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Influence of the breed on the physicochemical characteristics, fatty acid composition of sheep's milk and consumption of biologically active and trans fatty acids in the Rhodopes

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The presented study is aimed at establishing the physicochemical composition, the fatty acid profile of ewe's milk from 3 breeds grown in the Rhodopes. The qualitative assessment of the fat fraction was made based on the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids.

Biologically active fatty acids were synthesized to a different degree by ewe in milk and this was determined by their breed differences – the cis isomers of oleic acid have the highest concentration in MRB, omega-3 fatty acids – RTB, CLA in Karakachan breed, trans vaccenic acid – RTB and oleic acid in MRB. The evaluation of the parameter's lipid preventive score, atherogenic and thrombogenic index in the sheep's milk give us an idea of its usefulness, with the lowest values obtained for the lipid preventive score in the milk of the RTB from 14.40 to 15.86 g/ 100g product, the atherogenic index in the milk of the MRB from 1.47 to 1.81, the thrombogenic index in the milk of the Karakachan breed from 1.80 to 1.99, the ratio between hyper- and hypocholesterolemic fatty acids has the highest content in the milk of the MRB from 0.70 to 0.85.

The presented multidisciplinary research offers new knowledge in the field of healthy nutrition in humans, in particular the consumption of essential fatty acids.

The purpose of the research is to study and evaluate the content of biologically active and trans fatty acids in sheep milk from three breeds – Karakachan, Rhodope Tsigai and Middle Rhodope and to establish their daily consumption in humans through milk.

Keywords: ewe's milk; Rhodope Tsigai sheep; Middle Rhodope sheep; fatty acids; anticancerogenic substances (CLA); trans-, ω-3 and ω-6 FA

Abbreviations: FA – fatty acids; SFA – saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; TFA – trans fatty acids; CLA – conjugated linoleic acid; ω-3 – omega-3 fatty acids; ω-6 – omega-6 fatty acids; C18*:*1trans11 (VA) – vaccenic acid; FAME – fatty acids methyl esters; KB – Karakachan breed; RTB- Rhodope Tsigai breed; MRB – Middle Rhodope breed; TL – total lipids; LPS – lipid preventive score; AI – atherogenic index; TI – thrombogenic index; hH – hyper- and hypocholesterolemic; TS – total solids, SNF – solids-non-fat

Introduction

Obtaining an updated database for mountain areas with different geological structure, altitude and botanical composition of pasture vegetation, as well as differences in nutrition in the rearing of lactating sheep are an important prerequisite for clarifying the transfer of fatty acids and obtaining milk and milk products enriched with various biologically active and anticarcinogenic substances (ω-3, ω-6, C18:1trans11 and CLA). Research by a number of scientists is focused not only on the feeding regime of ruminants, but also on biochemical changes in the fatty acid fractions of ewe's milk and their importance for human nutrition (Angelov et al., 2012; Nuttelman, 2013; Tsvetkova et al., 2013; Salari et al., 2016; Mierliță et al., 2017). The rearing of Karakachan sheep in the conditions of free pasture grass – in the presence of a high percentage of leguminous and cereal plants and a low content of cartel (*Nardus stricta*), ensures a higher quality of milk fat in terms of biologically active and anti-carcinogenic substances – CLA, oleic and vaccenic acid, omega-3 and omega-6 fatty acids (Ojakova, 2012; Oraldi et al., 2013; Ramírez-Retamal & Morales, 2014; Shen et al., 2015). Milk fat provides the body with vital essential fatty acids. Ewe's milk is the main source of conjugated linoleic acid (CLA) and its amount varies depending on the breed, season and feeding regime (Angelov et al., 2012, 2013, 2014, 2017; Odzhakova, et al., 2012; Ivanova et al., 2014). Scientists, regulators and communicators have described the biological and health effects of fatty acids according to fatty acid class, but within each fatty acid class different members have specific actions and effects (Shingfield et al., 2013; Calder, 2015; Siurana & Calsamiglia, 2016; Gómez-Cortés et al., 2018; Shahidi & Ambigaipalan, 2018; Gebreyowhans et al., 2019; Manso et al., 2022). Despite the high levels of SFA in milk fat, sheep's milk plays an important role in human nutrition, as it is a source of biologically active substances – linoleic acid, conjugated linoleic acid (CLA), omega-3 and omega-6 fatty acids.

The purpose of the research is to study and evaluate the content of biologically active and trans fatty acids in sheep milk from three breeds – Karakachan, Rhodope Tsigai and Middle Rhodope and to establish their daily consumption in humans through milk.

Material and Methods

The research was carried out in the Research Centre of Stockbreeding and Agriculture, Agricultural Academy, 4700 Smolyan, Bulgaria with three breeds of Karakachan, Rhodope Tsigai and Middle Rhodope breed sheep. Each group includes 10 animals on the second lactation. The milk was collected in May, June and July (3x10). All samples were analysed for physicochemical and fatty acid composition. The physicochemical analysis includes the following indicators: fat, protein and lactose – BDS ISO 9622:2015; solids-non-fat (SNF) and total solids (TS) – BDS 1109:1989, ISO 9622. Extraction of total lipids was performed according to the method of Roese & Gottlieb (milk and milk products). Fatty acid methyl esters (FAME) were analysed using a Shimadzu-2010 gas chromatograph (Kyoto, Japan). The qualitative assessment of the fat fraction includes the following indicators: lipid preventive score, atherogenic and thrombogenic index (Ulbricht & Southgate, 1991), the ratio between hyper- and hypocholesterolemic fatty acids, trans fatty acids and the amount of saturated fatty acids (Regulation (EC) No 1924 /2006).

$$LPS = TL + 2xSFA - MUFA - 0.5\ PUFA \quad (1)$$

$$AI = 12{:}0 + 4\times14{:}0 + 16{:}0 / [\Sigma MUFAs + PUFA\ n6 + PUFA\ n3] \quad (2)$$

$$TI = (14{:}0 + 16{:}0 + 18{:}0)/ [0.5 \times \Sigma MUFAs + 0.5 \times PUFA\ n6 + 3\times PUFA\ n\text{-}3 + PUFA\ n3/ PUFA\ n6] \quad (3)$$

$$h/H = (C18{:}1n\text{-}9 + C18{:}1n\text{-}7 + C18{:}2n\text{-}6 + C18{:}3n\text{-}3 + C18{:}3n\text{-}6 + C20{:}3n\text{-}6 + C20{:}4n\text{-}6 + C20{:}5n\text{-}3 + C22{:}4n\text{-}6 + C22{:}5n\text{-}3 + C22{:}6n\text{-}3)/(C14{:}0 + C16{:}0) \quad (4)$$

Calculation of the daily intake of essential fatty acids using the Basket-method in the population living in the area of the town of Smolyan. Six women and six men participated in the experiment on 7 consecutive days for all subjects. The start of a duplicate experiment is independent of the day of the week (Table 1).

Table 1. Average daily consumption of milk by women and men in the region of Smolyan, ml

Milk		
Women	x	314.29
	sd	40.41
Men	x	294.05
	sd	69.03

The data were processed according to the methods of variation statistics using the statistical package of the EXCEL 2016 computer program. The reliability of the differences between the studied milks was established using the Student's t-test. The obtained results are presented in tabular form.

Results and Discussion

Ewe's milk has a higher content of fat, short and medium chain fatty acids compared to cow's milk (Olmedilla-Alonso et al., 2017). Inostroza et al. (2019) in the course of lactation of sheep found fat content from 5.76 to 7.07%, protein from 4.09 to 5.24%, lactose from 5.16 to 5.46%, total solids from 17.59 to 19.28%, SNF from 10.48 to 11.58%. Petrova et al. (2019) for milk from the Rhodope Tsigai breed, obtained the following results: fat – 7.71 to 7.91%, protein from 5.96 to 6.66%, lactose from 4.55 to 4.67%, dry matter from 19 to 19.92% and SNF from 11.71 to 12.46%.

The total fat in the studied milks of the Karakachan breed sheep ranged from 6.75 to 8.56%, for Rhodope Tsigai from 6.43 to 7.90% and for the MRB-breed from 6.48 to 9.01%, reared in the area of Smolyan. A significant change an increase in fat content, was found in the milk obtained from the Karakachan breed on May and July ($P \leq 0.01$) and between May and June ($P \leq 0.01$) and June and July ($P \leq 0.001$) in the MRB. The increase in milk fat on Rhodope Tsigai sheep during the lactation and between individual breeds are not statistically reliable. The amount of fat synthesized in the milk has the highest content in MRB sheep, followed by the Karakachan and the Rhodope Tsigai breed. The protein content of the milk at Karakachan sheep decreased from 6.48 to 6.11% ($P \leq 0.01$), while the protein content of the Rhodope Tsigai milk increased on June to 6.31% and decreased on July to 6.22%, while in MRB it increased slightly from 5.76% on May to 6.34% on June and remained on July – 6.32%. Lactose in milk decreased during the lactation in all three breeds of sheep, from 5.18 to 4.62% in the Karakachan sheep breed, from 5.02 to 4.67% in the Rhodope Tsigai breed, and from 4.83 to 4. 69% in MRB breed (Table 2). The concentration of SNF in the milk of the Karakachan breed sheep decreases reliably in the course of lactation from 12.87% on May to 11.87% on July ($P \leq 0.001$), in the milk of Tsigai it decreases insignificantly, and in the MRB increased slightly from 11.71 to 12.13%. Total solids in the studied milk from the Karakachan and the Tsigai breed of sheep increased slightly during the lactation, while it increased with a high degree of confidence ($P \leq 0.001$) in the milk from the MRB. The content of total solids has the highest value in MRB and the lowest in the milk Research Centre of Stockbreeding and Agriculture, Agricultural Academy, 4700 Smolyan, BulgariaTsigai breed.

The bioactive components of sheep's milk can make it a functional food. The fatty acid profile of ewe's milk consists of short- and medium-chain fatty acids, which have an important role in lipid malabsorption syndrome, but on the other hand it mainly contains SFA, such as lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) with a hypercholesterolemic effect, which increase the risk of heart diseases and compromise the consumption of sheep's milk and dairy products (Branciari et al. 2012; Chen & Liu, 2020; Satir et al., 2022). Recent studies have focused on the fatty acid content of milk and its various effects on human health. The polyunsaturated fatty acids (PUFA) in ewe's milk fat include linoleic (cis-9, cis-12 C18:2) and α-linolenic (cis-9, cis-12, cis-15 C18:3) acids, as well as -small concentrations of their isomers (Olmedilla-Alonso et al., 2017). Mono- and polyunsaturated fatty acids in sheep's milk may have a preventive effect on cardiovascular diseases, as determined by the atherogenic and thrombogenic index (Olmedilla-Alonso et al., 2017). Ewe's milk fat contains the highest levels of conjugated linoleic acid (0.65 g CLA/100 g FA) and of vaccenic acid, its physiological precursor (Olmedilla-Alonso et al., 2017).

Saturated fatty acids in the studied milk of sheep from different breeds raised on free pasture ranged from 61.67 to 66.31 g/100 g fat. The highest values for SFA were recorded in the milk obtained from the Rhodope Tsigai breed from 62.20 to 68.40 g/100 g fat, followed by the milk in Karakachan breed from 62.18 to 66.90 g/100 g fat and a MRB from 58 .94 to 63.46 g/100 g of fat (Table 3). Monounsaturated fatty acids have the highest concentration in milk obtained from MRB – 30.20 to 32.60 g/100 g fat, and polyunsatu-

Table 2. Physicochemical composition of sheep milk from Karakachan, Rhodope Tsigai and Middle Rhodope breed

Parameters	Karakachan breed			Rhodope Tsigai breed			Middle Rhodope breed		
	May	June	July	May	June	July	May	June	July
	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx	x±Sx
Fat, %	6.75±0.45 b**	7.79±0.79	8.56±0.86	6.43±0.31	7.52±0.67	7.90±0.11	6.48±0.47b**	9.54±0.28c***	9.01±0.56
SNF, %	12.78±0.38 a**,b ***	12.16±0.27	11.87±0.12	12.38±0.63	12.35±0.57	12.01±0.32	11.71±0.70	12.17±0.59	12.13±0.72
Protein, %	6.48±0.33b*	6.17±0.44	6.11±0.10	6.24±0.55	6.31±0.74	6.22±0.44	5.76±0.27	6.34±0.32	6.32±0.24
Lactose, %	5.18±0.17a*,b ***	4.87±0.25	4.62±0.04	5.02±0.26	4.93±0.30	4.67±0.21	4.83±0.20	4.71±0.15	4.69±0.16
TS, %	19.07±1.31	19.51±1.23	19.91±1.21	18.37±1.67	19.43±1.95	19.49±0.51	17.79 ± 0.40 a**,b ***	21.27 ± 1.91	20.70 ± 1.42

a – May/June; b – May/July; c – June/July; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001

rated fatty acids in milk from the Karakachan breed – 6.23 to 7.13 g/100 g fat. Of the MUFA, the spectrum of cis- and trans-isomers of oleic acid (C18:1) is the richest. The cis isomers during the analysed period have the highest values in MRB, on average 22.70 g/100 g fat, and the lowest in the Rhodope Tsigai breed – 19.28 g/100g fat, which is mainly due to a change in the content of oleic acid (C18:1cis9). Petrova et al. (2019), obtained a content of cis isomers in milk of MRB from 16.21 to 18.52 g/100 g fat and of oleic acid from 15.10 to 17.61 g/100 g fat in pasture-raised sheep. Interest in science represents the content of trans-fatty acids in milk. Trans isomers in milk have the highest content in the Rhodope Tsigai breed and range from 5.70 to 6.59 g/100 g fat, while in the Karakachan breed and in MRB they range from 5.34 to 6.74 and from 5.36 to 6.76 g/100g fat respectively. Petrova et al. (2019), found a content of trans fatty acids from sheep's milk at a MRB from 5.72 to 7.63 g/100 g fat. All trans isomers except vaccenic acid C-18:1t11 are considered "undesirable" due to their different degree of carcinogenicity. Vaccenic acid has a functional role in human nutrition, as it is a substrate for the synthesis of conjugated linoleic acids in the mammary gland under the action of the Δ9-desaturase enzyme. Its content was highest in the milk obtained from the Rhodope Tsigai breed – 3.87 g/100 g fat, followed by the sheep milk from the Karakachan breed – 3.76 and MRB- 3.26 g/100 g fat. Petrova et al. (2019) in the course of lactation, found a content of trans-vaccenic acid from 2.77 to 4.19 g/100 g fat. The total content of conjugated linoleic acid isomers is highest in the Karakachan breed – 2.00 g/100 g fat, followed by MRB- 1.81 g/100 g fat and Rhodope Tsigai- 1.77 g/100 g fat. The conducted research gives us information that sheep from the Karakachan breed synthesize the largest amount of anti-carcinogenic substances. The content of CLA in milk from Karakachan sheep was in the range of 1.73 to 1.84 g/100 g fat, in Rhodope Tsigai sheep was from 1.53 to 1.80 g/100 g fat and in MRB from 1.09 to 2 .58 g/100 g fat.

Omega-3 fatty acids in ewe's milk have the lowest concentration in MRB – 1.56 g/100 g fat and the highest in Rhodope Tsigai – 1.94 g/100 g fat. Omega-6 fatty acids in milk were lowest in MRB – 1.56 g/100 g fat and highest in Rhodope Tsigai – 1.94 g/100 g fat. The ratio between the two groups of fatty acids in sheep's milk is the lowest in the Rhodope Tsigai breed – 1.51 and the highest in the SRP – 1.64, but does not exceed the requirements for a healthy food product – over 5.

The qualitative assessment of the fat fraction was made based on the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids (Table 4). The lipid preventive score in the studied ewe's milk in different breeds varies from 12.45 to 17.50 g/100 g product. The studied sheep's milk from all groups is characterized by the highest value for LPS at the end of lactation – July, which is determined by the increase in the amount of fat in the milk and the increase in saturated fatty acids in the fat fraction. The atherogenic index gives the relationship between the sum of the main saturated fatty acids and unsaturated fatty acids, the former being considered pro-atherogenic (favouring the adhesion of lipids in the cells of the immune and circulatory systems) and the latter anti-atherogenic (inhibiting plaque aggregation and reducing the levels of esterified fatty acids, cholesterol, and phospholipids, thereby preventing the occurrence of micro- and macro-coronary diseases).

Table 3. Fatty acid composition of ewe's milk (g/100 g fat), n = 30

FA	Karakachan breed				Rhodope Tsigai breed				Middle Rhodope breed			
	X	SD	min	max	X	SD	min	max	X	SD	min	max
∑ CLA	2.00 a,b*	0.16	1.84	2.16	1.77	0.12	1.64	1.86	1.81	0.82	1.19	2.74
∑ C-18:1TFA	5.90	0.74	5.34	6.74	6.01	0.51	5.70	6.59	5.87	0.77	5.36	6.76
∑ C-8:1CFA	19.83a,b**	1.95	18.39	22.04	19.28	2.37	17.82	22.01	22.70	0.88	21.70	23.32
SFA	64.86a,b**	2.43	62.18	66.90	66.31c***	3.56	62.20	68.40	61.67	2.40	58.94	63.46
MUFA	27.95b**	1.46	27.02	29.63	27.16c***	2.29	25.36	29.73	31.09	1.31	30.20	32.60
PUFA	6.58a,b**	0.48	6.23	7.13	6.38c***	0.52	6.03	6.98	5.67	1.11	4.50	6.71
∑n-3	1.90b**	0.36	1.51	2.23	1.94c**	0.38	1.54	2.30	1.56	0.23	1.29	1.71
∑n-6	2.85	0.17	2.73	3.05	2.83	0.15	2.67	2.96	2.49	0.32	2.22	2.85
∑n-6/∑n-3	1.53	0.27	1.37	1.84	1.51	0.32	1.29	1.88	1.64	0.20	1.41	1.77
BFA	1.99	0.03	1.97	2.02	1.89	0.07	1.85	1.97	2.11	0.11	2.00	2.22
CLA	1.87	0.16	1.73	1.84	1.69	0.14	1.53	1.80	1.70	0.78	1.09	2.58
C-18:1t11	3.76	0.59	3.34	4.18	3.87	0.14	3.77	3.97	3.26	0.33	3.02	3.49
C-18:1c9	19.06b**	2.84	17.05	21.07	18.86c**	3.03	16.72	21.00	21.27	0.93	20.61	21.92

a – Karakachan/Tsigay; b – Karakachan/ Middle Rhodope Bread c – Tsigay / Middle Rhodope Bread; $^*P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

Table 4. Qualitative indicators of the fat fraction in sheep's milk, n = 30

FA profil	Karakachan breed				Rhodope Tsigai breed				Middle Rhodope breed			
	X	SD	min	max	X	SD	min	max	X	SD	min	max
LPS, g/100 ml milk	15.25	1.36	13.73	16.36	15.00	0.77	14.40	15.86	15.77	2.87	12.45	17.50
AI	1.85	0.17	1.65	1.99	2.00	0.29	1.66	2.19	1.68	0.18	1.47	1.81
TI	1.90	0.10	1.80	1.99	2.00	0.18	1.81	2.19	1.92	0.25	1.70	2.19
h/H	0.71	0.08	0.65	0.81	0.67	0.11	0.60	0.80	0.77	0.08	0.70	0.85
TFA, g/100 ml milk	0.45	0.03	0.42	0.48	0.44	0.02	0.42	0.45	0.49	0.13	0.35	0.61
SFA+TFA, g/100 ml milk	5.43	0.42	4.97	5.80	5.25	0.39	4.82	5.57	5.63	1.06	4.41	6.33

Thrombogenic index gives the tendency to form clots in blood vessels and is defined as the ratio between prothrombogenic (saturated fatty acids) and antithrombogenic (monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids) fatty acids. The thrombogenic and atherogenic index, as indicators, should not exceed 1.00, while the cholesterolemic index is above 1.00 (Ivanova & Hadzhinikolova, 2015). Diets rich in saturated fatty acids such as lauric (C12:0), myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) are strongly associated with an increased risk of atherosclerosis, obesity and coronary heart disease (Pilarczyk et al., 2015). According to the indices proposed by Ulbricht & Southgate (1991), lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) have an atherogenic character, and myristic (C14:0), palmitic acid (C16:0) and stearic acid (C18:0), thrombogenic character, while omega-3, omega-6 and monounsaturated fatty acids have antiatherogenic and antithrombogenic character. Scientific studies have found that feeding regime is the main factor that affects AI (Chen & Liu, 2020), but the stage of lactation, grazing season and some other factors in milk and milk products in dairy products also affect AI (Lauciene et al., 2019). The atherogenic index of the studied ewe's milk in different breeds of sheep varies from 1.68 to 2.19. The thrombogenic index maintains the trend of changes in the atherogenic index in sheep's milk, but with slightly higher values compared to it from 1.90 to 2.19. De Souza et al. (2015) found in cow's milk an atherogenic index- 4.10 and a thrombogenic index-5.17. Satir et al. (2022), found a thrombogenic index in sheep's milk from 0.57 to 1.57, and in the treatment of the ration with palm oil it decreased in the range of 1.05 to 1.14 and h/H -0.82 in the control group and h/H- 0.64 in the experimental one. The studied milks from sheep were characterized by a low cholesterolemic index (below 1.0), with the lowest values found in the Rhodope Tsigai breed – 0.67 and the highest content in the MRB – 0.77. The ratio between hyper- and hypocholesterolemic fatty acids found by Fernandez et al. (2007) in the Iberian ham is below 2.5, which is defined as favourable compared to the rest of the ham species investigated by them. Tonial et al. (2014) in two species of fish obtained values for AI-0.55-0.60; TI- 0.82-0.87 and h/H-1.56-1.63. Rozbicka-Wieczorek et al. (2015) found in sheep's milk from two breeds an atherogenic index-1.35 to 1.45 and a thrombogenic index- 1.63 to 1.67.

Naturally occurring trans fatty acids are important for human nutrition and are the subject of a number of scientific studies. Milk from different sheep breeds has an average TFA content from 0.44 to 0.49 g/100 ml milk. The obtained results for the sated samples give us reason to refer them to products with a low TMK content, according to Regulation (EC) No. 1924/2006. The content of saturated fatty acids in the studied milks from sheep of different breeds averaged in the range from 5.25 to 5.63 g/100 ml milk.

The calculation of the total consumption of fat through milk shows on the one hand the daily consumption of fat in women and men, and on the other hand the distribution of amounts of the main classes of fatty acids (Table 5). The results show some regularities that women consume on average three times daily more saturated and polyunsaturated fatty acids through milk compared to men, while monounsaturated was 3.6 times more by women compared to men. Intake of omega-3, omega-6, CLA, trans-vaccenic acid, trans and cis isomers of oleic acid was 3.6 times higher in milk intake by women compared to men (Table 5).

Recent studies have shown that MUFA consumption has beneficial effects in the short or long term by increasing or maintaining HDL and decreasing LDL cholesterol levels (Lopes et al., 2016). The main representative of monounsaturated fatty acids is oleic acid and its cis and trans isomers. Globally, the average intake of cis-MUFA varies from 3.5% of total energy in certain regions of China to about 22% in Greece. Oleic acid (C18:1c9) represents more than 92% of all consumed MUFA, while the other part of the cis isomers present in the diet is in an insignificant part. Oleic acid

Table 5. Daily consumption of fatty acids through milk in women and men, g/day

Fatty acids	Women		Men	
	X	SD	X	SD
SFA	15.70	1.01	4.32	0.11
MUFA	7.02	0.76	1.93	0.20
PUFA	1.52	0.67	0.42	0.04
∑ n-3	0.44	0.21	0.12	0.02
∑ n-6	0.67	0.26	0.18	0.01
n-6 / n-3	0.38	0.11	0.10	0.03
CLA 9c,11t	0.43	0.19	0.12	0.01
C-18:1t11 (vaccenic acid)	0.89	0.39	0.24	0.02
∑ C-18:1trans-FA	1.45	0.64	0.40	0.04
∑C-18:1cis-FA	5.03	0.95	1.39	0.02

binds to milk proteins such as α-lactalbumin and lactoferrin to form powerful anticancer complexes. These compounds have selective antitumor activity against malignant cancer cells, including Caco-2, HepG-2, PC-3, and MCF-7 tumor cells, which are non-toxic to normal cells (El-Fakharany et al., 2018). Omega-3 fatty acids improve metabolism, reduce obesity associated with metabolic disorders including chronic inflammation, insulin resistance and dyslipidaemia in humans (de Camargo Talon et al., 2015; Martínez-Fernández et al., 2015; Wang et al., 2012). Based on animal experiments and by extrapolation of the model, a "physiologically effective dose of CLA" for humans of 3-4 g/day is recommended according to WHO norms for consumption of 80 g/day fat, these amounts cannot be realized even with the use of foods rich in CLA. In theory, achieving such high levels of human consumption is only possible through the use of supplements. Several human studies have found that consumption of 3 to 6 g/day is a safe dose of CLA to produce beneficial health outcomes (Chen et al., 2012; Iwata et al., 2007; Blankson et al., 2000; Stanton et al., 1997, 2003). On the other hand, numerous studies have shown that higher doses of CLA can increase the accumulation of fat in the liver, which is a prerequisite for the development of metabolic syndrome and diabetes (Vyas et al., 2012; Jaudszus et al., 2010; Clément et al., 2002).

Conclusions

Rearing sheep of different breeds in the same conditions during the milking period leads to the synthesis of a different amount of fat, protein and lactose, which is determined by the breed. Milk from MRB has the highest fat content (from 6.48 to 9.54%), while milk from Karakachan sheep has the highest protein and lactose content, from 6.11 to 6.48% and from 4.62 to 5.18% respectively. A different amount of trans fatty acids was determined, which is determined by the breed and varies in the Karakachan breed from 5.34 to 6.74 g/100 g fat, in the Rhodope Tsigai breed from 5.70 to 6.59 g/100 g fat and in the Meddle Rhodope bread- 5.36 to 6.76 g/100 g fat. Biologically active fatty acids are synthesized in different degrees by sheep in milk and this is determined by their breed differences – the cis isomers of oleic acid have the highest concentration in MRB, omega-3 fatty acids – in Rhodope Tsigai, CLA – in Karakachan breed, trans-vaccenic acid in Rhodope Tsigai and oleic acid in MRB. The evaluation of the parameters lipid preventive score, atherogenic and thrombogenic index in the ewe's milk give us an idea of its usefulness, with the lowest values obtained for the lipid preventive score in milk of the Rhodope Tsigai breed from 14.40 to 15.86 g/100 g product, the atherogenic index in the milk of the MRB breed from 1.47 to 1.81, the thrombogenic index in the milk of the Karakachan breed from 1.80 to 1.99, the ratio between hyper- and hypocholesterolemic fatty acids has the highest content in the milk of the MRB breed from 0.70 to 0.85.

The investigated milk from all breeds of sheep were defined as a food product with a low content of TFA (from 0.35 to 0.61 g/100 ml milk) and a high content of SFA (from 4.41 to 6.33 g/100 ml milk). On average, women consume 3.6 times more biologically active and anti-carcinogenic fatty acids through milk than men.

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