and their F2 cross-breeds. Close values were observed for butyric acid (C4:0), the highest (5.30) was in cross-breeds in the second lactation (p>0.05).

Table 1. Saturated fatty acids, g/100 g (n=3)

Fatty acids	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
C4:0	4.66	0.19	4.40	0.31	4.23	0.67	5.30	0.68
C6:0	4.58*	0.30	3.07	0.46	3.31	0.36	3.55	0.54
C7:0	0.29	0.03	0.24	0.12	0.25	0.01	0.44	0.16
C8:0	3.22	0.83	3.40	0.48	3.41	0.74	3.55	0.40
C9:0	0.14	0.04	0.14	0.06	0.33	0.13	0.31	0.11
C10:0	10.63**	0.52	9.62*	1.17	7.34	0.36	6.79	0.17
C12:0	3.47	0.33	3.25	0.06	2.36	1.14	3.59	0.67
C13:0	-	-	0.5	-	0.74	0.30	0.63	0.27
C14:0	8.22	0.20	8.88	0.40	8.74	0.13	8.45	0.23
C15:0	0.65	0.23	0.72	0.34	0.89	0.35	0.83	0.19
C16:0	24.28	1.20	22.87	0.96	23.40	0.61	25.73	3.05
C17:0	0.86	0.10	0.86	0.10	1.74***	0.04	1.66***	0.25
C18:0	8.01	0.27	9.10	0.82	10.62*	1.21	10.04*	1.48

*p<0.05; ** p<0.01; *** p<0.001

The concentration of caproic acid (C6:0) was the highest in the first lactation of Tzigay sheep (at p<0.05) and the lowest in the second lactation (p<0.05) – 4.58 and 3.07, respectively. This is probably due to the difference of the feeding during the consecutive years**.** In enanthic acid (C:7) and nonylic acid (C:9) the values were low and without significant differences among the experimental groups and years. The level of caprylic acid (C8:0) in both breeds varied in the range 3.22-3.55 at p>0.05. In C10:0 the results were higher in the groups of Tzigay sheep in comparison with the cross-breeds, respectively with reliability p<0.01 for the first lactation (10.63 vs 7.34) and p<0.05 for the second lactation (9.62 vs 6.79), which is probably due to the breed effects (p>0.05).

Close values were measured in lauric acid (C12:0) for Tzigay sheep, with some variation in the F2 cross-breeds – from 2.36 in the first lactation to 3.59 in the second lactation. Traces of C13:0 were registered in Tzigay sheep, while in cross-breeds the content of that acid was minimal. The content of myristic acid (C14:0) in milk, had almost the same concentration in all groups of sheep. It was close to the values in the milk of Karakachan breed found by *Mihaylova et al., (2008)* (6.55+0.29 to 10.11+0.29) and lower than that in Srednostaroplaninska breed *(Gerchev et al., 2011)* (9.09+0.64 to 12.29+0.77).

The saturated fatty acids with an odd number of carbon atoms – C15:0 and C17:0 had higher levels in cross-breeds, with differences registered only in the last fatty acid (p<0.001). The palmitic acid (C16:0) was with the highest values in all of the four groups of sheep but no reliable differences were reported between the separate years. In stearic acid (C18:0), which determines to a certain degree the hardness of fat and its melting point, the tendency was similar to margaric acid (C17:0). Higher content of that fatty acid in the milk of cross-breed sheep was reported for first lactation with reliable difference p<0.05. The concentrations of saturated fatty acids C16:0, C17:0 and C18:0, in sheep milk of Tzigay breed and their cross-breeds were comparatively higher than those of Karakachanska breed *(Mihaylova, 2008),* respectively 21.02+0.49, 0.90+0.03 и 9.24+1.19 and lower, compared to the results for milk of Srednostaroplaninska breed *(Gerchev et al., 2011),* respectively 26.24+0.74, 2.27+0.19 and 13.03+0.10.

The content of unsaturated fatty acids C10:1, C12:1 and C14:1 in the milk fat in both groups was low with close values between the groups in the milking period of both lactations (Table 2).

Table 2. Unsaturated fatty acids (n=3)

Fatty acids	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
C10:1	0.23	0.03	0.39	0.08	0.23	0.03	0.39	0.08
C12:1	0.13	0.08	0.24	0.06	0.13	0.06	0.24	0.05
C14:1	0.28	0.08	0.41	0.13	0.28	0.08	0.41	0.13
C16:1	0.69***	0.05	0.45	0.16	0.54	0.20	0.33***	0.07
C18:1	25.32	0.90	21.92	1.71	25.32	0.90	21.92	1.71
C18:2	3.04	0.07	3.29	0.26	3.04	0.07	3.29	0.26
C18:3	1.35	0.16	1.80	0.09	1.50	0.02	1.91	0.35

*** p<0.001

The concentration of palmitic acid (C16:1) in the examined milk was higher in Tzigay breed in both lactations. The difference was significant at p <0.001 only between the group of Tzigay sheep at the first lactation and the group of cross-breeds at the second lactation. That was due to the feeding up of the animals in the beginning of the grazing period in the first lactation. The content of oleic acid (C18:1) in milk fat had close values and unidirectional tendencies (lower level at second lactation) in the period of lactation of sheep during both years. Typical for oleic acid (C18:1) is the high percent (particularly of some isomers) in the beginning of the grazing period. Many studies on milk, obtained during the grazing period *(Atti et al., 2006; Tsiplakou et al., 2006)* reported a positive relation between the concentration of C18:1 in milk fat and the amount of CLA, where C18:1 was a substrate in the synthesis of the latter.

In polyunsaturated fatty acids – linoleic (C18:2) and linolenic acid (C18:3) an increase was reported during the second lactation, which was unidirectional for both groups of sheep. We should mention that their values in milk fat depend mainly on feeding of animals, because they are not synthesized in their organism, as their lack cause a series of biological disorders in the organism. The found concentration of these two acids in the milk of Tzigay and their F2 cross-breeds was higher than this in Karakachan and Srednostaroplaninska sheep raised in the conditions of the Balkan Mountain *(Mihaylova et al., 2008; Gerchev et al., 2011)*.

The distribution of fatty acids in groups is shown in Table 3. The total amount of saturated fatty acids (SFA) during period of lactation was comparatively high, but in different directions and close values during the corresponding years.

Table 3. Groups of fatty acids in milk from sheep (n=3)

Fatty acid groups	Tzigay sheep				F2 cross-breeds (Tzigay x Chios)			
	Ist lactation		IInd lactation		Ist lactation		IInd lactation	
	x	Sx	x	Sx	x	Sx	x	Sx
Σ SFA	69.01	4.24	67.05	5.28	67.36	6.05	70.87	8.20
Σ MUFA	26.65	1.14	23.41	2.14	26.50	1.54	23.29	2.04
Σ PUFA	4.39	0.23	5.09	0.35	4.54	0.09	5.20	0.61
Σ C4:0-C11:0	23.52	1.91	20.87	2.60	18.87	2.27	19.94	2.06
Σ C12:0-C16:1	37.95	2.20	37.71	2.19	36.71	2.90	40.60	4.74
Σ C17iso-C25:0	37.58	1.50	36.97	2.98	42.82*	2.21	38.82*	4.05

* p<0.05

The high level of SFA in milk of Tzigay sheep and their cross-breeds corresponded with low values of MUSFA. The amount of monounsaturated fatty acids (MUSFA) was higher in the first year of lactation and decreased in next year in both groups of sheep. While in the content of polyunsaturated fatty acids (PUSFA) was reported the opposite tendency of lower content of the first lactation and increase in the next one. The high values of the ratio SFA/PUSFA in both years (15.71 and 13.17 in milk of Tzigay sheep and 14.83 and 13.62 in their F2 cross-breeds) showed good acidic stability in milk, which was better expressed in milk of Tzigay sheep. MUSFA have a preventive action against coronary and cardio-vascular diseases and the action of PUSFA is analogous, but they are more unstable to oxidation due to their greater non-saturation. PUSFA protect the membranes of cells from the oxygen radical almost as much as tocopherol and less than carotene.

The short-chain fatty acids had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids kept close concentrations in the milk of Tzigay sheep, while their content in the milk of F2 cross-breeds at the 1st lactation was higher compared to milk of Tzigay sheep at the 2nd lactation in reliability of the difference ($p<0.05$).

The biologically important ratio of PUSFA/SFA, or the so-called P/S ratio, in the sheep milk was low and varied in narrow limits – from 0.06-0.08 in Tzigay sheep to 0.7 in cross-breeds of Chios. The low values of these ratios showed that the breed had not had any significant influence over the content of particular acids, in case of grazing in one herd.

One of the criteria for evaluating the preventive value of milk is atherogenic index and the ratio omega 6/omega 3. Foods with a high index and ratio are considered to be harmful to human health *(Tsipakou and Zervas, 2008).*

Milk fat is usually considered to be proatherogenic, mainly because of the presence of a large amount of saturated fatty acids (mainly lauric, myristic and palmitic acids). The atherogenic index is a criterion for the level and interrelation of some fatty acids that may have atherogenic properties *(Mierlita, 2011).*

In this study, the atherogenic index was within 2.1-2.4 for Tzigay breed and 2.2-2.7 for cross-breeds of Chios (Figure 1). Our data were lower and close to those obtained by other authors for sheep milk *(Mierlita, 2012; de Renobles et al., 2012).*

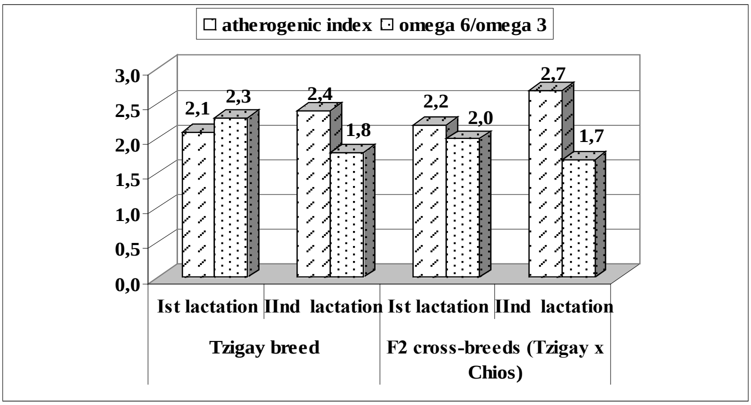

Figure 1. Atherogenic index and ratio omega 6/omega 3 of sheep milk

The ratio of omega 6/amega 3 fatty acids was not significantly different for both breeds over the years – respectively 2.3:1 for Tzigay breed and 2:1 for the cross-breeds of the first lactation and 1.8:1 for Tzigay and 1.7:1 for the cross-breeds of Chios of the second lactation. These data were very close to the ratio omega 6/amega 3 recommended for human health – from 2:1 to 4:1 *(Sretenovic et al., 2009)* and comparable to those reported by other authors for sheep milk *(Mierlita et al., 2011).*

Conclusions

The saturated fatty acids in milk of Tzigay sheep and their F2 cross-breeds had high values during both consecutive years, as they varied from 67.05% to 70.87%, with a lower content of myristic acid correspondingly 8.22-8.88%.

The total amount of polyunsaturated fatty acids in the examined milk for the two types of sheep was comparatively low with near concentrations (4.39-5.20%) in the period of the two years and maintained high values of the correlation SFA/PUSFA (15.71 and 13.17) and low values of the correlation PUSFA/SFA (0.06-0.08). Monounsaturated fatty acids, represented mainly by the oleic acid (C18:1) varied during both periods from 21.92% to 25.32% and appeared as a substratum in the synthesis of CLA.

The short-chain fatty acids (ΣC4:0-C11:0) had higher values in Tzigay sheep in comparison with F2 cross-breeds of Chios. The long-chain fatty acids (ΣC17iso-C25:0) maintained close concentration in the milk of Tzigay breed, while their content in the milk of F2 cross-breeds was increased.

Sastav masnih kiselina mlečne masti ovaca rase cigaja i njihovih F2 meleza sa rasom hios

G. Gerchev, N. Naydenova, S. Slavkova, G. Mihaylova

Rezime

Istraživanje je sprovedeno na zbirnim uzorcima mleka, koji su uzimani svakog meseca u periodu muže, od ovaca rase cigaja i njihovih F2 meleza sa rasom hios, gajenih u uslovima centralnih Balkanskih planina. Ekstrakcija masti uzoraka mleka je urađena po metodi Rouz-Gottlieb. Sastav masnih kiselina je određen na gasnom hromatografu sa jonizacionim detektorom i kapilarnim kolonama. Cilj istraživanja je bio da prati promene u sastavu masnih kiselina mlečne masti cigaja ovaca i njihovih F2 meleza.

Zasićene masne kiseline u mleku od dve grupe imale su visoke vrednosti tokom obe godina uzastopno, jer su varirale od 67,05% u mleku cigaja ovaca u

drugoj laktaciji do 70,87% kod njihovih F2 meleza. Sadržaj miristinske kiseline je bio 8,22-8,88% u mleku cigaja ovaca i 8,45-8,74% njihovih F2 meleza.

Ukupan sadržaj poli-nezasićenih masnih kiselina u ispitivanom mleku dva tipa ovaca je bio relativno nizak sa približnim koncentracijama (4,39-5,20%) u periodu od dve godine. Mleko od dve grupe je imalo visoke vrednosti korelacije SFA/PUSFA (15,71 i 13,17) i niske vrednosti PUSFA/SFA (0,06-0,08).

Mono nezasićene masne kiseline, zastupljene uglavnom kroz oleinsku kiseline (C18: 1) varirale su tokom oba perioda od 21,92% do 25,32% i pojavile su se kao supstrat u sintezi CLA.

Masne kiseline kratkog lanca (C4:0 - C11:0) su imale veće vrednosti kod cigaja ovaca u poređenju sa F2 melezima sa rasom hios. Masne kiseline dugačkog lanca (C17iso - C25:0) su imale približnu koncentraciju u mleku cigaja rase, dok je njihov sadržaj u mleku F2 meleza bio povećan.

References

MIHAYLOVA G., GERCHEV G., NAYDENOVA N. (2008): Changes in milk fat composition in Karakachan sheep reared in the region of the Central Balkan Mountains. Yubilee Scientific Conference '80 Years Agricultural Science in the Rhodopes', Smolyan, Proceedings, pp. 110-113

ATTI N., ROUISSI H., OTHMANE M.H. (2006): Milk production, milk fatty acid composition and conjugated linoleic acid (CLA) content in dairy ewes raised on feedlot or grazing pasture. Livestock Science, vol. 104, 1, 121-127.

DE RENOBALES M., AMORESA G., ARRANZB J., VIRTOA M., BARRÓNC L.J.R., BUSTAMANTEA M.A., RUIZ DE GORDOAA J.C., NÁJERAC A.I., VALDIVIELSOC I., ABILLEIRAC E., BELTRÁN DE HEREDIAB I., PÉREZ-ELORTONDOC F.J., RUIZB R., ALBISUC M., MANDALUNIZB N. (2012): Part-time grazing improves sheep milk production and its nutritional characteristics. Food Chemistry, 130, 90-96

GERCHEV G., MIHAYLOVA G., MASLEV TS. (2011): Fatty acid composition of fat in milk from sheep of Srednostaroplaninska breed from the region of the Central Balkan Mountains. Journal of Mountain Agriculture on the Balkans, vol. 14, 4, 630-639.

MIERLITA D. (2011): Effects of breed on milk fatty acid profile in dairy ewes, with particular reference to cis-9, trans-11 conjugated linoleic acid. South African Journal of Animal Science, vol. 41, 3, 223-231

MIERLITA D. (2012): Effect of feeding type (pasture vs. total mixed rations) of Turcana ewes on animal performance and milk fatty acid profile. Journal of Food, Agriculture & Environment, vol.10, (3&4): 815-818.

MILLS S., ROSS R. P., HILL C., FITZGERALD G. F., STANTON D C. (2011): Milk intelligence: Mining for bioactive substances associated with human health. International Dairy Journal, 21, 377-401.

SIGNORELLI F., CONTARINI G., ANNICHIARICO G., NAPOLITANO F., ORRU L., CATILLO G., HAENLEIN G., MOIOLI B. (2008): Breed differences in sheep milk fatty acid profiles: Opportunities for sustainable use of animal genetic resources. Small Ruminant Research, vol. 78, 1, 24-31.

SIMOPOULOS A. P. (2002): The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine Pharmacotherapy, 56, 365-379.

SANCHEZ 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., vol. 93, 1, 330-339.

SRETENOVIC L. J., PANTELIC V., NOVAKOVIC Z. (2009): Importance of utilization of omega-3 fatty acids in human and animal nutrition. Biotech. Anim. Husb., vol. 25, 5-6, 439-449.

TALPUR F. N. (2007): Fatty acid composition of ruminant milk, meat and dairy products of livestock in Sindh, Pakistan, Dissertation, University of Sindh, Jamshoro-Pakistan.

TSIPLAKOU E., MOUNTZOURIS K., ZERVAS G. (2006): The effect of breed, stage of lactation and parity on sheep milk fat CLA content under the same feeding practices. Livestock Science, vol. 105, 1, 162-167.

TSIPLAKOU E., ZERVAS G. (2008): Comparative study between sheep and goats on rumenic acid and vaccenic acid in milk fat under the same dietary treatments. Livestock Science, vol. 119, 1-3, 87–94.

ULBRICHT T. L. V., SOUTHGATE D. A. T. (1991): Coronary Heart- Disease – 7 Dietary Factors. Lancet, 338, 985-992.

WILLIAMS C. M. (2000): Dietary fatty acids and human health. Annales De Zootechnie, 49, 165-180.

Received 5 February 2015; accepted for publication 20 March 2015