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CLA CONTENT IN SHEEP MILK AND SHEEP DAIRY PRODUCTS

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The uniqueness of ruminant milk lipids is based on their high concentration of CLA. The purpose of our research was to find the CLA content in sheep milk and milk products manufactured from sheep milk. Proceeding from ancient Bulgarian traditions of developing mountain sheep breeding, milk from two groups of sheep for combined use during the pasture period (May–July) and three types of traditional milk products were studied. The CLA content in individual samples of sheep milk varied from 17.8 to 56.5 mg/g fat. We found that the milk of the Tsigay crosses contained a higher CLA content than the milk of the Karakachan crosses. A higher content of biologically important cis 9, trans 11 CLA isomer was found in the milk fat of the Tsigay crosses than in the Karakachan crosses (25.1 vs. 22.3 mg/g fat, respectively). The highest CLA concentrations in the milk products were found in white brined cheese (35.6 mg/g), followed by yoghurt (29.5 mg/g), and the lowest in yellow cheese (21.8 mg/g). In conclusion, the effect of individual and breeds' differences as well as that of the season on the CLA content in the sheep milk fat was found out.

Key words: sheep milk fat; fatty acids; CLA isomers; trans-11; cis-9 CLA; GC; Ag+-HPLC

СОДРЖИНА НА CLA ВО ОВЧО МЛЕКО И ОВЧИ МЛЕЧНИ ПРОИЗВОДИ

Уникатноста на млечните липиди кај преживарите е базирана на високата концентрација на CLA. Целта на нашите истражувања беше да се определи содржината на CLA во овчо млеко и во млечни продукти направени од овчо млеко. Следејќи ја бугарската традиција на развој на планинското овчарство, беа проучувани млекото од две групи овци за комбинирана употреба за време на пашниот период (мај–јули) и три типа традиционални млечни производи. Содржината на CLA во одделните примероци од овчо млеко вариraше од 17,8 до 56,5 mg/g маст. Ние откривме дека млекото од крстоските со цигаја содржи поголема количина на CLA од млекото на крстоските со каракачанска овца. Голема количина на биолошки важниот изомер *cis* 9 *trans* 11 CLA беше најден во млечната маст на крстоските со цигаја, а потоа и кај каракачанските крстоски (25,1 & 22,3 mg/g маст, соодветно). Висока концентрација на CLA во млечните продукти беше најдена во белото сирење (35,6 mg/g), потоа во јогуртот (29,5 mg/g) и најмала во жолтото сирење (21,8 mg/g). Како заклучок може да се каже дека индивидуалните и одгледувачки разлики, како и сезона се причина сезонската количина на CLA во овчата млечна маст да не биде пронајдена.

Клучни зборови: овча млечна маст; масни киселини; CLA изомери; *trans*-11, *cis*-9 CLA, GC, Ag^{+}-HPLC

1. INTRODUCTION

Sheep milk is both nutritious and delicious. The milk fat has a rich, bland, slightly sweet taste. Sheep milk is especially suitable for yoghurt and cheese production. Many aspects of sheep milk and milk products are unique. Certainly there are plenty of publications on sheep milk, but they often give only the main composition without identification of many specific unique favourable components for human nutrition.

Studies on milk fat have shown that milk fatty acid composition is of particular interest for consumers concerned about diseases (Jandal, 1996; Haenlein, 2001). The long chain unsaturated fatty

acids exert special physiological effects. Among these the conjugated linoleic acids (CLA) are of special interest (Belury, 1995). Comparative studies reported the following beneficial effects: anticarcinogenic, immune modulation, reduction of body fat, normalization of impaired glucose tolerance (Jahreis et al., 2000).

There are enormous differences in the milk CLA content of different ruminants (cow, goat, sheep). The highest CLA concentration among the collected bulk milk samples was found in ewe's milk – 1,2% of total fatty acid methyl esters (Jahreis et al., 2000). The CLA concentration in raw sheep milk varies in dependence on different factors, like breed, season (Secchiari et al., 2001), nutrition (Szumacher-Strabel et el., 2001). Higher levels of total CLA content in sheep bulk milk were found in the spring months compared to the summer ones (Secchiari et al., 2001).

In different dairy products, the CLA content ranges from 3 to 20 mg/g fat (Prandini et al., 2001). Certainly there are many factors, which affect the CLA content, such as kind, processing, cultures, aging (Lin et al., 1999).

Our first analyses of yellow cheese, produced in Bulgarian mountains from sheep milk, showed a high CLA content – about 24 mg/g fat (Mihaylova, 2002). In these areas ewe's milk and dairy sheep products play an important role in the survival of large groups of the population, which often have no other alternative source of income and food supplies.

The purpose of the present study was to determine the total fatty acids profile, including CLA isomers, in sheep milk from mountain regions and to evaluate the influence of some factors, like breed, season, altitude of pasture in sheep raw milk and different sheep dairy products.

2. MATERIALS AND METHODS

Samples

The survey was carried out using sheep milk, cheese and yoghurt. The study comprised two groups of eight ewes each during the milking period (May, June and July): Group I – crossbreed between the Karakachan ewes and the Blackhead Pleven rams (K×BHP) and Group II – crossbreed between the Tsigay ewes and the Blackhead Pleven rams (Ts×BHP). The selected animals had their second lactation and were located on the territory of the Rhodopes Mountains. Both groups were pastured under identical conditions with grass, typical of this region. The milk samples were collected during the morning and the evening milking in quantities aliquots to milk yield.

Three different samples of sheep yoghurt and two different kinds of ewe cheese (white brined cheese and yellow cheese) were collected from local dairy factories and three samples of bulk milk were taken from a dairy farm in May, June and July.

Chemical analysis

Milk samples were analyzed for dry matter, milk fat, protein and lactose by the Milko-scan 133B (Foss Electric, Denmark).

Lipid extraction from the milk was performed according to the Röse-Gottlieb method (Methodenbuch, Bd. VI. VDLUFA – Verlag, Darmstadt, 1985) using ethanol, diethyl ether and petroleum ether and dried over anhydrous sodium sulphate and then the solvent was evaporated in a rotary evaporator at 40°C.

Cheese fat and yoghurt fat were extracted according to Jahreis et al. (1997) from milk powder with Soxtherm 2000 using petroleum ether.

The FAME were prepared from the extracted fat. Briefly, aliquots (50 mg) of lipid extracts were dissolved in 2.5 ml n-hexane and the solution was methylated with Na-methylate, sufficient anhydrous $NaHSO_4.H_2O$ was added. A triacylglycerol was used as internal standard, which was added before the methylation. After centrifugation the supernatant was used for chromatography.

Analysis by the gas chromatography (GC): Analysis of FAME was carried out using two different GC procedures. This combination of both GC is necessary to resolve all fatty acids and their isomers.

For the fractionation of CLA isomers Ag^+-HPLC (Shimadzu, LC 10A) was used, equipped with solvent delivery system, an automatic sample injector, an UV detector and three silver-impregnated columns in series (each 4.6 mm i. d. × 250 mm stainless steal, 5 μm particle size, Varian-Chrompack International, Middleburg, The Netherlands).

The methyl esters were identified by reference standard.

Statistical analysis

All data was analyzed by the software package Statistica. The results are given as mean and standard deviation.

3. RESULTS

Milk yield and milk composition

The Tsigay crossbreed milking yield was much higher than that of the Karakachan crossbreed (Table 1), 854 ml/day vs. 737 ml/day, respectively ($P<0.05$). The daily secretion of milk fat and protein was higher for this crossbreed, too.

Table 1

Mean milk parameters from different crossbreeds

Parameters	Group I (n=24) x ± Sd	Group II (n=24) x ± Sd
Milk yield, ml/day	736.7 ± 275.4	854.4 ± 274.7
Fat yield, g/day	56.8 ± 20.18	62.6 ± 18.48
Protein yield, g/day	45.6 ± 16.49	54.4 ± 18.54
Dry matter, %	19.64 ± 0.95	19.39 ± 1.19
Fat, %	7.82 ± 0.71	7.48 ± 0.97
Protein, %	6.22 ± 0.26	6.34 ± 0.35
Lactose, %	4.70 ± 0.15	4.67 ± 0.14
Ash, %	0.91 ± 0.03	0.89 ± 0.03

Group I had higher dry matter and milk fat content, compared to Group II. There were no differences between both groups for protein, lactose and ash contents.

Milk composition during the lactation followed typical curves (Figure 1). Milk yield and fat yield decreased considerably towards the end of lactation.

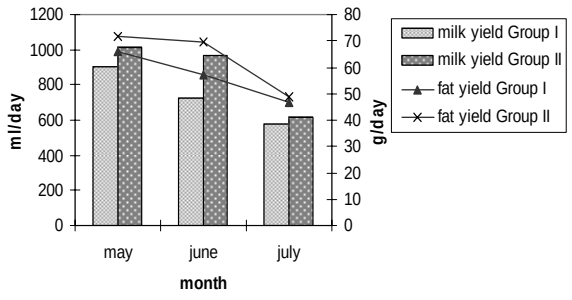

Fig. 1. Changes of milk yield and fat yield

Fatty acids composition, especially CLA content

The Karakachan crosses had higher SFA and MUFA contents, while the higher concentration of PUFA was established in milk fat of the Tsigay crosses (Table 2). Group II showed a higher CLA content than Group I. There were no differences in the content of trans-oleic acid.

Table 2

Groups of fatty acids (mg/g fat) in milk fat

Fatty acids	Group I (n=24)			Group II (n=24)		
	x ± Sd	min	max	x ± Sd	min	max
∑ SFA	671.1 ± 2.01	612.9	697.0	668.3 ± 3.38	608.1	726.0
∑ MUFA	246.8 ± 1.52	228.0	288.5	244.6 ± 2.35	198.3	290.3
∑ PUFA	82.2 ± 0.99	70.2	107.6	87.1 ± 1.26	68.6	115.5
∑ CLA	29.4 ± 0.65	17.8	49.1	31.9 ± 0.90	18.2	56.5
∑ C18:1 trans	68.3 ± 1.04	49.3	83.9	67.5 ± 1.51	48.1	108.8

Figure 2 shows the monthly variations of the CLA yield. The CLA yield in the milk of both groups varied with the month. The total CLA content increased from May to June, and after that decreased.

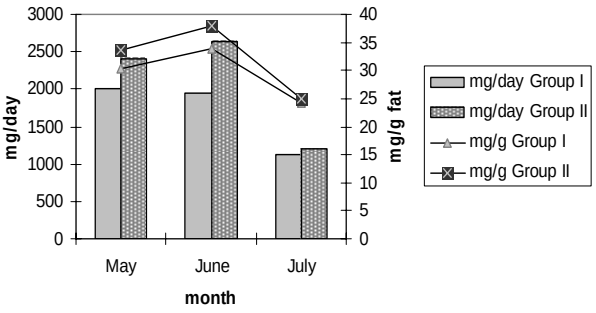

Fig. 2. Monthly variation in the CLA yield

The milk fat of the Tsigay cross had a higher content of cis-9, trans-11 CLA (Table 3), whereas only slight differences between two groups with regard to vaccenic, linoleic and linolenic acids were established.

During the pasture period higher variations were found in the cis-9, trans-11 CLA content of the Group II (Figure 3).

Table 3

Cis-9, trans-11 CLA and C18 fatty acids in milk, mg/g fat

Fatty acids	Group I (n=24)			Group II (n=24)		
	x ± Sd	min	max	x ± Sd	min	max
CLA cis-9, trans-11	22.3 ± 0.42	13.5	28.8	25.1 ± 0.77	13.7	43.6
C18:1 trans-11	32.0 ± 0.83	18.7	53.2	31.4 ± 0.93	17.3	46.9
C18:2 cis-9,12	20.7 ± 0.23	16.7	25.7	20.9 ± 0.28	16.5	27.5
C18:3 cis-9,12,15	15.2 ± 0.20	10.5	18.7	15.8 ± 0.24	10.4	19.8
ω-6 : ω-3 ratio	1.36 : 1			1.32 : 1		

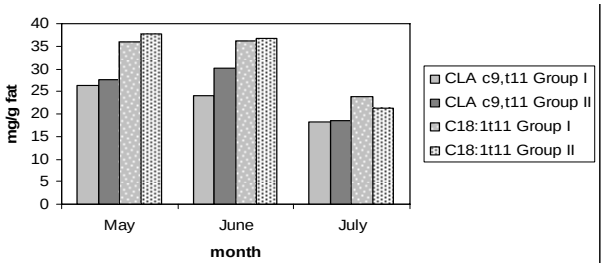

Fig. 3. Changes in CLA c9, tr11 and C18:1 tr11

The amount of CLA, found in different dairy products is shown in Table 4. Among all sheep dairy products white brined cheese had the highest CLA content. High levels of CLA were also found in raw bulk milk from sheep, kept on pasture, and in yoghurt. Yellow cheese had a relatively lower CLA content contrary to white cheese. Regarding yoghurt and cheese, produced from sheep milk, CLA content ranged from 19.4 to 37,2 mg/g fat.

Table 4

Mean CLA content (mg/g fat) in different types of dairy products

Products	∑ CLA	min	max
Raw milk	29.4	22.2	35.2
Yoghurt	29.5	27.9	32.2
White brine cheese	35.6	34.1	37.2
Yellow cheese	21.8	19.4	23.1

4. DISCUSSION

These results showed a higher milk yield of the Karakachan and the Tsigay crosses than the data of Kafedjiev et al. (1998) for the pure Karakachan and Tsigay breeds. Obtained values for the daily milk yield average value within the research period of both groups were higher than the results obtained by Stancheva et al. (1997) for Synthetic dairy crosses of ewes (Pleven Blackhead × Avasi × East-Friesian), where the authors have found variability of milk yield from 0.681 in May to 0.620 kg/day in July.

In studies of milk from the Karakachan sheep Petrova et al. (1998) found a higher content of milk fat in May, June and July, but a lower content of dry matter, protein and lactose, compared to the milk of the Karachakan crosses, studied by us. Comparing the results of Stancheva et al. (1997) on Synthetic dairy crosses, where milk fat varied from 6.18% in May to 7.27 and 8.05%, respectively in June and July, our data about milk of Group I were higher, but almost the same as the results for Group II in June (7.27%) and July (8.01%).

In a previous paper (Kafedjiev and Mihailova, 1998) we determined fatty acid composition in milk fat from two pure breeds in this region. The high content of the MUFA in milk fat from the Karakachan sheep (25.63%), compared with that from the Tsigay sheep breed (24.37%), was attributed to oleic acid – 22.24 vs. 20.62%, respectively. In the present study milk fat from the Karakachan crosses contains also a smaller amount of PUFA and more MUFA. The milk of the crosses of this research has a lower content of SFA than the milk of pure breed sheep, raised in the same region, and studied earlier. Milk of the two groups of crosses has a lower content of SFA than milk of Marishka sheep (70.93%), found by Dimov et al. (1997), and the latter differs in comparatively low content of RUFA (3.53%), but in a higher content of MUFA (28.88%) than the milk of crosses. Data of Prandini et al. (2001) on raw sheep milk shows a higher content of SFA

(71.37%) and MUFA (26.86%), but considerably lower content of PUFA (1.95%).

The major objective of this study was to examine the effect of breed and month during the pasture period on CLA concentration of sheep milk, and also to determine the CLA content of the typical Bulgarian cheeses and yoghurt. In this survey on the content of CLA in milk of two sheep crossees and dairy products an extraordinary high CLA content was found. The total CLA content in individual milk samples varied between 17,8 and 56,5 mg/g fat (Table 2). For both groups the highest CLA concentrations were found in June, and the lowest ones in July (Fig. 2). Milk of the Tsigay crosses contained 2.5 mg /g fat CLA more than the Karachakan crosses. However, the average values for the two groups of sheep were higher than those found by Sechinari et al. (2001) in milk from three milk breeds sheep and the results of Prandini et al. (2001) on raw sheep milk (8.67 mg/g fat).

The dynamics of daily production of the total CLA content was different for both groups of sheep. Since for Group II the tendency to variation of the average CLA content in milk fat and in daily secretion of CLA with produced milk was the same, for Group I the CLA secretion tended to decrease permanently. This is a result of the considerably bigger difference in milk yield between both groups in June (Figure 1).

The milk of the Tsigay crosses contains 11% more c9, tr11 CLA than that of the Karakachan crosses (Table 3). These sheep were also distinguished for a bigger variability of the isomer content in individual samples of milk, compared to Group I. Differences between breeds are also found by Secchiari et al. (2001). The authors have found similar values for breeds Garfagnina and Massese, but lower than Sarda compared to the first two.

Following the changes in c9, tr11 CLA content in milk during the pasture period, it seemed that in Group I the isomer content was the same in May and June, but lower in July. In Group II the content of c9, tr11 CLA was the highest in June, followed by the results in May and it was the lowest in July (Fig. 3). The results are similar to those of Secchiari et al. (2001) who found the content of c9, tr11 CLA in sheep milk higher in summer season than that in spring season, 1.66 and 1.10% respectively. The values, found by us, also result from the natural characteristics of the Rhodopes Mountains, where in spring and especially from the middle of May to the first half of June, the green forage has a richer content of PUFA compared to that in July (67.8% and 55.4% respectively – Mihaylova, 2002). Milk samples, collected in May and June, also differed in considerably higher content of tr11 C18:1 in milk fat, compared to those, collected in July.

Collected raw sheep milk had a considerably higher content of CLA than data by Prandini et al. (2001) about the raw sheep milk. The CLA content in raw sheep milk and in yoghurt was almost the same (Table 4). It seemed that the white brined cheese had a considerably higher CLA content (about 60%) than yellow cheese. Both cheeses have a long ripening period (about 2 months), but the white brined cheese is semi-hard cheese ripening in pickle, while the yellow cheese is hard cheese with chederization the coagulum and scalding in salt solution at temperature of 68–72ºC. Both types of cheese (white brined cheese and yellow cheese) had a higher CLA content than the different kinds of cheese, studied by Prandini et al. (2001). White brined cheese, produced in the Rhodopes Mountains, considerably exceeded in content of CLA the Feta cheese (Zlatanos et al., 2002). Comparing data about the content of CLA in cheese with our initial researches (Mihaylova, 2002), we should note that we found a higher CLA content in yellow cheese than that in the present research – 24.8 mg/g fat varying from 23.9 to 26.1 mg/g fat compared to white brined cheese the content of CLA was lower (21.3 mg/g fat).

5. CONCLUSIONS

During the last decade it was shown that CLA has a curing effect, as the scientific background is obtained only in animal models. A number of biological characteristics are well documented, while others are still disputable. The CLA concentration in milk varies, depending on a number of factors (type, breed, feeding, season etc.) and the influence of some of the factors is still not described well enough in scientific literature.

The obtained results from this first research about the content of CLA isomers in sheep milk and the typical Bulgarian milk products, manufactured from sheep milk, showed a complex influence of many factors. We observed an individual and breed related difference, on the other hand we observed a seasonal difference, respectively the

influence of the month, mainly regarding the fatty acid profile of pasture vegetation, including the altitude of pastures and the time when animals graze on them. In the beginning of the pasture period, sheep use low mountain and mountain pastures in the Rhodopes Mountains. The results show that the CLA content in sheep milk in spring (May-June) was higher than that in summer (July) and it was the same as in the data, found by Secchiari et al. (2001).

When we compare the results about dairy products, we have the impression that yellow cheese had a lower CLA content. However, the obtained data are lower than the results about the same product from preliminary studies (2001, unpublished data). Certainly, there are many factors that influence the CLA content in milk products, for example, type of the product, technology of production, used cultures, conditions and duration of ripening etc. On the other hand, the year of research and the geographical region also influenced the CLA content of dairy products. The classification of different factors will be subject to our next works.

REFERENCES

Belury, M. A. 1995. Conjugated dienoic linoleate: A polyunsaturated fatty acid with unique chemoprotective properties. *Nutr. Rev.*, 53, 83–89.

Dimov, D., M. Djorbineva, G. Mihailova. 1997. Milk composition and milk fat content of Splotch-faced Marishka sheep. *Animal Sciences*, 7–8, 133–136.

Acid Research. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and C. J. Nelson, editors. AOCS Press, Champaign, IL, Vol. 1, 215–225.

Jahreis, G., J. Kraft, F. Tischendorf, F. Schöne, C. von Loeffelholz. 2000. Conjugated linoleic acids: Physiological effects in animal and man with special regard to body composition. *Eur. J. Lipid Sci. Technol.*, 102, 695–703.

Jandal, J. M. 1996. Comparative aspects of goat and sheep milk. *Small Ruminant Research*, 22, 177–185.

Haenlein, G. F. W. 2001. Past, Present, and Future Perspectives of Small Ruminant Dairy Research. *Journal of Dairy Science*, 84, 2097–2115.

Kafedjiev, V., G. Mihailova. 1998. Fatty acid composition of milk and white brine sheep cheese. *Bulgarian Journal of Agricultural Science*, 4, 505–510.

Lin, H., T. D. Boylston, L. O. Luedecke, T. D. Shultz. 1999. Conjugated linoleic acid content of cheddar-type cheeses as affected by processing. *Journal of Food Science*, 64, 5, 874–878.

Mihaylova, G. 2002. Der Gehalt an konjudierten Linolsaeuren (CLA) in der Milch und in Milchproducten von Schafen in Abhaengigkeit von der rasse, der Ernaehrung und vom Laktionsverlauf, *Forschungbericht*, Bonn.

Prandini, A., D. Geromin, F. Conti, F. Masoero, A. Piva, G. Piva. 2001. Survey on the level of conjugated linoleic acid in dairy products. *Ital. J. Food Sci.* **13**, 2, 243–253.

Petrova, N., D. Nedelchev, D. Todorova. 1998. Milk yield and milk composition of the milk of Karakachan sheeps, *Animal Science*, 1, 86–92.

Secchiari, P., M. Mele, A. Serra, A. Buccioni, M. Antongiovanni, G. Ferruzzi, F. Paoletti, L. Andreotti. 2001. Conjugated linoleic acid (CLA) content in milk of three dairy sheep breeds. *Progress in Nutrition*, **3** (4), 37–42.

Stancheva, N., D. Todorova, N. Petrova. 1997. Changes in the milk yield, composition and properties of milk produced by Synthetic+Dairy ewes crosses, *Animal Science*, 3–4, 101–103.

Szumacher-Strabel, M., A. Potkanski, A. Cieslak. 2001. Effect of protected fat on fatty acid composition and conjugated linoleic acid level in meat and milk of sheep. *Arch. Tierz. Dummerstorf*, 44, Special Issue, 329–335.

Zlatanos, S., K. Laskaridis, C. Feist, A. Sagredos. 2002. CLA content and fatty acid composition of Greek Feta and hard cheeses. *Food Chemistry*, **78** (4), 471–477.