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INFLUENCE OF PARITY, DAYS POST-CALVING AND MILKING SEQUENCES ON THE FATTY ACID COMPOSITION OF MILK FROM ROMANIAN BUFFALOES

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Abstract

Demand for buffalo milk is on the rise, due to a shift in consumer choices towards healthier, more nutritious, and sustainable foods, this article aims to showcase the qualities of this product, particularly in terms of fatty acid (FA) composition. Romanian buffalo milk samples were collected from a buffalo farm in Mesendorf, Transylvania. The milk samples were individually collected at fixed intervals post-calving, across 3 milking sequences: beginning, middle and end of milking, from both primiparous and multiparous females, and analyzed in the laboratory. The study revealed that milking sequences significantly affected almost all FAs, and significant ($P<0.05$) interactions between days post-calving and milking sequences were observed for 6 out of the 14 fatty acids. Saturated fatty acids (SFA) averaged 74.29%, monounsaturated fatty acids (MUFA) averaged 23.43%, and polyunsaturated fatty acids (PUFA) had a mean of 2.28%. Milk Quality indices, like the Atherogenicity index (AI) or Thrombogenicity index (TI), among others, were calculated. Romanian buffalo milk of with an unsaturated to saturated fatty acid ratio of 0.35, shows a potentially healthy lipid profile.

Key words*: evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

Romanian water buffalo milk was, traditionally, very appreciated in the country. Following a few decades of decrease in interest, demand for buffalo milk is on the rise. This is due to a shift in consumer choices towards healthier and more nutritious food. Romanian buffalo, or Carpathian buffalo, as some call the breed, numbered more than 210.000 in 1989 (Onaciu, 2006), thereafter, the population plummeted, and in 2020 there were an estimated 14,000 left, most of which are found in Transylvania, where the research for this study was also conducted. The breed is part of the river buffalo subspecies (Minervino et al., 2020), and considered a Mediterranean river type. River buffaloes can be quite large, weighing between 450 and 1,000 kg, and yield consistently more milk than the swamp subspecies, which varies between 1,600 and 2,200 kg/lactation (Onaciu, 2006). Thus, most river breeds are raised primarily for dairy production in India, Pakistan, a few European countries, as well as western Asian, and South American countries. Buffalo milk is especially valued for its superior percentage of fat, protein, calcium, and other micronutrients, which contribute to its high nutritional qualities. The fat in buffalo milk differs from cow milk fat in terms of quantity, distribution of triglycerides, and also in its physical attributes from cow milk fat, notably due to a slightly higher

concentration of saturated lipids (Emakpor, 2024). The lack of carotene makes buffalo milk smooth and white (Emakpor, 2024). It is well known that buffalo milk is one of the richest types of milk due to its composition, especially because of the fat fraction (Menard, 2010). This aspect contributes to the milk's high energetic and nutritive value. In local rural areas, milk is often consumed raw, while available produce for urban areas are pasteurized, for microbiological safety reasons. Research shows that the consumption of water buffalo milk is sustainable from two perspectives: its antioxidant capacity surpasses that of cow milk and any other dairy species (Khan et al., 2017), thus being a healthwise choice, and these qualities, along with its fatty acid (FA) profiles, are not significantly altered by thermal procedures. Indeed, prolonged exposure to UHT procedures can cause significant alterations (Costa, 2011; Fan, 2023) but such processes are not practiced for buffalo milk in the country. Interesting aspects have been determined by previous research, for example, the calcium content is linked to the specific bone and skeletal development, which allows the buffalo calf to swim easier, as Zicarelli shows (2020). Buffalo calves have a lower intake capacity, at 2% of their live weight, as compared to bovine cattle, which have an intake of 2.4% of their live weight. This means that in the first months, buffalo calves ingest 16.76% less dry matter than bovine. Therefore, the quality of the milk is different, to balance meet such needs.

The synthesis of milk fatty acids (FAs) is particularly interesting, as mentioned by Çınar et al. (2019). FAs are produced primarily in the rumen from feed intake and as a result of microbial activity, and also in the mammary gland through de novo synthesis (Çınar, 2019; Liu et al., 2016) stated above, rumen bacteria convert polyunsaturated fatty acids (PUFA) into saturated stearic acid. This is why it can be found in ruminant animal-derived foods such as dairy products (Farah, 2007; Mansur, 2011). Buffalo milk may contain more saturated fatty acids (SFA) than bovine milk or even the food rations, because of the different populations of microorganisms in the water buffalo rumen (Sarfraz, 2013) PUFA, such as linoleic acid and conjugated linoleic acid (CLA) has also been shown to have potential health benefits in prevention of the mammary gland and skin tumours (Mansur, 2011; Paszczyk & Tonska, 2022). It seems that short chain fatty acids (SCFA) and medium chain fatty acids (MCFA) are produced by de novo synthesis in the mammary gland, while long chain fatty acids (LCFA) are produced out of circulating lipids. Also, some FAs are produced by both means (Liu et al., 2016). It can be noted that effects of lactation, namely Days post-calving, along with energy balance from food intake, has an impact on the FA profile of water buffalo's milk as well. Changes in milk FA composition during lactation, mostly at the beginning of lactation, can be brought about by physiological characteristics like de novo synthesis in mammary glands, ruminal synthesis or body fat mobilization (Hanuš et al., 2018; Mihavlova, 2007). The milking interval can affect the quality of milk, as Ratni et al. (2023) have shown. The time interval between morning milking and evening milking seems not to alter milk composition, but different milking time intervals can produce different milk compositions (Zicarelli, 2020). In the present article, milking sequences prove to be influential concerning FA percentages. Buffalo milk and products are good sources of CLA-PUFA, having a lot of potential as anti-carcinogenic, anti-adipogenic, anti-atherosclerotic and anti-diabetogenic properties (Ratni, 2023). Isomers of CLA, are transitional forms used by rumen bacteria to convert PUFA into saturated stearic acid. The concentration of CLA can be increased by optional dietary ma-nipulations like adding plant oils (Mingruo, 2012). Butyric acid, being a part of milk components, has also anti-carcinogenic and antimicrobial effect (Medhammar et al., 2011). The main FA profile in all buffalo milk is palmitic acid (C16:0), oleic acid (C18:1), myristic acid (C14:0) and stearic acid (C18:0), counted 74,61% of total FAs (Farrah, 2007). Also, it is richer in pentaenoic and tetraenoic, but less in trienoic and dienoic FAs than cow milk. Colostrum and mastitic milk has more cholesterol than normal milk (Sun, 2014, Farrah, 2007). Mihaylova & Peeva (2007) determined FAs concentrations from morning buffalo milk, using a gas chromatography and high performance liquid chromatography. From saturated fatty acids (SFA), the highest peak is that of palmitic acid (29.39%), myristic acid

(11.28%) and stearic acid (10.58%). Unsaturated fatty acids (UFA), the oleic acid was predominant having a concentration of (18.78%). The level of CLA was relatively low 0.38%, while the total amount of SFA in this milk sample was 72.15%, monounsaturated fatty acids (MUFA) 24.70% and PUFA 3.15%. FAs are crucial structural elements of biological membranes and a source of energy for organisms (Mihaylova & Peeva, 2007). They are regulators of oxidative stress, inflammation, lipid metabolism, glycemic control and other physiological processes (Mansur, 2011; Mihaylova & Peeva, 2007). If these processes malfunction, a multitude of metabolic syndrome disorders can occur, including cardiovascular disease. It seems that functional lipids that may have a positive impact on human health by lowering the risk of illnesses and enhancing the quality of life (Chen, 2004; Paszczyk & Tonska, 2022). Some FAs, like α-linolenic acid, linoleic acid, and oleic acid, have been found to be beneficial in the prevention, and treatment of cardiovascular disease, with positive effects on cardio-metabolic health, thus on overall health of people (Sun, 2014).

For a proper assessment of the qualities of water buffalo milk FAs, researchers calculate specific indices like the Atherogenicity index (AI), the Thrombogenicity index (TI), The S/P ratio - the Saturated to Polyunsaturated ratio, (S/P ratio), the Health-promoting index (HPI,), the - Desirable FAs (DFA)), sometimes referred to as the Hypocholesterolemic FAs, the OFA- Index of Hypercholesterolemic FAs, and others. All of these indices, together with the PUFA/SFA and n-6/n-3 PUFA ratios, are commonly used to evaluate the nutritional value of milk fat. Researchers consider that milk fat with high AI and TI values may be more likely to contribute to the development of atherosclerosis or coronary thrombosis in consumers. It seems that, milk with higher HPI ratios may have a positive effect in preventing cardiovascular diseases (Sarfraz, 2013; Paszczyk & Tonska, 2022). Thus, a higher proportion of PUFA in milk fat is desirable for human health. However, PUFAs can influence the technological properties of milk fat by improving the spreadability of dairy products, but they may also increase susceptibility to oxidation of dairy products, which can affect taste and shelf life (Paszczyk & Tonska, 2022). Additionally, PUFA can inhibit lipases that are important in the making of such cultured dairy product flavours (Mansur, 2011; Chen, 2024). Few studies address these milk quality indices for water buffalo, therefore, one of the objectives of the current research is to assess this issue from this perspective as well.

The present study aims to investigate the effects of days post-calving and milking sequences on the FA composition of milk from both primiparous and multiparous Romanian buffaloes, actual parity not being of importance, a topic that has not been previously explored in detail. Specifically, the research seeks to analyse the significant variations in FA profiles induced by the postpartum period and milking sequences and how these factors interact to influence the nutritional value of the milk. Additionally, the study aims to calculate and interpret key milk quality indices, such as the Atherogenicity index (AI), Thrombogenicity index (TI), Health-promoting index (HPI), and others, offering insights into the potential and sustainable health benefits of buffalo milk for consumers.

MATERIALS AND METHODS

Animals

The biological material is represented by 30 buffaloes of the Romanian buffalo breed (Figure 1) from the Transylvania region, consisting of lactating females grouped into two categories: 15 primiparous and 15 multiparous buffaloes, with a free box maintenance system. Actual parity not being essential for the study. The farm in question is situated in Meşendorf, Braşov county, with an agricultural surface of 682 hectares and, at the time, 145 water buffalo females in lactation. The farm is surrounded by pasture and beech tree forests, in a sub-montane zone. Lactating females do not graze in the pasture, but receive good quality hay and 3-4 kg of oat cereal plus minerals, administered when milked. Water is supplied on discretion for drinking, the pastures provide access to baiting ponds for the dry females and other physiological categories. Milking is done twice a day, mechanically in a small milking parlor with 8 posts per side.

Sampling

From the mentioned buffalo farm in the Transylvania region, 90 buffalo milk samples were collected from primiparous females and multiparous females. The milk samples intended for the analysis of FAs were collected individually on 3 milking sequences (sequence I - beginning of milking; sequence II - middle milking; sequence III - end of milking), at an interval of 42, 122 and 277 days post-calving, for both the primiparous and multiparous buffalo. The milking sequences were determined from the milking times which were previously recorded through a milking test, then equally divided. Milk was obtained from all four quarters as a single sample. The samples were taken in sterile tubes and frozen until the analysis was performed. All collected and analyzed samples met the quality standards for milk concerning somatic cell count (SCC) and total germ numbers TGN), in accordance with national and European legislation addressing animal health and the safety of food products of animal origin.

Gas chromatographic analysis of fatty acids

The analysis of milk FAs was performed using a Shimadzu GC-17A gas chromato-graph, which has a Chrompack column, with a diameter of 0.25 mm and a length of 25 m. An FID detector was used and the mobile phase is given by helium with 99.9% purity, and the stationary phase is given by a polyethylene glycol derivative, which was deposited in-side the column in the form of a thin film of 0.2 μm. Lipids were extracted from milk samples with chloroform and methanol according to the method of Bligh E.G. and Dyer W.J., 1959, with minor modifications regarding the volume of the sample and the solvents used. In Pyrex test tubes or transesterification, 100 μl of chloroform lipid extract is evaporated dry (by blowing with methane). To dissolve the lipids, 1 ml of benzene and 2 ml of 0.5 methanolic solution of sodium methoxide were added. It was heated at a temperature of 70ºC for 1 h. 100 μl glacial acetic acid and 1 ml of distilled water after cooling, were added. The ethyl esters of the FAs were extracted with petroleum ether (2×3 ml) in a separation funnel and the reunited ether extracts were anhydrofied with anhydrous Na_2SO_4, filtered and evaporated dry. The residue is chromatographed on a 60F 254 silica gel plate (Merck). Iodine is used as a visualization reagent and benzene is used as an eluent. Rf =0.5 for the methyl esters of FAs that are finally dissolved in hexane and gas-chromatographed.

Statistical Methods

The normal distribution of the values for each FA (4:0, 6:0; 8:0, 10:0; 12:0; 14:0; 14:1; 15:0; 16:0; 16:1; 17:0; 18:0; 18:1 and 18:2) was verified using the Shapiro-Wilk test. When necessary, in order to fulfil the assumption of normality, data were log transformed prior to analysis. The obtained results for each FA were expressed as mean ± standard deviation SD from data of five repetitions and analyzed with ANOVA model (JMP version 12, SAS Institute, USA) that included terms for milking sequences (M), days post-calving (D), separated by primiparous and multiparous. Significant differences among treatment means were further examined using Tukey's multiple range test at the 0.05 probability level. A p value of 0.05 was used as the threshold for statistical significance. The specific indices related to FA in food were calculated as to highlight the beneficial side of buffalo milk in regard to consumer health. In science, initially these indices were used to evaluate the negative effect of some FA, like C12:0, C14:0, and C16:0 on human health [20, 21]. Thus, the most important such indices were addressed in this study, such as the Atherogenicity index, the Thrombogenicity index, the Saturated to Polyunsaturated ratio, the Health-promoting index and a few others, as derived from literature. The mathematical deductions used were as follows:

The Atherogenicity index (AI) was calculated according to the Ulbricht equation [25]:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / (\sum MUFA + \sum(n\text{-}6) + \sum(n\text{-}3)); \quad (1)$$

Similarly, the Thrombogenicity index (TI) was calculated using the equation devised by Ulbricht & Southgate [22]:

$$TI = (C14:0 + C16:0 + C18:0)/((0.5 \times \sum MUFA + 0.5 \times \sum(n\text{-}6) + 3 \times \sum(n\text{-}3)) + (\sum(n\text{-}3)/\sum(n\text{-}6))); \quad (2)$$

The S/P ratio (Saturated to Polyunsaturated ratio) [25]:

$$S/P = (C14:0 + C16:0 + C18:0)/(\sum MUFA + \sum PUFA) \quad (3)$$

Health-promoting index (HPI) which is the inverse of the AI and indicates the potential health benefits of the fat:

$$HPI = (\sum MUFA + \sum PUFA)/(C12:0 + 4 \times C14:0 + C16:0) \quad (4)$$

Desirable Fatty Acids sometimes referred to as the Hypocholesterolemic Fatty Acids (DFA):

$$DFA = MUFA+PUFA+C18:0. \quad (5)$$

Index of Hypercholesterolemic fatty acids (OFA):

$$OFA = C12:0 + C14:0 + C16:0 \quad (6)$$

The ratio: $(C18:0 + C18:1)/C16:0$ (7)

The ratio between Poliunsaturated fatty acids and saturated fatty acids:

$$PUFA: SFA \quad (8)$$

RESULTS AND DISCUSSIONS

Influence of days post-calving and milking sequences on fatty acids (FAs) in milk from primiparous buffaloes

Milk from the primiparous group of water buffaloes had overall fat values from 7.01% to 7.58%% with a mean of 7.29% ± 0.06%. The average for each FA and the main factors affecting FAs and their interactions are reported in Table 1. Several FAs, including caproic acid (6:0), myristic acid (14:0), and palmitic acid (16:0), show an increase in concentration from 42 to 277 days postpartum. UFAs, except for oleic acid (C18:1), increased significantly during the postpartum period in the milk of primiparous Romanian buffaloes. Specifically, linoleic acid (C18:2) increased from 2.08 g/100 g fat to 2.25 g/100 g fat, palmitoleic acid (C16:1) increased from 1.24 g/100 g fat to 1.45 g/100 g fat, and myristoleic acid (C14:1) increased from 1.15 g/100 g fat to 1.21 g/100 g fat over the same period. The majority of FAs, with the exception of capric acid (10:0), heptadecanoic acid (17:0), and oleic acid (18:1), were significantly affected ($P<0.05$) by days post-calving (Table 1).

The milking sequences significantly ($P<0.05$) affected almost all FAs, whereas significant ($P<0.05$) interaction between days post-calving and milking sequences was found for 6 of the 14 fatty acids analysed. The highest values for each FA were obtained in the last milking sequence (Table 1).

Influence of days post-calving and milking sequences on fatty acids (FAs) in milk from multiparous buffaloes

Milk from the multiparous group of water buffaloes had overall fat values from 7.40% to 8.81%% with a mean of 7.98% ± 0.06%.

Data about the average concentrations for each FA in the milk of multiparous buffaloes, alongside the main factors influencing these FAs and their interactions are reported in Table 2. Milking sequences had a significant effect ($P<0.05$) on nearly all FAs, except for palmitoleic acid (C16:1) and heptadecanoic acid (C17:0). Importantly, the highest concentrations of FAs were observed during the last milking sequence, consistent with the findings for primiparous buffaloes (Table 2). Days post-calving significantly affected only butyrate (C10:0), lauric acid (C12:0), and palmitic acid (C16:0) ($P<0.05$). Notably, while no significant interactions were found between days postpartum and milking sequences for most FAs, there was a significant effect observed for lauric acid (C12:0).

Table 1. Effect of days post-calving and milking sequences on FAs (g/100 g fat) in milk from primiparous buffaloes

	Fatty acid - primiparous													
	4:0	**6:0**	**8:0**	**10:0**	**12:0**	**14:0**	**14:1**	**15:0**	**16:0**	**16:1**	**17:0**	**18:0**	**18:1**	**18:2**
						Days post-calving (D)								
D 42	4.74b	2.69b	2.16b	3.41a	2.86c	11.11a	1.15b	1.48c	27.61b	1.24b	1.01a	10.56b	19.77a	2.08b
	(0.40)	(0.35)	(0.36)	(0.31)	(0.59)	(0.52)	(0.08)	(0.09)	(0.94)	(0.09)	(0.06)	(0.72)	(0.67)	(0.13)
D 122	5.01a	3.12a	2.26ab	3.29a	3.96a	11.35b	1.16ab	1.55b	28.02b	1.32b	1.11a	11.17a	19.5	2.16ab
	(0.24)	(0.36)	(0.43)	(0.48)	(0.33)	(0.57)	(0.08)	(0.09)	(0.92)	(0.15)	(0.18)	(0.29)	(0.54)	(0.17)
D 277	4.84ab	3.17a	2.46a	3.36a	3.37b	11.54a	1.21a	1.62a	28.81a	1.45a	1.08a	10.97a	19.68	2.25a
	(0.35)	(0.36)	(0.27)	(0.45)	(0.83)	(0.48)	(0.06)	(0.06)	(0.69)	(0.08)	(0.10)	(0.61)	(0.43)	(0.19)
						Milking sequence (MS)								
MS 1	4.83a	2.77b	2.28a	3.38ab	3.54a	11.46b	1.16a	1.53b	27.84b	1.28b	1.06a	10.84a	19.48b	2.15ab
	(0.37)	(0.37)	(0.44)	(0.47)	(0.80)	(0.48)	(0.06)	(0.09)	(1.05)	(0.14)	(0.08)	(0.63)	(0.55)	(0.21)
MS 2	4.87a	3.09a	2.16a	3.18b	2.95b	10.97ab	1.17a	1.51b	27.76b	1.32b	1.03a	10.78a	19.42b	2.09b
	(0.39)	(0.34)	(0.25)	(0.35)	(0.72)	(0.54)	(0.09)	(0.10)	(0.84)	(0.13)	(0.08)	(0.54)	(0.53)	(0.12)
MS 3	4.9a	3.11a	2.45a	3.49a	3.68a	11.58a	1.19a	1.61a	28.86a	1.41a	1.11a	11.09a	20.05a	2.24a
	(0.29)	(0.45)	(0.36)	(0.37)	(0.55)	(0.42)	(0.08)	(0.09)	(0.64)	(0.11)	(0.19)	(0.66)	(0.34)	(0.17)

The values are mean of fifteen samples (n=15, standard error of the mean in parentheses). Different letters within a column denote significant differences ($p<0.05$).

Figure 1. Romanian Buffalo females at the farm in Mesendorf (own source)

Table 2. Effect of days post-calving and milking sequences on FAs (g/100 g fat) in milk from multiparous buffaloes

	Fatty acid - multiparous													
	4:0	**6:0**	**8:0**	**10:0**	**12:0**	**14:0**	**14:1**	**15:0**	**16:0**	**16:1**	**17:0**	**18:0**	**18:1**	**18:2**
	Days post-calving (D)													
D 42	4.87a	3.35a	2.25a	3.48a	2.86c	11.53a	1.19a	1.64a	27.92b	1.29a	1.03a	10.71a	19.68a	2.13a
	(0.42)	(0.35)	(0.38)	(0.39)	(0.42)	(0.42)	(0.09)	(0.13)	(1.11)	(0.17)	(0.26)	(0.65)	(0.51)	(0.19)
D 122	4.78a	3.43a	2.12a	3.18b	3.96a	11.5a	1.21a	1.57a	29.15a	1.33a	1a	10.81a	19.57a	2.17a
	(0.33)	(0.34)	(0.29)	(0.30)	(0.40)	(0.41)	(0.09)	(0.15)	(0.71)	(0.16)	(0.23)	(0.58)	(0.59)	(0.17)
D 277	4.91a	3.41a	2.35a	3.34ab	3.32b	11.54a	1.18a	1.61a	28.51ab	1.35a	1.08a	10.91a	19.77a	2.17a
	(0.47)	(0.31)	(0.40)	(0.31)	(0.35)	(0.42)	(0.10)	(0.14)	(1.26)	(0.15)	(0.24)	(0.62)	(0.42)	(0.17)
	Milking sequence (MS)													
MS 1	4.64b	3.11b	1.99b	3.14b	3.54a	11.42b	1.15b	1.58ab	27.81b	1.32a	0.97a	10.37b	19.39b	2.05b
	(0.38)	(0.21)	(0.20)	(0.30)	(0.42)	(0.43)	(0.08)	(0.14)	(1.22)	(0.17)	(0.28)	(0.65)	(0.58)	(0.15)
MS 2	4.90ab	3.60a	2.34a	3.43a	2.95b	11.39b	1.18b	1.55b	28.58ab	1.32a	1.03a	10.91a	19.67ab	2.11b
	(0.43)	(0.26)	(0.32)	(0.32)	(0.34)	(0.41)	(0.10)	(0.12)	(1.13)	(0.15)	(0.26)	(0.49)	(0.51)	(0.16)
MS 3	5.02a	3.78a	2.40a	3.43a	3.68a	11.75a	1.26a	1.68a	29.19a	1.33a	1.1a	11.13a	19.96a	2.30a
	(0.33)	(0.29)	(0.41)	(0.35)	(0.35)	(0.28)	(0.07)	(0.11)	(0.60)	(0.15)	(0.16)	(0.42)	(0.18)	(0.11)

The values are mean of fifteen samples (n=15, standard error of the mean in parentheses). Different letters within a column denote significant differences ($p<0.05$).

Table 3. Assessment of Milk Fat Quality Indices in primiparous and multiparous Romanian buffaloes

n	**AI**	**TI**	**S/P**	**HPI**	**DFA**	**OFA**	**(C18:0 + C18:1)/ C16:0**	**USFA/ SFA**	**PUFA/ SFA**	**MUFA/ SFA**
Primiparous (P)	3.16	2.57	2.07	0.32	37.38	45.49	1.09	0.35	0.031	0.32
Multiparous (M)	3.21	2.60	2.09	0.31	36.92	45.78	1.07	0.34	0.030	0.31

AI - Atherogenicity Indices; TI - Thrombogenicity Indices; The S/P ratio - Saturated to Polyunsaturated ratio; HPI - Health-promoting index; DFA - Desirable FAs sometimes referred to as the Hypocholesterolemic FAs**;** OFA- Index of Hypercholesterolemic Fas.

Milking sequences exert a significant effect on almost all FAs analysed, with the highest concentrations consistently observed in the last milking sequence. Interaction effects between days post-calving and milking sequences are evident for various FAs. Overall, these findings highlight the significant variation in FA composition in milk from primiparous and multiparous buffaloes in relation to postpartum period and milking sequences. The analysis revealed significant correlations between the studied factors and concentrations of certain FAs, as well as lack of correlation in others, providing a detailed perspective on the influence of these variables on buffalo milk composition. For example, several FAs in buffalo milk, such as caproic acid (6:0), caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0) and linoleic acid (18:2) showed a significant increase in concentration across lactation periods, 42 to 277 days postpartum (Table 2). Furthermore, the data analysis highlighted significant correlations between milking sequences and the concentration of most FAs.

Milk Fat Quality Indices in primiparous and multiparous Romanian buffaloes

Probably the most interesting part of this research is the determination of the principal Milk Fat Quality Indices relative to the results obtained earlier concerning FA profiles in milk from both first lactating females and buffalo females with more than one lactation. These indices illustrate the impacts of FA consumption for human health. The results indicate that there are no statistically significant differences in the calculated indices between the two categories (primiparous and multiparous buffaloes), as shown in Table 3. While subtle differences were observed, they do not reach statistical significance. For instance, the AI index, which shows the relationship between the sum of primary saturated FAs and that of pro-atherogenic unsaturated ones, had a value of 3.16 for primiparous and of 3.21 for multiparous females. TI index resulted in a value of 2.57 for the first category and 2.60 for the second as can be seen in the table. Health-promoting index and DFA index proved to be in a good part of the typical range, with means of 0.31 and 37.16, respectively. The ratios between Saturated and Polyunsaturated FAs, with the specific derivations, are revealed to have values within normal range, without significant differences between the two mentioned categories of lactating buffalo females.

Although numerous studies have examined the FA composition of buffalo milk, data on how factors like days post-calving and milking sequences affect the FA profiles of primiparous and multiparous buffaloes have not been previously explored. In this current research, a total of 14 FAs of different saturation levels were identified in buffalo milk fat, of which 10 were SFA and 4 were USFA.

Out of the identified FAs, 74.29% were SFA, 23.43% were MUFA, and 2.28% were PUFA. The five most abundant FAs in Romanian water buffalo milk were palmitic acid (29.91%), oleic acid (20.77%), stearic acid (11.45%), myristic acid (12.06%), and butyric acid (5.13%), which together accounted for more than 75% of the total FAs.

Some FAs show an increase in concentration from 42 to 277 days postpartum because of physiological changes in the animal. As stated, UFAs, except for oleic acid, increased during the postpartum period in the milk of primiparous Romanian buffaloes. This is significant since an increase in UFAs is considered to have positive effects on human health, especially cardiometabolic health, as suggested by previous studies (Paszczyk & Tonska, 2022; Ulbricht & Southgate, 1991; Khalili & Kourimská, 2022). Others, such as Kris-Etherton et al. (1999) and Mensink et al. (2003), cited by Sun et al. in 2014, consider that a diet rich in PUFAs and MUFAs reduces low-density lipoprotein cholesterol (LDL) and that the replacement of SFA with UFA will reduce the risk of coronary artery diseases (Sun, 1999). This context is relevant for the working hypothesis of our re-search related to benefits of Romanian buffalo milk.

Therefore, data showed that in Romanian buffalo milk, the average percentage of Omega-6 (linoleic acid) was 2.16%, a notable level compared to other breeds. This is relevant because PUFAs, such as Omega-6 and Omega-3, are essential FAs that cannot be synthesized by the human body and therefore need to be obtained through diet, as shown by Paszczyk & Tonska (2022).

An important aspect that proved significant is that days post-calving (Tables 1, 2) and milking sequences also significantly affected the majority of FAs, with the highest values for each FA obtained in the last milking sequence.

For multiparous females, days post-calving significantly affected only butyrate (C10:0), lauric acid (C12:0), and palmitic acid (C16:0) ($P < 0.050$). Between days postpatum and milking sequences, no significant interactions were found for most FAs, with the exception of lauric acid (C12:0). The research highlighted significant correlations between milking sequences and the concentration of most FAs.

As indicated by the Milk Fat Quality Indices calculated in Table 3, the results reassert the potential benefits that buffalo milk could have for consumers. The mean AI value recorded in this study was 3.18, which, while not particularly low, is still considered favorable given the high-fat content of buffalo milk. Similarly, the mean TI value of 2.58 reflects a balanced composition. The Saturated to Polyunsaturated ratio of 2.08 is also a positive value. The HPI, which is the inverse of the AI, the health-promoting index had a value of 0.31 -

good, but still relatively low. The HH indices, namely the DFA and OFA or Hypocholesterolemic index and Hypercholesterolemic index had mean values of 37.16 and 45.63 respectively. Even the rest of indices (C18:0 + C18:1)/C16:0, with a mean of 1.08, USFA: SFA with 0.35, PUFA:SFA with 0.031 and MUFA:SFA with 0.32, all exhibit good ratios of UFA.

The potential benefits of these indices are very much supported by existing literature. For example, the AI is widely regarded as a dietary risk indicator for lipids that may contribute to cardiovascular diseases (Paszczyk, 2022; Ulbricht & Southgate, 1991). The TI highlights the possibility of forming clots in the blood vessels (Paszczyk, 2022) and points to the relationship found between pro-thrombogenic (saturated) and anti-thrombogenic FAs (MUFAs, (n-6) PUFAs, and (n-3) PUFAs), as shown by Paszczyk (2022). Furthermore, as established by the equations used here, all UFA - regardless of the number, position, or configuration of their double bonds - are effective in reducing atherogenic risk (Hanuš et al., 2018). As clearly emphasized by Paszczyk & Tonska (2022), diets that incorporate products with lower AI and TI values may help reduce the risk of coronary heart disease, owing to their favorable nutritional profiles.

CONCLUSIONS

Milk Quality Indices like the Atherogenicity index (AI), the Thrombogenicity index (TI), The S/P ratio - Saturated to Polyunsaturated ratio, the Health-promoting index HPI, the - Desirable FAs (DFA) sometimes referred to as the Hypocholesterolemic FAs, the OFA- Index of Hypercholesterolemic FAs, and others calculated showed positive values situated in the good part of the typical range concerning each index. They strongly point at the health benefits of buffalo milk, and indeed great potential by improving feed and husbandry technology for buffalo.

These findings provide new insights that can guide breeding and dietary interventions to further enhance milk quality related to different milking sequences, lactation period and parity of the buffaloes, particularly emphasizing the potential to optimize the processing of specific dairy products. For instance, the last fraction of milk, which exhibits the highest concentration of fat and FAs, could be especially valuable for the production of high-fat dairy products, while the first fraction, which contains lower fat and fatty acid content, may be better suited for low-fat dairy options or other applications such as animal feed or ingredient formulation. Future research related to milk processing technology related to these aspects could prove of great value.

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