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Preliminary studies on the fatty acid composition of buffalo milk

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Abstract

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The production of environmentally friendly and healthy buffalo milk and dairy products has been a lasting trend in recent years, due to consumers' increasingly demanding consumption of quality and safe food.

A study was carried out on the physicochemical and fatty acid composition of buffalo milk from Bulgarian Mura breed during the winter season of two farms.

The milk yield in the study groups of animals decreased from November to January. The fat content of the Makak farm group decreased from 7.82 to 6.81%, while that of the Tsar Kaloyan farm group decreased from 7.51% on November to 7.22% on December and again increased on January to 7.71%, while the protein content of the milk did not change significantly and was relatively constant in both groups of animals. High statistical significance was found in the analyzed milk from two groups of buffaloes from Bulgarian mura breed on December ($P \leq 0.001$) with respect to the indicators of solids non-fat residue and total solids.

The saturated fatty acids in milk obtained from the Makak farm decreased during the study period from 75.08 to 68.54 g / 100 g fat, while monounsaturated and polyunsaturated fatty acids increased respectively from 25.68 to 30.62 g / 100 g fat and from 2.52 to 2.74 g / 100 g fat. The content of biologically active omega-3 and omega-6 fatty acids is relatively constant, while the amount of CLA increases from 0.27 to 0.40 g / 100 g fat. The saturated fatty acids in milk obtained from Tsar Kaloyan farm during the November and December are stable and increase on January to 71.80 g / 100 g fat. Monounsaturated fatty acids increase in December to 28.13 and decrease to 24.98 g / 100 g fat in January. Polyunsaturated fatty acids decrease from 3.74 on December to 2.84 g / 100 g on January. The content of biologically active omega-6 fatty acids is relatively constant, while the amount of omega-3 fatty acids decreases on December and increases on January. The CLA in the analyses buffalo milk significantly decreased ($P \leq 0.01$) from 1.03 on December to 0.63 g/ 100 g fat on January.

Keywords: buffalo milk; physicochemical parameters; fatty acids

Introduction

Milk and dairy products are an integral part of the human diet and occupy a special place in the nutritional balance, along with other animal and plant foods, as it contains all necessary for the human organism nutrients in an optimal proportion. The production of quality buffalo milk and dairy products with increased content of useful trans fatty acids, as well as biologically active substances and anticancerogenic substances depends primarily on the composition of the pasture grass, the biodiversity and the vegetation stage of the individual plant species, the breeding differences, the rainfall and the climatic particularities of the area. Enriching the ruminants with nutritional resources rich of linoleic and alpha linoleic acid in the pasture grass condition leads to in in milk especially of biologically active fatty acids – omega-3, omega-6, CLA, trans and cis fatty acids and decreases the amount of saturated fatty acids. (Auldist et. al., 1998; Walsh and Thomson, 1998; Tripathi, 2014).

The buffalo milk is superior to cow's milk in fat content (6.6 to 8.8%) and protein (3.6-5.4%), which determines the high total solids and nutritional value (Hamad and Baiomy 2010, Simões et al., 2013, O'Brien and Guinee, 2016, Khedkar et al., 2016). The buffalo milk has a very high fat content, which is twice as high as that of cow's milk.

Studies carried out by Penchev et al. (2016) on conventional feeding of buffaloes, the Bulgarian Murra's breed and the Black- Chariot cows (Figure 2) show that for both species the highest percentage is C16:0, followed of C18:1 and C14:0. Higher levels of C17:0, C18:0 and C18:1 have been found in buffaloes, although only 15% and with low credibility ($P \leq 0.05$). Monounsaturated FA (MUFA) is higher in buffalo milk than cow's milk at the expense of polyunsaturated fatty acids.

The aim is to study the physicochemical and fatty acid composition of buffalo milk of the Bulgarian Mura breed during the winter season on two farms.

Material and Methods

Individual milk samples were investigated in the November-January period (3x8 pieces) of buffalo from Bulgarian Mura breeds during the winter season from two farms Makak and Tsar Kaloyan for physicochemical and fatty acid composition. Physicochemical studies were performed using the Ecomilk automatic analysis system.

The extraction of total lipids was carried out by the Roese-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH3ONa, Merck, Darmstadt) and drying with $NaHSO_4.H_2O$. Fatty acid methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on a CP 7420 capillary column (100 m x 0.25 mm i.d., 0.2 µm film, Varian Inc., Palo Alto, CA). Hydrogen is used as the carrier gas, and as a make-up gas – nitrogen. Four-step furnace mode is programmed – the column's initial temperature is 80°C / min, maintained for 15 minutes, then increased by 12ºC / min to 170ºC and maintained for 20 minutes, followed by a further increase of 4ºC / min 186ºC for 19 minutes and up to 220ºC with 4ºC / min until the process is complete.

The data were processed using the statistical package using the statistical package of the EXCEL 2013 computer program.

Results and Discussion

The average daily milk yield from buffaloes studied in both farms has a low confidence trend (Table 1). The fats in the studied buffalo milk of the Bulgarian Mura breed in winter range from 7.82 with a downward trend to 6.81% in the Makak farm from 7.51 to 7.71% with an upward trend in the Tsar Kaloyan farm. Protein content is relatively constant in both groups of animals. The total solid in buffalo milk from f. Makak rises to 19.08% on December and drops again to 17.56% on January, while for milk from f. Tsar Kaloyan declined to 17.52% on December and grow up to 17.92% on January.

The results were obtained for physicochemical compositions identical to those of Kashwa, 2016. Statistical reliability of results obtained for milk yield, solids nonfat and total

Table 1. Physicochemical composition of milk

Month	Farm		Milk yield	Fat,%	SNF,%	TS, %	Density	Protein, %
November	Makak	X	6.74b*	7.82	10.90	18.72c*	34.63	4.16
		SD	2.26	1.07	1.07	1.30	0.67	0.05
	Tsar Kaloyan	X	5.99c*	7.51	11.05b*	18.56a*	34.23	4.13
		SD	1.45	0.51	0.87	0.90	1.07	0.08
December	Makak	X	5.34	7.86	11.22e**	19.08e**	34.44	4.17
		SD	2.12	0.94	0.37	1.01	0.74	0.02
	Tsar Kaloyan	X	6.23	7.22	10.34	17.52	34.10	4.16
		SD	0.69	0.63	0.46	0.62	0.80	0.05
January	Makak	X	4.07	6.81	10.75	17.56	34.23	4.16
		SD	2.21	0.97	0.95	1.40	0.90	0.03
	Tsar Kaloyan	X	4.73	7.71	10.22	17.92	34.23	4.19
		SD	1.21	1.27	0.58	1.46	0.79	0.05

a – November / December; b – November / January; c – December / January; d – november f. Makak / november f. Tsar Kaloyan, e – December f. Makak / December Tsar Kaloyan f – January f. Makak / January f. Tsar Kaloyan, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

solid without residue and dry matter both during the study period and between samples analyzed by individual farms.

The investigated buffalo milk from Bulgarian Mura breed in two different farms during the winter period gives us information on the quality of milk as a raw material for dairy production. Saturated fatty acids in milk obtained from f. Makak significantly reduced from 75.08 to 68.54 g/ 100 g fat, while approx. f.Tsar Kaloyan increased from

Table 2. Fatty acid groups (g / 100g fat) in buffalo milk

Fatty acid groups	Makak						Tsar Kaloyan					
	November		December		January		November		December		January	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
∑CLA	0.30b,e*d,f***	0.11	0.41	0.16	0.44	0.11	1.16a,b**	0.36	0.69	0.26	0.67	0.08
TFA	1.87b*	0.40	2.26	0.66	2.51	0.55	4.11b**,c*	1.68	4.36	2.03	1.67	0.21
CFA	21.04b***.c,d,f**e*	2.86	22.48	1.57	25.62	1.44	19.45	8.98	21.53	2.97	20.32	2.79
SFA	75.08b***,c**	3.77	72.33	2.20	68.54	2.03	69.98	9.40	69.86	3.74	71.80	3.88
MUFA	25.68b***,c,f**	2.73	27.38	1.82	30.62	1.58	26.50c*	7.84	28.13	2.93	24.98	2.72
PUFA	2.52f***	0.28	2.47	0.23	2.74	0.34	3.74a*	1.24	2.55	0.82	2.84	0.35
∑n-3	0.40b,c,d**	0.05	0.39	0.07	0.48	0.05	1.00a*,b**	0.52	0.50	0.22	0.80	0.14
∑n-6	2.04 d**,f***	0.20	1.91	0.09	2.07	0.26	1.64	0.47	1.53	0.35	1.45	0.17
∑n-6/Σn-3	5.17b**,c,d,e*,f***	0.59	5.02	0.71	4.29	0.38	2.95c*	3.15	3.60	1.69	1.83	0.19
Branched FA	1.95b*d,e,f,***	0.47	2.30	0.38	2.44	0.34	4.14a,c***	0.51	3.27	0.28	4.54	0.66
CLA	0.27b*d,f***	0.11	0.36	0.17	0.40	0.11	1.03a,b**	0.33	0.60	0.25	0.63	0.07

a – November / December; b – November / January; c – December / January; d – november f. Makak / november f. Tsar Kaloyan, e – December f. Makak / December Tsar Kaloyan f – January f. Makak / January f. Tsar Kaloyan, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 3. Saturated fatty acids (g / 100g fat) in buffalo milk

SFA	Makak						Tsar Kaloyan					
	November		December		January		November		December		January	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
C-4:0	7.18e*	1.26	6.83	0.91	6.12	0.93	6.69a,b*	0.99	5.50	0.87	5.83	0.63
C-6:0	2.94	0.70	2.63	0.45	2.57	0.59	2.56	0.43	2.16	0.36	2.31	0.27
C-7:0	0.01	0.00	0.01	0.01	0.01	0.00	0.02	0.03	0.01	0.01	0.01	0.00
C-8:0	1.23e*	0.41	1.09	0.21	1.12	0.33	0.97	0.19	0.81	0.17	0.94	0.16
C-9:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C-10:0	2.09	0.69	1.91	0.40	1.92	0.54	1.60	0.31	1.47	0.37	1.72	0.37
C-11:0	0.02	0.01	0.02	0.00	0.02	0.01	0.03	0.06	0.01	0.01	0.02	0.00
C-12:0	2.39e**	0.73	2.24	0.41	2.21	0.53	1.99	0.69	1.58	0.31	1.94	0.35
C-13:0	0.07	0.01	0.07	0.01	0.08	0.01	0.07b,c*	0.02	0.07	0.01	0.09	0.02
C-14:0	11.39d*	2.01	11.28	1.49	10.31	1.58	9.35b**,c*	1.33	9.63	1.40	11.39	1.30
C-15:0	0.37d*,e,f***	0.04	0.01	0.00	0.01	0.00	0.03b,c***	0.02	0.04	0.01	0.07	0.01
C-16:0	34.74b,c,d**f***	3.59	33.99	1.18	29.99	2.40	28.75a**,b***,c*	3.26	34.13	3.11	38.49	3.40
C-17:0	0.44d,e,f***	0.08	0.41	0.06	0.45	0.08	1.02a**	0.11	0.84	0.08	0.94	0.14
C-18.0	11.81f***	4.40	11.47	2.14	13.32	3.50	15.98b**,c***	6.50	12.67	2.61	7.34	1.29
C-20:0	0.20e*	0.06	0.16	0.04	0.19	0.04	0.19	0.16	0.31	0.13	0.20	0.04
C-21:0	0.05 d**,e,f***	0.01	0.04	0.02	0.05	0.02	0.15	0.07	0.13	0.03	0.10	0.02
C-22:0	0.06b*,d,e,f***	0.02	0.06	0.02	0.08	0.01	0.22	0.08	0.21	0.03	0.19	0.04
C-23:0	0.03c*,d,e,f***	0.02	0.02	0.01	0.03	0.02	0.14	0.07	0.11	0.04	0.10	0.03
C-24:0	0.04e***,f*	0.02	0.02	0.01	0.02	0.02	0.07	0.04	0.10	0.03	0.06	0.04
C-25:0	0.01e**,f***	0.01	0.01	0.01	0.01	0.01	0.05	0.07	0.07	0.03	0.05	0.01
C-26:0	0.01d**	0.02	0.02	0.03	0.01	0.01	0.09a*,b**,c*	0.07	0.02	0.01	0.01	0.01

a – November / December; b – November / January; c – December / January; d – november f. Makak / november f. Tsar Kaloyan, e – December f. Makak / December Tsar Kaloyan f – January f. Makak / January f. Tsar Kaloyan, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Table 4. Monounsaturated fatty acids (g / 100g fat) in buffalo milk

MUFA	Makak						Tsar Kaloyan					
	November		December		January		November		December		January	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
C-10:1	0.12e***	0.06	0.10	0.02	0.10	0.03	0.35c**	0.84	0.05	0.01	0.09	0.02
C-12:1n1	0.03b*,d**	0.01	0.03	0.01	0.04	0.01	0.04	0.01	0.03	0.02	0.04	0.01
C-14:1n5	0.14e,f*	0.08	0.09	0.02	0.12	0.03	0.13c*	0.06	0.12	0.03	0.19	0.06
C-15:1n5	0.20a,b,d,e*	0.25	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
C-16:19tr	0.24b*f**	0.05	0.25	0.03	0.28	0.03	0.38	0.35	0.23	0.02	0.20	0.04
C-16:1n7	1.83d,e**	0.58	1.96	0.36	1.72	0.51	1.15b,c**	0.39	1.35	0.21	2.04	0.50
C-16:2n4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00
C-17:1n7	0.19e**,f***	0.03	0.17	0.03	0.17	0.03	0.69	0.89	0.31	0.11	0.34	0.04
C-16:3n4	0.00	0.01	0.00	0.01	0.01	0.01	0.05	0.09	0.05	0.07	0.00	0.00
C-18:1t4	0.02b*f**	0.01	0.03	0.01	0.03	0.02	0.02c**	0.02	0.03	0.01	0.01	0.00
C-18:1t5/6/7	0.27a,b**f***	0.06	0.36	0.04	0.40	0.08	0.49c*	0.46	0.48	0.33	0.17	0.02
C-18:1t9	0.21b,f**	0.05	0.24	0.07	0.32	0.08	0.67	1.02	0.94	1.24	0.17	0.02
C-18:1t10	0.26b**f***	0.06	0.30	0.05	0.34	0.05	0.36c*	0.43	0.56	0.43	0.12	0.02
C-16:4n1	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.00	0.00	0.00	0.00
C-18:1t11	0.66d,e***	0.20	0.85	0.44	0.92	0.31	2.21b,c***	0.74	2.01	0.21	0.96	0.10
C-18:1c9	20.25b***,c,f**	2.75	21.49	1.62	24.58	1.33	18.76	8.88	20.67	2.88	19.70	2.74
C-18:1t15	0.24e*,f**	0.10	0.20	0.05	0.20	0.03	0.17	0.16	0.14	0.04	0.13	0.04
C-18:1c11	0.37f*	0.17	0.45	0.06	0.49	0.10	0.43	0.17	0.47	0.12	0.37	0.07
C-18:1c12	0.21d,f***	0.07	0.24	0.04	0.26	0.05	0.06a,b*	0.06	0.17	0.09	0.08	0.04
C-18:1c13	0.04	0.02	0.06	0.02	0.04	0.02	0.04	0.04	0.06	0.06	0.04	0.06
C-18:1t16	0.21a,e*,b*,f***	0.06	0.28	0.04	0.30	0.07	0.19b*,c**	0.08	0.21	0.06	0.12	0.04
C-18:1c14	0.03	0.01	0.03	0.02	0.02	0.01	0.05	0.03	0.03	0.02	0.03	0.02
C-18:1c15	0.14a,e**,b,f***	0.03	0.20	0.03	0.23	0.04	0.11c*	0.06	0.14	0.01	0.11	0.03
C-20:1n9	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.02	0.01	0.02
C-22:1n11	0.01	0.02	0.01	0.02	0.02	0.02	0.05	0.10	0.01	0.02	0.01	0.00
C-22:1n9	0.01d,e*,f**	0.01	0.01	0.02	0.02	0.02	0.05	0.04	0.04	0.02	0.05	0.01
C-24:1n9	0.00d**	0.01	0.00	0.00	0.00	0.01	0.03b**	0.02	0.01	0.02	0.00	0.01

a – November / December; b – November / January; c – December / January; d – november f. Makak / november f. Tsar Kaloyan, e – December f. Makak / December Tsar Kaloyan f – January f. Makak / January f. Tsar Kaloyan, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

68.98 to 71.80 g/ 100 g fat. Monounsaturated fatty acids in buffalo milk increase from 25.68 g / 100 g fat to 30.62 g / 100 g fat on the first farm, while in the second they increase to 28.13 g / 100 g fat on December and decrease to 24.98 g / 100 g fat on January. Polyunsaturated fatty acids decrease on December to 2.47 g / 100 g fat and on January increase to 2.7 g / 100 g fat in milk from f. Makak, with the milk from f. Tsar Kaloyan found the same trend of 2.55 g / 100 g fat and 2.84 g / 100 g fat respectively. Trans fatty acids in buffalo milk obtained in f. Makak rises over the period under consideration 1.87 to 2.51 g / 100 g fat, while for milk from f. Tsar Kaloyan, whose values are twice as high, are relatively stable on November and December (4.11 and 4.36 g/ 100 g fat), and have fallen significantly on January to 1.67 g / 100 g fat. Cis fatty acids in milk from f. Makak increased significantly from 21.04 to 25.62, while at f. Tsar Kaloyan increase from 19.45 to 20.32 g / 100 g fat. Cis fatty acids are significantly higher in milk than f. Makak versus milk from f. Tsar Kaloyan during the period under consideration (Table 2).

The total amount of conjugated linoleic acid was highest in January in milk obtained from f. Makak – 0.44 g / 100 g fat, while in milk from f. Tsar Kaloyan received the highest value for November – 1.16 g/ 100 g fat. The total content of conjugated linoleic acid is significantly lower in milk than f. Makak vs. f.Tsar Kaloyan. The concentration of CLA in the tested buffalo milk from the first farm increased significantly from 0.27 to 0.40 g / 100 g fat, while in the second it decreased significantly from 1.03 to 0.63 g / 100 g fat and were significantly higher than those by f. Makak (Tables 2 and 5). Omega-3 fatty acids are significantly higher in milk obtained from f.Tsar Kaloyan, and omega-6 fatty ac-

Table 5. Polyunsaturated fatty acids (g / 100g fat) in buffalo milk

PUFA	Makak						Tsar Kaloyan					
	November		December		January		November		December		January	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
C-18:2t9,12	0.03d**	0.01	0.04	0.01	0.04	0.02	0.07	0.03	0.05	0.01	0.04	0.02
C-18:2c9,12	1.60d,e**,f***	0.15	1.48	0.10	1.60	0.24	1.05	0.41	1.07	0.29	1.00	0.10
gC-18:3n6	0.07a,b*,d**,e,f***	0.03	0.04	0.02	0.04	0.02	0.21c***	0.13	0.12	0.02	0.19	0.03
aC-18:3n3	0.14e*,b,d**,c,f***	0.03	0.14	0.01	0.19	0.02	0.47a*,c**	0.26	0.24	0.10	0.42	0.05
CLA9c,11t	0.27b*,d,f***	0.11	0.36	0.17	0.40	0.11	1.03a,b**	0.33	0.60	0.25	0.63	0.07
CLA10t,12c	0.00	0.00	0.01	0.01	0.00	0.00	0.05a,e*,b,c**	0.04	0.02	0.01	0.00	0.01
C-18:4n3	0.08c*,d,f**	0.02	0.08	0.01	0.09	0.02	0.17	0.08	0.12	0.05	0.17	0.05
CLA9c,11c	0.03	0.01	0.04	0.01	0.03	0.01	0.06	0.08	0.05	0.03	0.03	0.01
CLA9t,11t	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.00	0.00
C-20:2n6	0.03	0.01	0.02	0.01	0.02	0.01	0.03	0.03	0.03	0.03	0.03	0.01
C-20:3n6	0.01d**,f***	0.01	0.02	0.02	0.02	0.01	0.06a*,c**	0.04	0.03	0.02	0.05	0.01
C-20:4n6	0.08f*	0.01	0.06	0.03	0.08	0.02	0.09	0.17	0.04	0.02	0.05	0.02
C-20:3n3	0.12b*,c,e**	0.02	0.11	0.02	0.15	0.02	0.11a,c*	0.06	0.06	0.04	0.11	0.04
C-20:5n3	0.01d*	0.02	0.01	0.01	0.01	0.01	0.03a*,b**	0.02	0.01	0.01	0.00	0.01
C-22:2n6	0.01e*	0.01	0.00	0.00	0.01	0.01	0.07	0.12	0.02	0.02	0.01	0.01
C-22:5n3	0.04d,f*	0.03	0.05	0.05	0.03	0.02	0.19a*	0.16	0.06	0.04	0.09	0.05
C-22:6n3	0.00	0.00	0.00	0.00	0.01	0.01	0.02b*	0.02	0.02	0.02	0.01	0.01

a – November / December; b – November / January; c – December / January; d – November f. Makak / november f. Tsar Kaloyan, e – December f. Makak / December Tsar Kaloyan f – January f. Makak / January f. Tsar Kaloyan, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

ids are significantly lower. The ratio of omega-6 to omega-3 fatty acids decreased during the study period in milk from f. Makak whiles at the milk of f.Tsar Kaloyan the highest on December and declined on January.

Butyric acid (C-4: 0) is higher in milk from f. Makak also varies with a tendency to decrease from 7.18 to 6.12 g / 100g fat, and at f.Tsar Kaloyan from 6.69 to 5.83 g / 100 g fat. The content of C-6: 0 in milk from both farms decreased as follows from 2.94 to 2.57 g / 100 g fat at f. Makak and from 2.56 to 2.31 at f.Tsar Kaloyan. The trend is similar in the case of caprylic acid (C-8: 0), lauric acid (C-12: 0), palmitic acid (C-16: 0) and stearic acid (C-18: 0) (Table 3).

Oleic acid in the studied buffalo milk from Bulgarian Mura breed have trends to increase from 20.25 to 24.58 g / 100 g fat at f. Makak and 18.76 to 20.76 g / 100 g fat at f.Tsar Kaloyan. Vaccenic acid in milk obtained from f. Makak grows slightly from 0.66 to 0.92 g / 100 g fat and is significantly lower than the milk obtained from f.Tsar Kaloyan, keeping its concentration relatively constant on December and decreasing to 0.96 g / 100 g fat on January (Table 4).

The linoleic acid in the studied buffalo milk from Bulgarian Mura breed in both farms varied within narrow limits, with higher concentrations found at f. Makak. Gamma and alpha linoleic fatty acids are significantly higher in milk obtained from f.Tsar Kaloyan (Table 5).

A number of authors have obtained identical results for fatty acid groups following the supplementation of buffaloes with different plant species and the incorporation of oilseeds (Danków et al., 2015; Månsson, 2008; Gantner et al., 2015; Myers, 2007).

Conclusion

Buffalo milk, obtained from Bulgarian Mura breed in two farms had a high quality in terms of physicochemical parameters. Milk is used with a high content of biologically active fatty acids – trans, cis and omega-3. Essential substances are contained in buffalo milk with balanced and reduced properties and do not exceed certain values for a healthy source of omega-3 and omega-6 fatty acids.

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