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Content of biological active and anticancerogenic substances in the fat fraction of ewe's milk and dairy products and their consumption in humans

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Abstract

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The presented study is addressed to the growing market interest to the ewe's milk as a natural source of essential components, like conjugated linoleic acids (CLA) ω-3 and ω-6 polyunsaturated fatty and vaccenic acid, described to be effective substances for prevention of different human diseases. The work was focused on the transfer of certain organic components (fatty acids) in the food chain "plants–animal products-men" in the endemic regions of the Rhodope Mountain.

The submitted multidisciplinary investigation offers new knowledge in the field of healthy nutrition of sheep raised in mountain regions and the dynamic changes of organic nutrients (saturated and unsaturated fatty acids) in the meadow vegetation during the grazing period.

With the detailed mapping of the pasture areas, an evaluation of exactly distribution of unsaturated and saturated fatty acids in the meadow vegetation as important substrate for the following transformation in the animal organism in form of biological active, isomers – conjugated fatty acids (CLA), vaccenic acid, ω-3 and ω-6 polyunsaturated fatty acids can be provided. The detailed study of essential fatty acids, especially its derivatives – conjugated linoleic acids (CLA) and natural trans fatty acids in the ewe's milk took an important place in the investigation.

The dynamic changes in the available precursors in plant species affect the MUFA and PUFA-profiles of ewe's milk and provide additional information to clarify the mechanism of CLA-synthesis and the content of natural trans fatty acids in raw milk.

Settlement the human organism with biologically active and anticancerogenic substances can not be fully achieved by the consumption of milk and dairy products, so it is necessary to use rational nutrition and supplements/food additives/ based on omega-3, omega-6 and CLA.

Keywords: ewe's milk; fatty acids; anticancerogenic substances (CLA); trans-, ω-3 and ω-6 FA; consumption in humans

Introduction

Milk and dairy products are an integral part of the human diet and occupy a particular place in the nutritional balance, among other animal and plant foods, that it contains all nutrients necessary for the human body in optimal proportions (Dimov et al., 1975; Georgiev et al., 1995). Compared to cow's, human's and goat's milk, sheep's milk is superior to milk protein, ten essential amino acids, monounsaturated fatty acids and linoleic acid, calcium, magnesium, iron, zinc, thiamine, riboflavin, vitamins B6, B12, D. Sheep milk has a higher titratable acidity (22-26°C) of cow's milk (15-

18°C), due to the higher protein and mineral content in it. Characteristics of ewe's milk and dairy products depend on a significant number of factors. They are related both to the chemical, biochemical and microbiological properties of the raw materials as well as to the dairy technology used. Other undoubtedly important factors besides those mentioned are the genetic and physiological features of the animals. Breed differences have a strong impact on the milk yields and the duration of lactation in ruminants and in sheep in particular. Ewe milk and its derivatives are of outstanding interest in all European countries. The production of quality sheep's milk and dairy products with increased content of anticancerogenic and biologically active substances depends above all on the composition of the pasture grass, the botanical diversity and the vegetation stage of the individual plant species, the rainfall and the climatic characteristics of the area (Angelow et al., 1996; Odjakova et al., 2001; Todorova et al., 2000).

There is a close link between the botanical composition of pastures and the phenological stages of individual grass associations on changes in the fatty acid composition of meadow phytocoenoses. The botanical composition of the pastures in the study by Tsvetkova & Angelov (2011) were assesses the botanical diversity of the main plant classes during grazing in the region of Smilyan (Western Rhodopes) and traces the changes in the fatty acid composition of the grass during the different subperiods altitude of pastures.

Ewe's milk and dairy products (Kelsey et al., 2003) are among the main sources of CLA. The highest concentrations of CLA-derivatives were detected in the milk and meat in sheep. The content of CLA is very volatile. The concentration of conjugated fatty acids in the raw ewe's milk and dairy products vary depending on several factors: species, season and diet. Jahreis et al., (1999, 2000) found in sheep milk reliably higher levels of CLA compared to other species. Kelly et al. (1998), Parodi (1999, 2004, 2005) and Lock et al. (2004) found that inhibits CLA carcinogenesis in experimental animals (NRC, 1996) and may reduce the tumours, as well as is given as a cytostatic against existing tumour cells. The studies of Sugano et al., (1998) showed that CLA inhibits atherosclerosis and has anti-inflammatory properties. Mihailova et al. (2003, 2004, 2008) found that the concentration of CLA in ewe milk ranges between from 3 to 4 g/100g milk fat. Sheep and goat milk is superior to cow's content on bioactive substances (Mihailova, 2004; Bode et al., 2003). In spite of the high SFA levels in milk fats, sheep milk plays an important role in the human nutrition as it is a source of biologically active substances – linolic acid, conjugated linolic acid (CLA), omega-3 and omega-6 fatty acids.

The presented study addresses the growing interest in the sheep milk and dairy market as a natural source of essential components: conjugated linoleic acid (CLA), ω-3 and ω-6 polyunsaturated fatty acids and vaccinic acid, presented as effective substances for prevention of various human diseases. The present work focuses on the transfer of some organic components (fatty acids) to the food chain "plant-animal production – man" in the endemic mountain regions of the Rhodope Mountains.

Materials and Methods

The plant samples were collected from the region of the Middle Rhodopes during the period from May to July and standard 2 × 2 m sites were used, the floristic composition of the pastures was determined, the extraction of total lipids was done by the method of Bligh & Dyer (AOAC, 1959) with chloroform and methanol in a 1: 2 ratio.

The milk were studied for bioactive components in milk fat from three sheep breeds in second lactation (n = 18) – Middle Rodopean Bread (MRB), Karakachan Bread (KB) and Rhodopean Tsigay Bread (RTB). White brined cheese was manufactured by milk from Middle Rodopean Bread, Karakachan Bread and Rhodopean Tcigay Bread (n = 6). The extraction of total lipids was carried out by the method of Roese-Gottlieb, by diethyl and petroleum ether and consequent methylation with the aid of sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO_4.H_2O$. The fatty acids methyl esters (FAME) was analysed with the aid of gas chromatograph Shimadzu-2010 (Kioto, Japan) equipped with flame-ionizing detector and automatic injection system (AOC-2010i). The analysis was carried out on a capillary column CP 7420 (100 m × 0.25 mm i.d., 0.2 μm film, Varian Inc., Palo Alto, CA). As carrier gas was used hydrogen and as make-up gas – nitrogen. A regime of the furnace was programmed at four steps – the initial temperature of the column – 80°C/min., which is maintained for 15 min, after which is increased by 12°C/min up to 170ºC and is maintained for 20 min, follows new increase by 4°C/min up to 186°C for 19 min and up to 220°C c by 4°C/min until the end of the process.

The study was conducted with six men and six women for seven days is based on the calculation of the average daily consumption of milk and dairy products from the human population inhabiting the Smolyan (Middle Rhodopes region). The daily intake of essential fatty acids is calculated using the Basket method and Duplicate method.

The data were processed by the method of variation statistics with the statistical package of EXCEL 2010 software. The validity of the differences between the studied groups was established by the Student's t-test.

Results and Discussion

The axonometric assessment of the pastures surveyed shows the distribution of the dominant plant species at an altitude of 1000 to 1200 m in the region of the Middle Rhodopes. The highest percentage of wheat grasses is 36.20%, followed by the leguminoses by 35.75% and the different grass – 28.05%. The favorable ratio between the three classes is a prerequisite for a high level of monounsaturated and polyunsaturated fatty acids in the pasture grass (Table 1).

Table 1. Botanical composition (%) of natural pasture areas of the Middle Rhodopes region

№	Plant species	g	%
1	*Trifolium incarnatum* L.	215.42	30.21
2	*Lolium perenne* L.	38.85	5.45
3	*Bromus mollis* L.	39.59	5.55
4	*Festuca fallax* Thuil.	4.28	0.60
5	*Trifolium repens* L.	23.21	3.26
6	*Poa pratensis* L.	69.35	9.73
7	*Pteridium aquilinium* (L.) Kuhn.	11.96	1.68
8	*Vicia villosa* Roth.	16.25	2.28
9	*Nardus stricta* L.	93.98	13.18
10	Other species	200.00	28.05
		713.00	100.00

Table 2. Fatty acid composition g/100g fat of pasture grass depending on the vegetation

FA	Vegetation period		
	15 May	15 June	15 June
14:0	1.07	1.37	1.51
15:0	0.28	0.48	0.47
16:0 DMA	0.76	0.37	0.41
16:0	20.67	21.91	22.18
16: 1ω-9	0.50	0.31	0.19
16:1ω-7	2.29	2.32	2.24
17:0	0.20	0.22	0.25
17:1	0.37	0.41	0.48
18:0	2.13	2.56	2.86
18:1ω-7	2.53	3.58	7.88
18: 1ω-9	0.45	0.66	2.06
18:2	14.05	17.49	25.53
18:3	53.02	46.37	31.38
20:0	0.60	0.76	0.88
22:0	0.57	0.55	1.00
24:0	0.51	0.64	0.68
Σ SFA	26.79	28.86	30.24
Σ MUFA	6.14	7.28	12.85
Σ PUFA	67.07	63.86	56.91

Note: FA – fatty acids, SFA – Saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – polyunsaturated fatty acids

The data show that the progression of vegetation changes the concentration of the main groups, increasing the amount of saturated and monounsaturated fatty acids (SFA and MUFA) at the expense of polyunsaturated (PUFA). Of particular interest is the dynamics of changes in the linoleic acid, which is a substrate for CLA synthesis (anticancerogenic activity) in the rumen of ruminants. The concentration of fatty acid 18: 2 increases twice from May to July and reaches a maximum by 25.53 g/100 g fat (Table 2).

Determining the fatty acid spectrum of grass associations in the middle and the end of the study period would contribute to a final assessment of the fatty acid dynamics in the grass associations of the area under consideration. An increase in saturated fatty acid (SFA) concentrations from May to July is observed for the reference period of 26.79 g/100 g fat to 30.24 g/100 g fat. This is associated with an increase in myristic (C14:0) palmitic (C16:0) stearic acid (C18:0) and C20:0. C22:0 in the investigated grass associations. The total amount of monounsaturated fatty acids (MUFA) increases from 6.14 to 12.85 g/100 g of fat, i.e. we have a two fold increase in content at the end of the review period. The oleic acid content (C18: 1ω-7) is increased three fold in the grassland at the end of the pasture period. It should be noted (Table 2) that the level of polyunsaturated fatty acids (PUFA) in the period from May to July constitutes a significant part of the total lipid content in natural meadows from 67.07 to 56.91 g/100 g fat.

Linolenic (18: 3 ω-3) fatty acid decreased during the period under review from 53.02 to 31.38 g/100 g fat at the expense of an increase in the concentration of linoleic (18: 2 ω-6) acid from 14.05 to 25.53 g/100 g fat. High concentrations of PUFA throughout the vegetation period provide essential fatty acids to free pasture grass animals as well as to stimulate CLA synthesis in milk lipids. The differences in concentrations of SFA, MUFA and PUFA as well as changes in the ratios of the essential fatty acids confirm the fact that this affects the fatty acid profile of the milk lipids. The similar results have been received by Tsvetkova & Angelov (2011).

The milks were studied from three breeds of sheep during the natural pasture period provide information for the synthesis of biologically active components that depend on breed differences. The CLA have a highest value in Karakachan breed – 3.77 and lowest in Middle Rodopean breed sheep – 2.38 g/100 g fat during the dairy period (Table 3).

The established a low statistical reliability in the total content of CLA and CLA in milk between Middle Rodopean breed and Rhodopean Tsigay. The high content of omega-

Table 3. Biological active fatty acids (g/100g fat) in ewe's milk from three breed

FA	MRB	KB	RTB
ΣCLA	3.04±0.18	3.95±1.74	3.59±0.04
CLA	2.38±0.20	3.77±1.59	2.95±0.04
Σ ω-3	1.73±0.01	2.03±0.37	1.83±0.00
Σ ω-6	3.20±0.01	2.64±0.27	3.42±0.03
Σω-6/Σω-3	1.86±0.01	1.32±0.10	1.88±0.02
C-18:1c9	15.85±0.11	18.10±5.75	15.10±0.38
C-18:1tr11	4.12±0.39	6.69±2.20	4.18±0.07

Note: MRB – Middle Rodopean Bread, KB – Karakachan Bread, RTB – Rhodopean Tsigay Bread, FA – fatty acids, CLA – conjugated linoleic acids, ω-3 – omega-3 fatty acids, ω-6 – omega-6 fatty acids

3 fatty acids were found in Karakachan breed – 2.03 g/100 g fat, until omega-6 is well secured milk from Rhodopean Tsigay. The quantitative proportion between the two groups of essential fatty acids ranged from 1.32 in Karakachan breed to 1.88 in the Rhodopean Tsigay.

Statistical confidence in omega-3 is found between breeds Middle Rodopean Breed and Rhodopean Tsigay and omega- 6 between Middle Rodopean Breed and Rhodopean Tsigay and between Karakachan Breed and Rhodopean Tsigay. Oleic acid (18: 1cis9) were synthesized in ewe's milk in the highest quantities in Karakachan breed – 18.10 g/100 g fat and lowest in the representatives of the Rhodopean Tsigay – 15.10 g/100 g fat. There were not statistically significant differences between the three species in oleic acid and vaccenic acid. The vaccenic acid in the studied breeds was highest in Karakachan breed (6.69 g/100 g fat) and lowest in Middle Rodopean Breed of ewe's (4.12 g/100 g fat).

Production of the white brine cheese leads to minor changes in the fatty acid composition of fat, as is applied a low temperatures to obtain it and not suffer significant changes due to processes of oxidation and isomerization. CLA had the lowest concentration in white brined cheese from the milk of Middle Rodopean Breed – 2.94 g/100 g fat and highest in Karakachan breed – 2.93 g/100 g fat. The similar results for CLA and biologically active substances have been received by a number of authors (Mihailova et al., 2003, 2004, 2008; Bode et al., 2003; Sugano et al., 1998; Jahreis et al., 1999, 2000).

Table 4. Biological active fatty acids (g/100g fat) in white brine cheese from three breed

FA	MRB	KB	RTB
ΣCLA	2.94±0.02	3.53±0.06	3.56±0.04
CLA	2.36±0.02	2.93±0.03	2.92±0.04
Σ ω-3	1.87±0.02	1.80±0.01	1.83±0.02
Σ ω-6	3.23±0.12	3.45±0.03	3.40±0.03
Σω-6/Σω-3	1.73±0.09	1.89±0.02	1.86±0.02
C-18:1c9/	14.87±0.35	14.86±0.26	14.83±0.38
C-18:1tr11	3.88±0.10	4.16±0.05	4.13±0.07

Note: MRB – Middle Rodopean Bread, KB – Karakachan Bread, RTB – Rhodopean Tsigay Bread, FA – fatty acids, CLA – conjugated linoleic acids, ω-3 – omega-3 fatty acids, ω-6 – omega-6 fatty acids

The omega-3 fatty acids are lowest in Karakachan and highest in Middle Rodopean Breed of ewe's (Table 4), while the omega- 6 are in highest concentration in cheese of Karakachan Breed – 3.45 g/100 g fat and lowest from Middle Rodopean Breed – 3.23 g/100 g fat.

The ratio between omega-6 and omega-3 fatty acids doesn't exceed two and the lowest is in cheese from Middle Rodopean Breed – 1.73 and highest in cheese from Karakachan Breed- 1.89. Oleic acid in the analysed cheeses range from 14.83 to 14.87 g/100 g of fat, but due to technological process its content decreases as the loss in technological treatments range from 1 to 3%. The vaccenic acid in white brined cheese have a lowest concentration from Middle Rodopean Breed – 3.88 g/100 g fat and highest in cheese from Karakachan breed 4.16 g/100 g fat.

The results of the study show significant differences in the daily intake of milk and cheese from different participants. On average, women consume 11.8% more raw milk and 22% less cheese than men (Table 5).

Table 5. Daily consumption of milk and cheese according to sex (g/day)

Female	Milk	Cheese	Male	Milk	Cheese
1	329	29	1	243	43
2	300	50	2	243	64
3	86	14	3	214	14
4	300	29	4	257	29
5	343	29	5	343	36
6	286	86	6	171	114
X	274	39	X	245	50
Sd	95	25	Sd	52	32

Minimum – Maximum

Calculation of the total fat consumption through milk and cheese shows on one hand the daily consumption of fats in females (20.17 g/day) and, on the other hand, the distribution of the relative amounts of the major classes of fatty acids (Table 6).

The results show some regularities that female consume significantly more saturated fatty acids (53.3%) over the milk compared to SFA via cheese (20.1%) on average daily. The MUFA intake of milk is twice as much as cheese (3.73 g/day and 1.17 g/day). The same conclusion, but a higher degree of PUFA intake between milk and cheese is found. The differences reach 310% (499 mg/day through milk and 161

Table 6. Daily consumption of fatty acids through milk and cheese at female (mg/day)

Milk	Female	
FA-profil	Average	min–max
SFA	10 563	3315–13223
MUFA	3 733	1172–4673
PUFA	499	157–624
Σ ω-3	90	28–113
Σ ω-6	373	117 – 467
Σ ω-6 / Σ ω-3	4	4
CLA 9c.11t	36	11–44
C-18:1t11(vaccenic acid)	104	33–130

Cheese	Female	
FA-profil	Average	min–max
SFA	4 053	1455–8937
MUFA	1 165	418–2569
PUFA	161	58 –356
Σ ω-3	33	12–74
Σ ω-6	109	39–241
Σ ω-6 / Σ ω-3	3	3
CLA 9c.11t	30	11–65
C-18:1t11 (vaccenic acid)	35	13–77

Note: FA – fatty acids, SFA– Saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – polyunsaturated fatty acids, CLA – conjugated linoleic acids, ω-3 – omega-3 fatty acids, ω-6 – omega-6 fatty acids

Table 7. Daily consumption of fatty acids through milk and cheese at male (mg/day)

Milk	Male	
FA-profil	Average	min–max
SFA	9 445	6 592 ± 13 223
MUFA	3 338	2 330 ± 4 673
PUFA	446	311 ± 624
Σ ω-3	81	56 ± 113
Σ ω-6	333	233 ± 467
Σ ω-6 / Σ ω-3	4	4
CLA 9c.11t	32	22 ± 44
C-18:1t11(vaccenic acid)	93	65 ± 130

Cheese	Male	
FA-profil	Average	min–max
SFA	5 196	1 455 ± 11 846
MUFA	1 494	418 ± 3 406
PUFA	207	58 ± 472
Σ ω-3	43	12 ± 98
Σ ω-6	140	39 ± 319
Σ ω-6 / Σ ω-3	3	3
CLA 9c.11t	38	11 ± 87
C-18:1t11 (vaccenic acid)	45	13 ± 102

Note: FA – fatty acids, SFA – Saturated fatty acid, MUFA – Monounsaturated fatty acid, PUFA – polyunsaturated fatty acids, CLA – conjugated linoleic acids, ω-3 – omega-3 fatty acids, ω-6 – omega-6 fatty acids

mg/day through cheese). The intake of CLA and C-18: 1t11 varies in a small range for milk and cheese.

Calculation of total fat consumption through milk and cheese in male reaches to 20.13 g/day. The distribution of relative amounts of the major classes of fatty acids follows the same trend as for female (Table 7). Men consume insignificantly less SFA daily through milk (46.9%) compared to SFA consumed by cheese (20.1%). MUFA intake of test subjects through milk is twice as high as that obtained with cheese (3.34 g/day and 1.49 g/day). The same conclusion can be made for the intake of MUFA, ω-6 and vaccinic acid.

The comparative study on fat consumption by milk and cheese in female and male (20.17 g/day compared to 20.13 g/day) shows slight differences within 1-2 percent.

Based on animal experiments and by extrapolation of the model a "physiologically effective of CLA dose" is recommended for humans of 3-4 g/day according to the WHO consumption norms of 80 g/day of fat these amounts can not be realized even with the use of CLA-rich foods. Theoretically achieving such high levels of human consumption is only conceivable through the use of supplements (Table 8).

Table 8. Comparative study for daily consumption of fatty acids in humans

Milk + cheese	Female	Male	%*
FA-profil	mg/day	mg/day	
SFA	14616	14641	100.2
MUFA	4898	4832	98.7
PUFA	660	653	98.9
Σ ω-3	123	124	101.0
Σ ω-6	482	473	98.1
Σ ω-6 / Σ ω-3	3.9	3.8	–
CLA 9c.11t	66	70	106.0
C-18:1t11 (vaccenic acid)	139	138	99.2

Note: *female = 100%; male = X%; FA – fatty acids; SFA – Saturated fatty acid; MUFA – Monounsaturated fatty acid; PUFA – polyunsaturated fatty acids; CLA – conjugated linoleic acids; ω-3 – omega-3 fatty acids; ω-6 – omega-6 fatty acids

Conclusions

The content of CLA in milk of three different breeds of ewe's reared on the natural pasture range from 2.38 to 3.77 g/100 g fat. A production of white brine cheese doesn't have significant changes on the amount of conjugated linoleic acid. Essential fatty acids in milk from different breeds of sheep are balanced and are maintained during processing technology of white brine cheese.

Settlement the human organism with biologically active and anticancerogenic substances can not be fully achieved by the consumption of milk and dairy products, so it is necessary to use rational nutrition and supplements/food additives/ based on omega-3, omega-6 and CLA.

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