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Fatty-acid composition of buffalo milk under intensive and pasture farming

Мастнокиселинен състав на биволско мляко при интензивно и пасищно отглеждане

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ABSTRACT

With the aim to assess the fatty-acid profile of buffalo milk from intensive and pasture farming system, the study included two farms. Farm 1 assigned 9 non-grazing buffaloes raised on green fodder or maize silage, and Farm 2 – 8 buffaloes on pasture until November and hay in winter. Individual samples of milk, taken in 7 monthly test days from August to February, were subjected to the Roese-Gottlieb lipid analysis. Analyses of variance were carried out per each fatty acid (FA), including the effects of farming, test day, milk yield and fat content. Farming system was established to be significant source of variation of all individual monounsaturated and polyunsaturated FAs (PUFA) and total PUFA. All PUFAs, except C20:3n3 and C20:2n6, showed better values in the milk from the buffaloes on pasture – more than 2-fold difference in total conjugated linoleic acids (0.913%) and rumenic acid (0.829%) in particular, in alpha-linolenic (0.145%) and gamma-linolenic (0.502%) acid, and in omega-3 FAs (n3), rendering n6/n3 ratio definitely lower (1.99). This applies also to greater extent to trans-C18:1 (4.027%) and vaccenic acid (2.323%) in particular, and to lesser to atherogenicity (2.44) and thrombogenicity (3.21) index. While C18:4n3 was found to increase, vaccenic and gamma-linolenic acid decline throughout grazing season, as well as conjugated linoleic acids with the exception of a peak in December. C20:5n3, C22:5n3 and C20:3n6 are characterized by such even more pronounced peak.

Keywords: buffalo, grazing, atherogenicity, omega ratio, trans-FA

РЕЗЮМЕ

С цел оценка на мастнокиселия състав на биволското мляко от интензивна и пасищна система за отглеждане, в проучването бяха включени две ферми. От ферма 1 бяха взети 9 биволици без паша, хранени със зелена маса или царевичен силаж, а от ферма 2 – 8 биволици на пасищно отглеждане до ноември и на сено през зимата. Индивидуалните проби мляко, взети в 7 месечни тестови дни от август до февруари, бяха подложени на липиден анализ по метода на Roese-Gottlieb. Бяха проведени анализи на варианса за всяка мастна киселина (МК), включвайки ефектите на ситемата на отглеждане, тестовия ден, млечността и маслеността. Беше установено, че системата на отглеждане е достоверен източник на вариране на всички отделни мононенаситени и полиненаситени (ПНМК) МК, както и ∑ПНМК. Всички ПНМК, с изключение на C20:3n3 и C20:2n6, показват по-добри стойности в млякото от биволиците на паша – повече от двукратна разлика в сумата от конюгираните линолови киселини (0,913%) и в частност в C18:2c9t11 (0,829%), в алфа-линоленова (0,145%) и гама-линоленова (0,502%) киселина, както и в омега-3 (n3), правейки съотношението n6/n3 определено по-ниско (1,99). Това се отнася до голяма степен до транс-C18:1 (4,027%), в частност C18:1t11 (2,323%), и в по-малка до индекса на атерогенност (2,44) и тромбогенност (3,21). Докато C18:4n3 се повишава, C18:1t11 и гама-линоленовата

киселина намаляват с напредване на пасищния сезон, както и конюгираните линолови киселини с изключение на пика през декември. C20:5n3, C22:5n3 и C20:3n6 се характеризират с подобен, още по-силно изразен пик.

Ключови думи: биволи, паша, атерогенност, омега съотношение, транс-МК

INTRODUCTION

Milk and dairy products constitute considerable portion of human diet and that is why there is definite criticism against their saturated nature and hence the negative effects on human health (Givens and Shingfield, 2006). In this context, according to FAO, saturated fatty acids (SFA) should provide no more than 10% of total calories (FAO/WHO, 2003). But on the other hand, though it cannot compete with other foods (e.g. fish) for the content of omega-3 fatty acids, as a ruminant product milk is a dominant provider of the also beneficial trans-isomers. It contains highly desirable oleic acid and conjugated linoleic acid, proved to have anti-cardiovascular, anti-carcinogenic, anti-atherogenic, anti-obesity, anti-diabetic effects and stimulation of immune system (Belury, 2002; Parodi, 2004; Dilzer and Park, 2012). According to some authors, there is also protective effect of some SFA's (Knopp and Retzlaff, 2004; Dabadie et al., 2005).

The activity of Δ9-desaturase enzyme, which takes main part in the synthesisis of those trans isomers (Bauman et al. 2006), and the rate of ruminal biohydrogenation of unsaturated fatty acids (UFA) are responsible for the expression of the effect of system of feeding on fatty acid (FA) profile of milk (Shingfield et al., 2005; Kalač and Samková, 2010), observed by many authors (Dhiman et al., 1999; Fernandes et al., 2007). This is relevant to the policy of the World Health Organization (FAO/WHO, 2003) to urge producers to improve the lipid profile of the foods of ruminant origin via extensive farming, in view of the generally low consumption of CLA below the necessary levels for reducing cancer risks (Dhiman et al. 1999).

The milk of the water buffalo (*Bubalus bubalis*) is a delicacy product marked with higher content of lactose, protein, ash and Ca, and vitamins A and C, as well as with the presence of biliverdin, bioactive pentasaccharide and gangliosides, which are not present in bovine milk (Abd El-Salam and El-Shibiny, 2011). Its fat content is double higher but cholesterol is lower, and the lipid globules are more numerous but smaller in size, as compared to cow milk (Zicarelli, 2004; Abd El-Salam and El-Shibiny, 2011). The fatty-acid profile was found to be similar to that of cow milk in determined by previous study (Penchev et al., 2016), also establishing the effect of diet, as it has been done with buffaloes abroad (Fernandes et al., 2007; Gagliostro et al., 2015; Pegolo et al., 2017).

Most buffalo farms' management in Bulgaria is based on pasture resources for the summer feeding, but still there are such that should rely on intensive system. In dairy cows the effect of grazing on an array of beneficial fatty acids was observed by many authors (Stockdale et al., 2003; Gorlier et al., 2012; Rego et al., 2016). Nevertheless, in the bubaline species such research is scarce, with the exception a study chiefly on SFA and ratios within a dissertation (Mihaylova, 2007).

The aim was to study the fatty-acid profiles of buffalo milk from intensive and pasture farming system, with an emphasis on the levels of beneficial individual acids and categories.

MATERIAL AND METHODS

For the aim of the study, 9 and 8 lactating buffalo cows of the *Bulgarian Murrah* breed were assigned respectively from farm 1 (FM_I) and farm 2 (FM_P). The housing system on both farms is tie stalls – with exercise yard on FMI and pasture throughout the day (in a National Reserve) from April to October on FM_P.

The daily diet on FM_I from July to October involves 18 kg green foliage, 4 kg wheat straw, and 4 kg compound feed per capita, and from November the green roughage is replaced by 20 kg maize silage (inoculant 11CFT by Pioneer Hi-Bred International, Inc., containing *Lactobacillus buchneri* and *Lactobacillus casei*). On FM_P until October the buffaloes are fed 2 kg wheat straw, and

3.4 kg concentrate to supplement the pasture grazed, and from November – 3 kg alfalfa hay, 5 kg wheat straw, 4 kg compound feed, and 4 kg dried fodder beet chips.

The concentrate feed for both herds provides 1629 kcal energy and 96 g digestible protein and has the following composition: wheat – 15%, barley – 12%, corn – 56%, wheat bran – 10%, sunflower oilcake – 5%, dicalcium phosphate 0.6%, salt – 0.4%, and chalk – 1%.

The buffaloes from both farms were selected to have calved within a close range – from June 6th to June 25th; and allotted by the analogue method regarding parity. Seven monthly test days were carried out per each month from August to February. Each test day was in the beginning of the respective calendar month – within the first three days. The milk samples were taken from the morning milking of each animal within each farm and frozen.

The lipid analysis was carried out at the Laboratory of the Department of Food Technology, Institute of Cryobiology and Food Technology, Sofia. The extraction of total lipids was carried out by the Rose-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, 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 (100m x 0.25mm i.d., 0.2µm film, Varian Inc., Palo Alto, CA). Hydrogen was used as the carrier gas, and as a make-up gas - nitrogen. Four-step furnace mode was programmed - the column's initial temperature is 80 ° C / min, maintained for 15 minutes, then increased by 12°C/min to 170°C and maintained for 20 minutes, followed by a further increase of 4°C/min 186°C for 19 minutes and up to 220°C with 4°C/min until the process is complete.

The overall spectrum of the lipid analyses includes 22 SFAs, 24 MUFAs, 20 PUFAs, and 8 isomers. The content of FAs was expressed as a percentage of total fat in milk (%)

ANOVA was carried out per each of the thirty-five fatty acids of importance using the software products LSMLMW and MIXMDL (Harvey, 1990), under the following linear model:

$$Y_{fgjk} = \mu + H_f + TD_g + MY_i + FP_k + e_{fgjk},$$

where µ is the overall mean of the trait (FA, group of FAs, or FA index); H_f , TD_g, MY_i, and FP_k –the fixed effects of farming system (f= 1...2), test day (f= 1...7), test-day milk yield (f= 1...4), fat percentage (f= 1...4) respectively; and efgjk – the residual effect.

The atherogenicity (IA) and thrombogenicity (IT) indices were calculated using the equations developed by Ulbricht and Southgate (1991) as follows:

IA = (C12:0 + 4*C14:0 + C16:0) / UFA;

IT= (C14:0 + C16:0 + C18:0) / (0.5*MUFA + 0.5*n6 + 3*n3 + n3/n6).

RESULTS

As the results of the ANOVAs in Tables 1, 2 and 3 indicate, farming system was found to explain most of the variability of the majority of fatty acids obtained by the lipid analysis.

Total SFA constitute 72.41% in the buffalo milk from FM_I and 69.81% from FM_P (Table 1), the effect of farm being significant at P<0.05. Farming system shows to have no significant effect on stearic C18:0. Same with the predominant SFA, palmitic C16:0 (P<0.05), while the effect on lauric C12:0 is significant at P<0.01. On myristic C14:0 this effect is highly significant (P<0.001) – the value for FM_P buffaloes being by 11.6 percent relatively lower than FM_I. On the SFAs with 6, 8 and 10 carbon atoms farm is also highly significant source of specific variance, and test-day milk yield also plays effect. All they have higher values on FM_I, compared to FM_P. The odd fatty acids are minor representatives of SFA, margaric C17:0 being the only SFA with significantly higher value on FMP, with most pronounced effect of farming (F= 648.4, P<0.001). Pentadecanoic C15:0 is not influenced by farming but together with C4:0 are the only SFAs dependable on test day.

Table 1. Effect of farming system (F-value with level of P-value, F/P), respective LSM-estimates by farms, and of the factors test day (TD), test-day milk yield (MY) and fat percentage (F%) on SFA

Fatty acids	Farm					TD	MY	F%
	F/P[a]	FM_I		FM_P		Level of P-value		
		n	LSM ± SE	n	LSM ± SE			
C4:0	5.95 *	54	7.02 ± 0.161	54	6.47 ± 0.169	*	NS	NS
C6:0	15.36***	55	2.73 ± 0.074	54	2.31 ± 0.079	NS	**	NS
C8:0	23.42***	55	1.145 ± 0.040	54	0.870 ± 0.043	NS	*	NS
C10:0	19.50***	55	1.96 ± 0.071	54	1.51 ± 0.076	NS	*	NS
C12:0	11.18 **	55	2.24 ± 0.086	54	1.83 ± 0.092	NS	NS	NS
C14:0	12.02***	55	11.18 ± 0.262	54	9.88 ± 0.279	NS	**	NS
C15:0	3.23 NS	51	0.087 ± 0.024	54	0.027 ± 0.024	***	NS	NS
C16:0	2.37 NS	55	33.42 ± 0.725	54	31.84 ± 0.772	NS	*	NS
C17:0	648.40***	55	0.432 ± 0.014	54	0.923 ± 0.014	NS	NS	*
C18.0	2.87 NS	55	11.94 ± 0.669	53	13.56 ± 0.721	NS	NS	NS
∑SFA	4.26 *	55	72.41 ± 0.88	54	69.81±0.94	NS	*	NS

[a] Levels of significance of P-value: * – P<0.05; ** – P<0.01; *** – P<0.001; NS – P>0.05

Butyric is significantly affected by farming (P<0.05) but the difference between the two herds is small.

Total MUFA are not affected by farming system, the mean content being 27.52% of total fat (Table 2). Oleic C18:1 (isomers *cis-9*, *trans-12* and *trans-13*), as the main representative of MUFA, is little higher in FM_I buffaloes (LSM= 21.99) compared to FM_P (LSM= 20.27), the effect of farm being significant at P<0.05. Similarly with C18:1 cis-isomers (P<0.05) and C16:1n7 (P<0.01), the latter being the only MUFA affected by day of testing. Contrarily, trans-isomers are much higher on FM_P, system of farming being a solid source of specific variance (P<0.001).

Vaccenic C18:1trans11 (Table 2) is also in keeping with the general trend of superiority of FM_P buffaloes – a 3.2-fold difference supported by highly significant effect of farming system (P<0.001). It is also affected by test day (P<0.05), expressed in slight increase on FMI, and in generally considerable decrease on FMP (Figure 1), which renders the superiority of this herd especially great in the active grazing season.

Table 2. Effect of farming, respective LSM-estimates by farms, and the factors test day (TD), milk yield (MY) and fat percentage (F%) on MUFA

Fatty acids	Farm					TD	MY	F%
	F/P	FM_I		FM_P		Level of P-value		
		n	LSM ± SE	n	LSM ± SE			
C16:1n7	16.50***	55	1.707 ± 0.075	54	1.276 ± 0.080	**	NS	NS
C18:1 [a]	4.29 *	55	21.99 ± 0.584	54	20.27 ± 0.622	NS	*	NS
C18:1t11	96.42***	55	0.732 ± 0.114	54	2.323 ± 0.121	*	NS	NS
∑C18:1t	35.55***	55	2.188 ± 0.217	54	4.027 ± 0.231	NS	*	NS
∑C18:1c	5.09 *	55	22.69 ± 0.808	54	20.10 ± 0.861	NS	NS	NS
∑MUFA	0.04 NS	54	27.61 ± 0.66	51	27.43 ± 0.61	NS	*	NS

[a] Sum of C18:1 *cis-9*, C18:1 *trans-12* and C18:1 *trans-13*

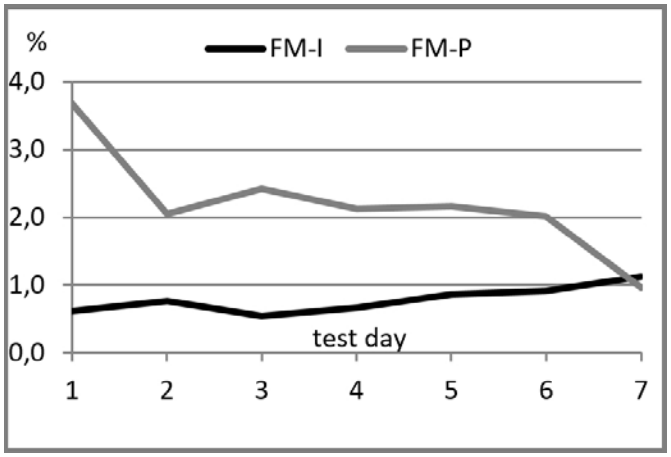

Figure 1. Dynamics of C18:1t11 by test day as per intensive (FM-I) and pasture (FM-P) farming (via descriptive statistics)

Table 3 presents fifteen of all registered PUFAs. The effect of farming on the sum of all PUFAs is highly significant (P<0.001), in the buffalo milk from FM_P (3.297%) being by 38 percent higher compared to FM_I, Highest is the portion of rumenic C18:2 cis-9, *trans-11* – constituting 88.9 and 90.1% of all conjugated linoleic acids (CLA) on FM_I and FM_P respectively. The effect of farm on it is most prominent (P<0.001), defining a 2.7-fold superiority of FM_P buffaloes (0.829%), as compared to FM_I. This trend is observed also in CLA *trans-10*, *cis-12* with even much bigger difference (14 fold) and also high F-value, while the other CLA *cis-9*, *cis-11* is not affected. C18:2t10c12 was absent in the milk of 7 buffaloes on FM_I and 6 on FM_P, and C18:2c9c11 – respectively in 3 and 2 buffaloes (Table 3). The other trans-isomer of C18:2 (*trans-9*, *trans-12*) is affected by farm at marginal significance (P<0.05) with little higher value in the FM_P buffaloes.

Of the six registered omega-3 (n3) FAs, γ-linolenic C18:3 (GLA) is most presented and the most highly affected by farming (P<0.001), expressed in 3.4-fold higher LSM-estimate in the buffaloes from FM_P (Table 3). All other FAn3 are also significantly affected by farm, with higher concentrations in the milk from FM_P, except for C20:3. The most presented FAn6 is α-linolenic C18:3 (ALA) on which the effect of farm is very well expressed

Table 3. Effect of farming, respective LSM-estimates by farms, and the factors test day (TD), milk yield (MY) and fat percentage (F%) on PUFA

Fatty acids	Farm					TD	MY	F%
	F/P	FM_I		FM_P		Level of P-value		
		n	LSM ± SE	n	LSM ± SE			
C18:2t9,12	6.34 *	55	0.038 ± 0.003	54	0.049 ± 0.003	NS	NS	NS
C18:2c9t11	113.9***	55	0.304 ± 0.031	54	0.829 ± 0.034	NS	NS	NS
C18:2t10c12	32.54***	48	0.0024±0.0041	48	0.0337±0.0044	NS	NS	NS
C18:2c9c11	1.94 NS	52	0.0348±0.0061	52	0.0465±0.0063	NS	NS	NS
C18:3n6	39.64***	55	0.058 ± 0.010	54	0.145 ± 0.010	NS	NS	NS
C18:3n3	94.35***	55	0.149 ± 0.025	54	0.502 ± 0.027	**	NS	NS
C18:4n3	43.20***	55	0.067 ± 0.007	52	0.130 ± 0.007	**	NS	NS
C20:2n6	4.79 *	55	0.020 ± 0.002	54	0.028 ± 0.003	NS	NS	NS
C20:3n6	24.73***	55	0.0139±0.0031	54	0.0357±0.0033	*	NS	NS
C20:4n6	4.47 *	54	0.0460±0.0103	51	0.0772±0.0111	NS	***	NS
C20:3n3	14.00***	55	0.118 ± 0.005	54	0.089 ± 0.006	NS	NS	NS
C20:5n3	10.30 **	52	0.0055±0.0021	48	0.0152±0.0023	*	NS	NS
C22:2n6	11.71***	53	0.0017±0.0073	49	0.0344±0.0080	NS	*	NS
C22:5n3	19.12***	55	0.034 ± 0.008	54	0.084 ± 0.009	*	*	**
C22:6n3	15.46***	49	0.0051±0.0019	52	0.0159±0.0020	NS	NS	NS
∑PUFA	38.23***	55	2.385 ± 0.104	54	3.297 ± 0.110	NS	*	NS

(P<0.001) defining 2.5-fold higher value in the FM_P buffaloes, as it was found to be also in the other n6.

Like CLA*c9t11* and CLA*t10c12*, total CLA in the buffalo milk is strongly affected by farming (P<0.001), expressed in 2.7-fold higher levels in the buffaloes on FM_P (Table 4). Similar is this effect on total FAn3 (P<0.001) with similar superiority of FM_P milk (2.2 fold). In the same time, total FAn6 is by 13.8 percent higher on FM_I (P<0.001), which renders the n6/n3 ratio considerably higher on this farm – 5.00 versus 1.99.

On the atherogenicity and thrombogenicity indices the effect of farm is significant at P<0.05 and the values lower in the FM_P buffaloes – respectively 2.44 and 3.21 versus 2.85 and 3.68 on FM_I.

The effect of test day has been established to be significant in four of the FAn3, graphically given in Figure 2. The curve of C18:3 for FM_P is more dynamic and much higher in August and declining down to January. In contrast, C18:4 has very low levels in August and practically unchangeable after October, especially on FM_I, hence the non-significant effect of this factor on total FAn3 (Table 3). Considering C20:5 and C22:5, noteworthy is the rather high peak in December on FM_I. Obviously this peak is the most of the difference between the two farms in these two n3 FAs, and the most of the effect of test day.

Table 4. Effect of farming, respective LSM-estimates by farms, and the factors TD, MY and F% on groups of FA

Fatty acids	Farm					TD	MY	F%
	F/P	FM_I		FM_P		Level of P-value		
		n	LSM ± SE	n	LSM ± SE			
∑CLA	138.4***	55	0.342 ± 0.034	54	0.913 ± 0.036	*	NS	NS
∑n3	76.29***	55	0.377 ± 0.037	54	0.831 ± 0.038	NS	NS	NS
∑n6	13.53***	55	1.885 ± 0.044	54	1.657 ± 0.047	NS	*	NS
IA	6.31 *	54	2.85 ± 0.115	51	2.44 ± 0.123	NS	**	NS
IT	6.16 *	54	3.68 ± 0.131	51	3.21 ± 0.140	NS	*	NS

IA – Index of atherogenicity; IT – Index of thrombogenicity

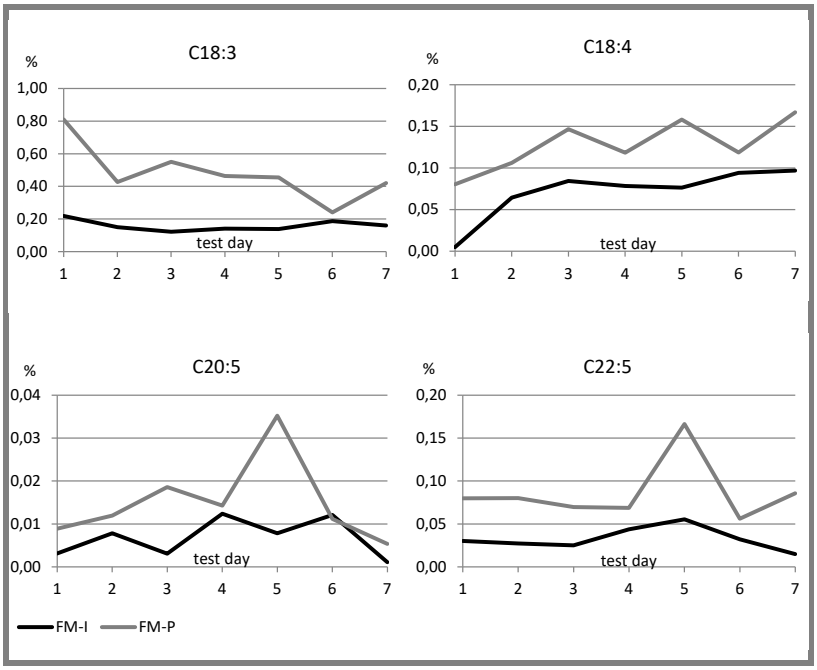

Figure 2. Dynamics of n3 FAs by test day as per intensive (FM-I) and pasture (FM-P) farming (via descriptive statistics)

Of all FAn6, only C20:3 is affected by test day (P<0.05), Figure 3 indicating more inconsistent dynamics on FMP with highest value again on test day five.

The dynamics of total CLA (Figure 4) are represented with similar peak on fifth test day on FM_P, but only as an exception of the general decline from August to February. The effect of test day on total PUFA is marginally non-significant (P= 0.059, not tabulated). For the FM_P buffaloes it is expressed in a curve (Figure 5) similar to the curve of ∑CLA, the content of which (Table 4) being actually only 28% of the content of total PUFA (Table 3).

The processed productivity records of the buffalo cows from the two farms taken on the respective test dates for lipid analysis are given in Table 5. The data show that, compared to FM_I, the FM_P animals have generally better milk yield from first to seventh month of lactation (averagely by 13 percent) and slightly lower fat content in milk.

Table 5. Milk yield and fat content on the two farms by test day (x ± Sx)

Test day month	FM_I			FM_P		
	n	Milk, kg	Fat, %	n	Milk, kg	Fat, %
August	8	9.05 ± 0.49	7.13 ± 0.23	8	9.51 ± 0.72	7.70 ± 0.30
September	9	7.97 ± 0.54	6.90 ± 0.20	8	9.80 ± 0.87	7.04 ± 0.27
October	9	7.71 ± 0.58	7.84 ± 0.41	8	8.53 ± 0.59	7.04 ± 0.26
November	9	6.74 ± 0.75	7.82 ± 0.36	8	7.26 ± 0.69	7.78 ± 0.19
December	7	5.34 ± 0.80	7.86 ± 0.35	8	5.99 ± 0.51	7.51 ± 0.18
January	7	4.07 ± 0.84	6.81 ± 0.37	7	6.23 ± 0.26	7.22 ± 0.24
February	6	4.40 ± 0.65	9.32 ± 0.67	7	4.73 ± 0.46	7.71 ± 0.48
Mean	55	6.66 ± 0.34	7.61 ± 0.17	54	7.55 ± 0.33	7.43 ± 0.11

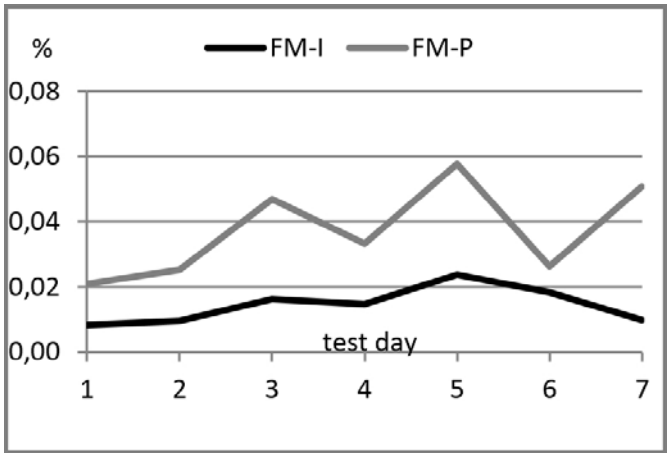

Figure 3. Dynamics of C20:3n6 by test day as per intensive (FM-I) and pasture (FM-P) farming (via descriptive statistics)

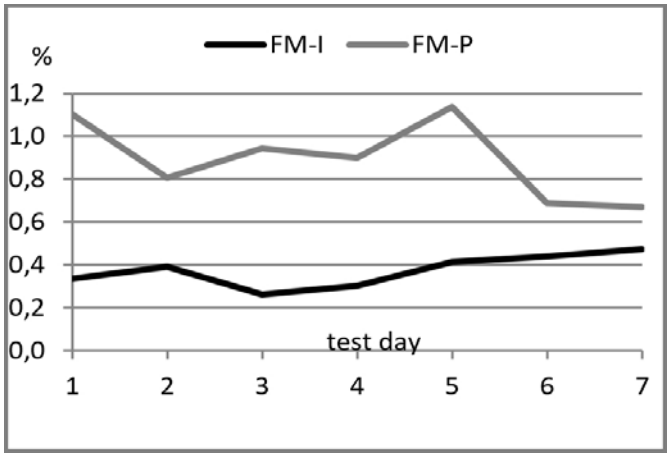

Figure 4. Dynamics of ∑CLA by test day as per intensive (FM-I) and pasture (FM-P) farming (via descriptive statistics)

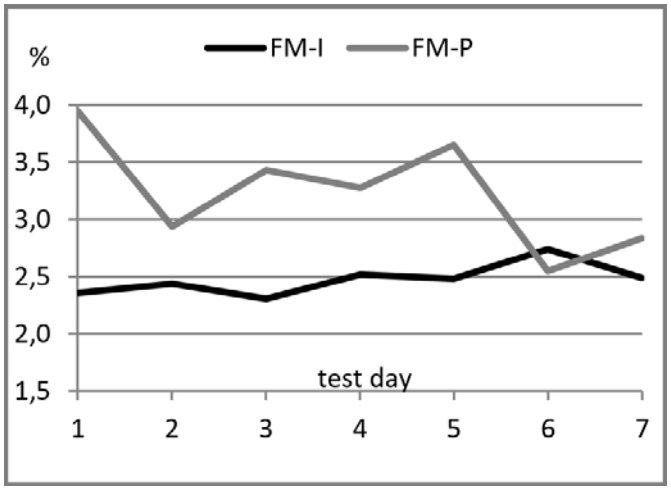

Figure 5. Dynamics of ∑PUFA by test day as per intensive (FM-I) and pasture (FM-P) farming (via descriptive statistics)

DISCUSSION

The milk of the intensively bred buffaloes from this study was found to have lower CLA, PUFA, higher n6/n3 ratio, similar ALA, but higher oleic acid, compared to the reports of Pegolo et al. (2017) on the Mediterranean Italian and of Kushwaha et al. (2018) on the Bhadawari breed in India. It has also lower PUFA but higher trans-C18:2, similar trans- and cis-C18:1 (Qureshi et al., 2012) but also much lower SFA (Sharma et al., 2000), compared to Murrah buffaloes in India. In previous studies with the same herd (FMI) were found little lower index of atherogenicity and thrombogenicity but also much worse omega ratio thanwas established by this study (Mihaylova and Peeva, 2007; Tzankova and Dimov, 2003; Penchev et al., 2016).

In comparison to two other herds of Bulgarian Murrah under grazing conditions (Naydenova, 2005), results of this study concerning pasture farming (FM_p) showed that the most atherogenic C14:0 and the C12:0 are lower but C16:0 is higher, MUFA is lower but PUFA is definitely higher. Compared to studies in buffaloes under extensive conditions in Colombia (Bustamante et al., 2017) and Brazil (Gagliostro et al., 2015), the beneficial FAs in milk from pasture in present study are generally lower, while compared to the buffaloes of the Mediterranean type in Romania (Vidu et al., 2015) they are better.

More noteworthy is that the studied milk from the two herds of buffaloes revealed principally different levels of the important fatty acids, demonstrating the effect of management, in particular the positive influence of fresh pasture on the beneficial vaccenic acid, CLA, ALA, FAn3 (resp. n6/n3 ratio) and PUFA, as established by Stockdale et al. (2003) and Rego et al. (2016). The effect of grazing is associated with better Δ9-desaturase activity (Shingfield et al., 2005; Kalač and Samková, 2010), as well as with enhanced growth of specific bacteria in the rumen, due to the higher content of soluble sugars in fresh plants compared with preserved forages (Kelly et al., 1998; French et al., 2000). This stimulates CLA production (like Δ9d) and prevents the reduction of vaccenic to stearic acid (Nudda et al., 2005), and also leads to increase of FAn3 (Chilliard et al., 2001; Dewhurst et al., 2006). Both conjugated and omega-3 FAs have important health-related functional properties, like anti-cardiovascular, anti-carcinogenic and other effects (Belury, 2002; Dilzer and Park, 2012), and have been established to derive from ALA (Leaf, 2008; Barceló-Coblijn and Murphy, 2009). But while FAn3 have low concentrations in livestock products, CLA, in particular rumenic acid, in the human diet can be obtained predominantly from milk and meat of ruminant origin (Lawson et al., 2001; Vargas-Bello-Pérez and Garnsworthy, 2013).

The role of CLA is also to mitigate the atherogenic effect of the dominating palmitic acid (Clandinin et al., 2000). Alike it, anti-atherogenic properties are attributed also to the butyric acid (Givens and Shingfield, 2006) that has shown comparatively high levels in the studied buffalo herds. Margaric acid, which also represents the level of bacterial synthesis in the rumen (German and Dillard, 2006), and which is associated with high density lipoproteins, neutralizing the atherogenic acids (Parodi, 2009), was found to be much higher on the farm with pasture (FM_p).

Better rumen biohydrogenation process is implied also in the established much higher levels of trans-isomers of oleic acid in the milk produced from pasture (Dabadie et al., 2005; Vargas-Bello-Pérez and Garnsworthy,

2013). These higher levels also predetermine the higher concentrations of rumenic CLA (Chilliard et al., 2000; Lock and Garnsworthy, 2002), justifying the correspondence of the well expressed effect of farming system regarding C18:1 and C18:2 trans isomers observed in this study. Something more, a definite portion of vaccenic from the human diet is converted into rumenic acid (Turpeinen et al., 2002).

The dynamics observed here are not commensurate with the improvement of trans C18:1, CLA and PUFA with the advance of lactation in dairy buffaloes (Sharma et al. 2000; Verdurico et al., 2012; Pegolo et al., 2017). This improvement has been found to be not very prominent, presumably because negative energy balance in this species is not so strongly expressed (Pegolo et al., 2017; Golla et al., 2019), which, to our field observations, in the Bulgarian Murrah is even weaker. Hence, here the mentioned effect of test day is attributed to the alterations in forage recourses, all the more that this factor concerns mostly the herd raised on pasture, while on FM_l the changes are negligible.

The decrease of the concentrations of some FAs in milk with the advance of the grazing season, especially regarding vaccenic acid, GLA and partially CLA and PUFA, can be attributed to the changes in the botanical and phenological condition of the grassland (Ferlay et al., 2008; Gorlier et al., 2012) and its protein content (Elgersma et al., 2003). The drop in CLA and PUFA has the exception of the peak in December, which was found to be much more prominent in C20:5n3 and C22:5n3, presumably due to the shift from autumn pasture to quality alfalfa hay with possible effect on rumen microflora, observed in lactating ewes by Dervishi et al. (2012) but not proved elsewhere. Also, feeding fodder beetroot to dairy cows has shown controversial results (Fleming et al., 2018), though molasses beet enhanced ALA, GLA and CLA (O'Callaghan et al., 2019).

More importantly, the comparison of the test-day curves of the two farms – especially until November and regarding chiefly vaccenic acid, GLA, CLA, PUFA – supports the effect of pasture very well.

CONCLUSIONS

The substantial differences between the farms, especially during grazing season, demonstrate in the buffalo the impact of management, and in particular the positive effects of pasture on all important (from consumers' viewpoint) fatty acids in milk – expressed to greater extent in the beneficial trans-FA, like rumenic and vaccenic, omega-6/omega-3 ratio, total MUFA and PUFA, and to a lesser extent in atherogenicity and thrombogenicity index. This is based on the effect of farming system that was established to explain significant portion of the variation of all individual MUFAs and PUFAs, and of total PUFA.

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