

COMPARATIVE STUDY ON FATTY ACID CONTENT IN MILK OF TSIGAY AND KARAKACHAN SHEEP

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Summary

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The study on fatty acid content was performed in bulk sheep milk obtained from Tsigay and Karakachan sheep raised in the Sredna Stara Planina region. Fatty acids were separated by means of a system of 2 gas chromatographs, necessary for the complete separation of the different fatty acids and their isomers. The obtained data showed that the milk of Karakachan sheep was with a higher dry matter, milk fat and total protein content than that of Tsigay sheep. The former breed yielded milk with more short-chain fatty acids compared to the latter one. The Tsigay milk was richer in oleic acid (C18:1 c9) – 20.14%, but with less vaccenic acid content (C18:1 tr11) – 2.73% compared to Karakachan sheep milk (17.46% and 3.40% respectively). Polyunsaturated fatty acids content in Karakachan sheep milk was higher than that in Tsigay sheep milk (7.83 vs 7.26 g/100 g fat). The average content of conjugated linoleic acid (CLA) in milk obtained from Tsigay sheep and Karakachan sheep was 26 and 29 mg/g fat respectively.

Key words: CLA, cis- and trans-isomers, fatty acids profile, sheep milk

INTRODUCTION

The milk of ruminants is unique by respect to the content of biologically active substances. Milk fat is especially remarkable in this connection. The various fatty acids composing milk fat are with various characteristics. They determine the biological and the nutritive values of milk and its appropriateness for manufacturing dairy products.

The studies on sheep milk have shown that the composition of milk fat is particularly important for consumers with regard to the positive health effects (Babayan and Rosenau, 1991; Jandal, 1996; Haenlein, 2001). Therefore, sheep milk is considered not only as foodstuff, but as a delicacy too. For many people, sheep milk

and sheep dairy products are necessary as alternative of cow's milk from a medical point of view (Haenlein, 2001). At the same time, ruminant milk fat contains more saturated fatty acids – a risk factor for coronary cardiac diseases (Brisson, 1986) and is with a relatively poor content of polyunsaturated fatty acids that are known to decrease this risk (Davignus *et al.*, 1997).

During the last years, a special attention is paid on conjugated linoleic acid (CLA), a mixture of positional and geometric isomers of linoleic acid (C18:2n-6). The reports of many authors demonstrate antioxidant and anticarcinogenic properties of CLA in experimental models

of diseases (Jiang *et al.*, 1996; Parodi, 1997) as well as immunomodulating effect (Cook *et al.*, 1993), reducing body weight effect (Jahreis *et al.*, 2000) etc. The studies of Knekt *et al.* (1996) in women showed that the daily intake of 55 mg CLA via milk reduced breast cancer.

Until now, the data about the content of cis- and trans-isomers of fatty acids in sheep milk fat and CLA levels in milk and dairy products made of milk from Bulgarian sheep breeds, are insufficient. Previous studies of ours (Mihaylova, 2002) demonstrated that CLA content in traditional dairy products (white brine cheese and kashkaval), made of mixed milk from sheep raised in the Dospat region, was relatively high (about 21–25 mg/g milk fat).

The aim of the present study was to determine the fatty acid profile of milk obtained from Tsigay and Karakachan sheep, raised in Sredna Stara Planina.

MATERIALS AND METHODS

Experimental animals

The study was performed in the Sredna Stara Planina region during the grazing period (April–July) in 2 groups of 50 ewes each between 1st and 5th lactation: group I – truebred (introduced) Tsigay and group II – Karakachan breed. Both groups were balanced by respect to the age, time of parturition and conditions of breeding. Until March, the animals were raised in a stable, fed concentrated forage and hay and during the summer period – were grazing on pastures.

Milk samples

The study comprised only the lactation period, when, according to the Instruction of Production Control (Anonymous,

2003), four control examinations were performed. Milk samples were obtained each month from the pooled milk of all 50 animals in the group, after mixing the yield from the evening and the morning milking observing the rules of milk sampling. Altogether 4 samples from bulk milk of each group were collected for the 4-month period. Each sample was divided into two parts, one for chemical analysis¹ and the other – for fatty acids determination².

Analyses

The quantitative determination of milk fat, dry matter, total protein and lactose was done on a Milko-scan 133B (Foss Electric, Denmark). Ash content was measured after burning of the sample.

The fatty acids content of milk was assayed as followed:

- Extraction of milk fat by the method of Rose Gottlieb using diethyl and petroleum ether, evaporation of solvents on a vacuum-rotary evaporator and freezing of separated fat at –18 °C until analysed.
- Synthesis of methyl esters of fatty acids using sodium methylate (CH₃ONa) and triacylglycerol (C23:0) as an in-

¹ Chemical analyses of milk composition were made in the Laboratory of the Institute of Mountain Animal Husbandry and Agriculture, Troyan, Bulgaria.

² The extraction of milk fat from crude milk was done in the Laboratory of the Department of Animal Breeding, section Ruminants and Dairy Production at the Faculty of Agriculture – Stara Zagora. The analyses of milk fatty acids content were made in the laboratories of the Department of Physiology of Nutrition at the Institute of Nutrition, University of Jena, Germany.

ternal standard, added prior to the methylation of fat.

Methyl esters of fatty acids were analysed on two different gas chromatographs (GC) aiming to obtain a better separation of oleic acid isomers (C18:1).

- The first GC analysis was carried out for fatty acid separation. A Shimadzu GS equipment (17A Shimadzu, Kyoto, Japan) with flame ionization detector (FID) and automated injection system (AOC-5000) was used. The analysis was performed on a capillary column (DB-225 MS, J & W Scientific, Folsom, USA; 30 m × 0.25 mm, I. d.; 0.2 µm film) and H₂ as a carrier gas.
- The second GC analysis helped to separate the cis- and trans-isomers of C18:1 on a capillary column CP-Sil 88 (Chrompack, Middelburg, The Netherlands; 100 m × 0.25 mm, I. d.; 0.2 µm film).

The data obtained by summarizing the results of both analyses, were statistically processed by the Student's t-test (Statistica for Windows 4.3, StatSoft Inc., 1994).

RESULTS AND DISCUSSION

The data about dry matter, milk fat, total protein, lactose and ash content in milk during the lactation period of both groups of sheep is shown in Table 1. The results indicated higher values in all parameters with the exception of lactose in Karakachan sheep milk compared to that of the Tsigay breed. Yet, the differences were not statistically significant ($p>0.05$). The milk fat and total protein content in both groups were lower than those, reported by Odjakova *et al.* (2002a; 2002b) for the same breeds, but raised in the Rodopi mountain region. Having studying individual samples from Karakachan sheep, raised in Petrohan mountain, Petrova *et al.*

Table 1. Composition of milk (%). Data are presented as mean ± SD (n=4)

Parameters	Tsigay breed	Karakachan breed
Dry matter	18.43±1.654	19.22±1.264
Milk fat	6.67±1.354	7.29±1.125
Total protein	5.83±0.715	6.04±0.474
Lactose	5.00±0.361	4.93±0.278
Ash	0.93±0.058	0.96±0.037

Table 2. Saturated fatty acids, g/100 g fat. Data are presented as mean ± SD (n=4)

Acids	Tsigay breed	Karakachan breed
C4:0	3.56±0.21	3.62±0.56
C6:0	2.78±0.31	3.03±0.24
C8:0	2.25±0.35	2.47±0.08
C10:0	5.90±0.96	6.62±0.55
C11:0	0.06±0.02	0.07±0.02
C12:0	3.07±0.32	3.35±3.04
C13:0	0.09±0.01	0.09±0.02
C14:0	8.71±1.08	9.11±1.24
C15:0	1.29±0.14	1.31±0.22
C16:0	21.40±1.04	21.37±0.86
C17:0	0.86±0.13	0.81±0.08
C18:0	11.25±0.65	10.42±0.64
C20:0	0.30±0.04	0.28±0.05
C21:0	0.10±0.02	0.10±0.03
C22:0	0.16±0.02	0.16±0.04
C24:0	0.08±0.02	0.08±0.02
C25:0	0.02±0.00	0.02±0.00

(1998) reported higher dry matter, milk fat and total protein content during the pasture period than we did in the present study.

The data for saturated fatty acids confirmed that the content of caproic, caprylic, capric, lauric and myristic acids was higher, and that of stearic acid - lower

in group II than in group I, but the differences were not statistically significant (Table 2). The concentration of butyric and palmitic acids was the same in both groups. The total content of caproic, caprylic and capric acids in Karakachan sheep milk was 12.12% vs 10.93% in Tsigay sheep. The biggest inter-group difference was observed in stearic acid concentration – it was by 0.8% less in Karakachan sheep milk than in Tsigay sheep milk. The content of forementioned saturated fatty acids, except for lauric acid, was higher than that observed by Kafedjiev and Mihailova (1998) for the same sheep breeds, raised in the Rodopi mountain.

Table 3. Monounsaturated fatty acids, g/100 g fat. Data are presented as mean $\pm$ SD (n=4)

Acids	Tsigay breed	Karakachan breed
C10:1	0.21 $\pm$ 0.03	0.21 $\pm$ 0.05
C12:1	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02
C14:1	0.18 $\pm$ 0.06	0.18 $\pm$ 0.06
C16:1	0.97 $\pm$ 0.06	0.97 $\pm$ 0.06
C17:1	0.17 $\pm$ 0.03	0.35 $\pm$ 0.04
C18:1 tr4	0.02 $\pm$ 0.00	0.01 $\pm$ 0.00
C18:1 tr5	0.03 $\pm$ 0.03	0.02 $\pm$ 0.01
C18:1 tr6/7	0.24 $\pm$ 0.05	0.27 $\pm$ 0.04
C18:1 tr9	0.26 $\pm$ 0.05	0.27 $\pm$ 0.03
C18:1 tr10	0.27 $\pm$ 0.07	0.41 $\pm$ 0.26
C18:1 tr11	2.73 $\pm$ 0.51	3.40 $\pm$ 0.91
C18:1 tr12	0.34 $\pm$ 0.14	0.35 $\pm$ 0.13
C18:1 tr13	0.77 $\pm$ 0.44	0.87 $\pm$ 0.40
C18:1 tr15	0.30 $\pm$ 0.14	0.32 $\pm$ 0.13
C18:1 tr16	0.48 $\pm$ 0.15	0.49 $\pm$ 0.15
C18:1 cis9	20.14 $\pm$ 2.61	17.46 $\pm$ 1.61
C18:1 cis11	0.42 $\pm$ 0.04	0.46 $\pm$ 0.05
C18:1 cis12	0.16 $\pm$ 0.05	0.15 $\pm$ 0.06
C18:1 cis13	0.07 $\pm$ 0.02	0.07 $\pm$ 0.02
C18:1 cis15	0.24 $\pm$ 0.07	0.25 $\pm$ 0.06
C20:1 n9	0.10 $\pm$ 0.05	0.05 $\pm$ 0.01

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Table 3 presents the content of mono-unsaturated fatty acids. It shows clearly that the amount of the two principal isomers of oleic acid (C18:1) – C18:1tr11 and C18:1c9, varied significantly between the milk of both breeds. In the milk of Karakachan sheep, the vaccenic acid (C18:1 tr11) content was by 20% higher and that of oleic acid (C18:1 c9) – by 13% lower. The differences were not significant. The highest vaccenic acid content in the milk of both breeds was measured in May (4.72% and 3.47% in Karakachan and Tsigay sheep respectively). The other isomers were not at variance.

The data for polyunsaturated fatty acids (Table 4) showed an insignificantly higher linoleic acid (C18:2 cis 9, 12) concentration in Karakachan sheep milk.

Table 4. Polyunsaturated fatty acids, g/100 g fat. Data are presented as mean $\pm$ SD (n=4)

Acids	Tsigay breed	Karakachan breed
C18:2 tr 9,12	0.68 $\pm$ 0.24	0.73 $\pm$ 0.20
C18:2 cis 9,12	1.90 $\pm$ 0.43	2.03 $\pm$ 0.82
C18:3 cis 6,9,12	0.17 $\pm$ 0.04	0.17 $\pm$ 0.05
C18:3 cis 9,12,15	1.33 $\pm$ 0.28	1.37 $\pm$ 0.23
C18:4 cis 6,9,12,15	0.03 $\pm$ 0.01	0.04 $\pm$ 0.00
C20:2n6	0.02 $\pm$ 0.00	0.01 $\pm$ 0.01
C20:3n6	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01
C20:4n6	0.14 $\pm$ 0.02	0.14 $\pm$ 0.03
C20:3n3	0.01 $\pm$ 0.00	0.01 $\pm$ 0.00
C20:5n3	0.12 $\pm$ 0.02	0.10 $\pm$ 0.01
C22:3n3	0.07 $\pm$ 0.05	0.07 $\pm$ 0.05
C22:5n3	0.16 $\pm$ 0.02	0.16 $\pm$ 0.02
C22:6n3	0.08 $\pm$ 0.03	0.07 $\pm$ 0.01

Table 5. Branched fatty acids, g/100 g fat. Data are presented as mean $\pm$ SD (n=4)

Acids	Tsigay breed	Karakachan breed
C13iso	0.04 $\pm$ 0.03	0.03 $\pm$ 0.02
C14iso	0.17 $\pm$ 0.04	0.17 $\pm$ 0.04
C15iso	0.44 $\pm$ 0.17	0.41 $\pm$ 0.11
C15aiso	0.74 $\pm$ 0.15	0.74 $\pm$ 0.14
C16iso	0.33 $\pm$ 0.06	0.35 $\pm$ 0.03
C17iso	0.51 $\pm$ 0.22	0.35 $\pm$ 0.24
C17aiso	0.55 $\pm$ 0.07	0.54 $\pm$ 0.05
C18iso	0.07 $\pm$ 0.02	0.06 $\pm$ 0.01

Table 6. Groups of fatty acids, g/100 g fat. Data are presented as mean $\pm$ SD (n=4)

Acids	Tsigay breed	Karakachan breed
Σ SFA	64.76 $\pm$ 2.78	65.58 $\pm$ 2.65
Σ MUFA	27.98 $\pm$ 2.45	26.58 $\pm$ 1.86
Σ PUFA	7.26 $\pm$ 1.00	7.83 $\pm$ 1.04
Σ C4:0-C11:0	14.78 $\pm$ 1.80	16.04 $\pm$ 0.65
Σ C12:0-C16:1	37.44 $\pm$ 2.94	38.11 $\pm$ 2.94
Σ C17iso-C25:0	47.77 $\pm$ 3.26	45.85 $\pm$ 2.83
Σ C18:1 trans	5.43 $\pm$ 1.47	6.41 $\pm$ 1.73
Σ CLA	2.60 $\pm$ 0.21	2.93 $\pm$ 0.57

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CLA = conjugated linoleic acid

Except for C17iso, the branched fatty acids content between groups was not different (Table 5).

The content of the principal groups of fatty acids is presented in Table 6. The saturated and polyunsaturated fatty acids content in milk tended to be higher in group II whereas that of monounsaturated fatty acids –in group I. Karakachan sheep milk was with a higher short-chain fatty acids content (16.04 g/100 g fat) com-

pared to Tsigay sheep milk (14.78 g/100 g fat). Polyunsaturated fatty acids content in Karakachan sheep milk was higher than that in Tsigay sheep milk (7.83 vs 7.26 g/100 g fat).

Dimitrov *et al.* (2001) observed a higher saturated fatty acids content in the milk of South-Bulgarian Corriedale (69.2%) and their crossbreds with local Stara Zagora rams (68.5%) than we found out in our study. The milk of both studied breeds was richer in polyunsaturated fatty acids compared to the results of Kafedjiev and Mihailova (1998) about the milk of same breeds, but raised in the Rodopi mountain region.

The summarized amount of trans-isomers of oleic acid and the conjugated linoleic acid (CLA) content in milk tended to be higher in group II than in group I by 1% and 0.3% respectively. The highest concentrations of CLA were measured in May for both groups: 3.53% in milk of Karakachan sheep and 2.84% in milk of Tsigay sheep. The CLA levels during the various months correlated positively with the highest amounts of C18:1 tr11. According to Jahreis *et al.* (1999), CLA levels are the highest during the grazing period of ruminants and also the highest in sheep milk than in milk from other species. The CLA values reported by Prandini *et al.* (2001) in crude sheep milk and by Secchiari *et al.* (2001) in mixed milk from three dairy ovine breeds (Garfagnina, Massese and Sarda) are lower than ours.

The data from Tables 1 and 6 suggest that in 100 g milk from sheep, raised in the Sredna Stara Planina region, there were about 170–210 mg CLA (for Tsigay and Karakachan sheep respectively). Although the differences between both studied breeds were not statistically significant, they should not be underestimated

because of the fact that the interpreted average values were for a 4-month lactation period, a prerequisite for significant intra-group variations and relatively high standard deviations.

CONCLUSIONS

The milk of Karakachan sheep was with a higher dry matter, milk fat and total protein content than that of Tsigay sheep.

Tsigay sheep milk was richer in oleic acid (C18:1 c9) – 20.14%, but with a lower vaccenic acid content (C18:1 tr11) – 2.73% compared to Karakachan sheep milk (17.46% and 3.40% respectively).

Karakachan sheep milk was with a higher short-chain fatty acids content (16.04 g/100 g fat) compared to Tsigay sheep milk (14.78 g/100 g fat).

Polyunsaturated fatty acids content in milk of Karakachan sheep was higher than that in the milk of Tsigay sheep (7.83 vs 7.26 g/100 g fat).

The average content of conjugated linoleic acid (CLA) in milk obtained from truebred Tsigay and Karakachan sheep was 26 and 29 mg/g fat respectively.

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