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Fatty acid profile of buffalo milk produced in southern Brazil

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ABSTRACT. Considering that buffalo milk is one of the richest in composition, mainly due to its fat fraction, the objective of this research communication was to determine the detailed fatty acid (FA) profile of buffalo milk produced in southern Brazil. Samples were collected from three farms that represent 100% raw buffalo milk producers of dairy products. Properties A and C had only one milking during the lactation period, and farm B had two milking. Farms A and B provided pasture and grain supplements, and farm C, provided only green pasture to the animals. A total of nine FA was identified: six saturated, two monounsaturated, and one polyunsaturated (conjugated linoleic acid - CLA). This study is the first to report the FA profile, including desirable fatty acids (DFA) like monounsaturated, polyunsaturated fatty acids, and stearic acid in buffalo milk from southern Brazil. The farms tested used different food management practices, as well as pasture management, showing that green pasture increases the fatty acid profile in buffalo milk. In addition, buffalo milk represented a good source of DFA for humans and opens a new field for the dairy industry that can explore the control of its FA composition, mainly through feed management.

Keywords: chromatography-flame ionization detector; fatty acid; milk; animal handling.

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Introduction

Worldwide, consumer attention has started to focus on foods and their health impacts. An important component of food in this regard is the fatty acid (FA) profile (Bhat & Bhat, 2011). Whereas saturated fatty acids (C12 – C16) and trans fatty acids are reported to be positively correlated with atherosclerosis and coronary heart disease (Molkentin, 1999), conjugated linoleic acids (CLA) have been shown to have anticarcinogenic, antiobesity, antidiabetic, and antihypertensive properties in humans (Koba & Yanagita, 2014).

Buffalo milk is among the richest milk from a compositional point of view, with fat constituting its main differential component (Khedkar, Kalyankar, & Deosarkar, 2016). Since the 90s, there has been an increase in the number of industries manufacturing products derived from buffalo milk in Brazil, which is responsible for using most of the milk produced (Sales et al., 2018). However, there is still scarce scientific literature on buffalo milk's physicochemical and hygienic parameters. Recently, we published a paper on the physicochemical and complete microbiological characteristics of buffalo milk used as raw material for dairy products in southern Brazil (Godinho et al., 2020). This was the first study addressing this range of parameters in this region in a study period of over one year, which is creating gains for the dairy industry and for the consumer.

Regarding the composition of FA, many studies have been carried out on bovine milk fat due to its potential beneficial or negative effects on human health (Micinski et al., 2012). However, we cannot extrapolate these findings to buffalo milk, which has its characteristic FA composition (Ménard et al., 2010). To our knowledge, most studies aimed at evaluating the FA profile of buffalo milk have been carried out during the lactation stage (Caldeira, Ferrão, Fernandes, Magnavita, & Santos, 2010; Pegolo et al., 2017; Tonhati et al., 2011) or in relation to the final dairy product (Gulzar et al., 2019). This study focused on the whole milk from the tank entirely intended for the dairy industry. Therefore, the aims of this study were (1) to determine by gas chromatography-flame ionization detector (GC-FID) the detailed FA profile of buffalo milk produced in southern Brazil; and (2) to evaluate the contribution of seasonality, management, and feeding as potential sources of variation affecting the profiles of the identified milk FA traits.

Material and methods

Farms and milk samplings

Three farms were chosen for this study, for the collection of milk samples. These properties represent 100% of the official buffalo milk market, which produces dairy products in Rio Grande do Sul (RS). The characteristics of these farms are listed in Table 1.

Table 1. Characteristics of the farms.

	Farm A	Farm B	Farm C
Location coordinates	30°36'41"S, 51°34'44"W, 600m	30°11'29"S, 52°22'25"W, 100m	29°56'40"S, 50°59'31"W, 26m
Total / lactating animals	305 / 127	400 / 70	76 / 14
Volume of milk/week	2314 L	5819 L	618 L
Milkings/day	1	1 (Jun 2017 – Dec 2017) 2 (Dec 2017 – Aug 2018)	1
Mastitis cases	rare	rare	sometimes
Animal nutrition	corn silage and pasture	corn silage, rice bran, and soybean meal; oat and ryegrass pasture (winter), Sudan grass pasture (summer)	native field and cultivated pasture

To represent the seasonal variation in this study, samples were taken over a year (from June 2017 to August 2018). A total of 12 milk samples were considered: each of the three farms in each of the four seasons: spring, summer, fall, and winter. Milk was sampled from the cooling tank after homogenizing the contents, representing all the milkings of the farm animals at different stages of lactation. Samples were maintained frozen until analysis.

Fatty acid composition

The fatty acid (FA) composition of milk was determined by gas chromatography-flame ionization detector (GC-FID) adapted from American Oil Chemists' Society (AOCS, 2009) since the milk samples contain polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA), which could be analyzed by high-temperature gas chromatography. Free FA was extracted with a 0.5 N NaOH-methanol solution, followed by the addition of hexane for FA separation. Then, the sample (1°µL) was injected on the GC-FID (3400 CX, Varian, CA, USA) equipped with a CP-Sil 88 capillary column (50 m × 0.25 mm × 0.25 µm). The analysis followed the conditions: initial temperature of 140°C and final temperature of 185°C, heating rate of 1°C min.; detector temperature of 185°C; hydrogen as carrier gas at 1 mL $min.^{-1}$ flow rate. FA was identified by comparison of the component retention time with a standard mix of FA code 18919 (Supelco), and quantified by area normalization, through the Work Station Software. The results were expressed as area percentages (%). All samples obtained by various techniques under different operational conditions or solvents were analyzed by GC-FID.

From the data on FA composition, the following indices of lipid quality were determined:

1) Desirable Fatty Acids (DFA) = MUFA + PUFA + Estearic (C18:0);

2) Index of Atherogenicity (IA): indicates the relationship between the sum of the main SFA and that of the main classes of unsaturated, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (Garaffo et al., 2011). The following equation was applied:

$$IA = \frac{\text{Lauric (C12: 0)} + \text{[4 x Myristic (C14: 0)]} + \text{Palmitic (C16: 0);}}{\Sigma\,\text{MUFA} + \Sigma\,\text{PUFA}}$$

3) Index of Thrombogenicity (IT): shows the tendency to form clots in the blood vessels. This is defined as the ratio of pro-thrombogenic to anti-thrombogenic fatty acids (MUFA, PUFA ω6, and PUFA ω3) (Garaffo et al., 2011). The following equation was applied:

$$IT = \frac{\text{[Miristic (C14: 0)} + \text{Palmitic (C16: 0)} + \text{Estearic (C18: 0)],}}{\text{(0.5 x MUFA)} + \text{(0.5 x ω6)} + \text{(3 x ω3)} + \text{(ω3/ω6)}}$$

where; ω6 represents the omega 6 FA and ω3 represents omega 3 FA.

Statistical analysis

The experimental model used was a completely randomized design in a factorial arrangement (3 x 4), and the statistical model referring to the variables was:

$Y_{ijk} = \mu + T_i + E_j + TE_{ijk+} \varepsilon_{ijk}$,

where Y_{ijk} represents the dependent variables (different FA); μ is the mean of all observations; T_i is the effect of treatments (farms A, B, and C); E_j is the season of the year (spring, summer, fall, and winter); TE_{ijk} is the effect of the interaction of treatments x season of the year and ε_{ijk} is the residual effect (error). Analysis of the results was performed using the statistical software R-project® (R Core Team, 2016).

Results and discussion

The composition of buffalo milk has been studied in several countries and variations have been reported. In addition to distinct methods of analysis, some divergences indicate variability among herds, management, environmental conditions, and seasonality. The results presented here provide a nutritional evaluation of the FA profile of buffalo milk from southern Brazil using gas chromatographic analysis. A total of nine FA was detected: six SFA, two MUFA, and one PUFA. Their concentrations were expressed in grams 100 grams^{-1} of total fatty acids as reported for each farm in Table 2.

Table 2. Fatty acids area percentage (g 100 g^{-1}) of buffalo milk in different farms.

Fatty Acids	Farm			p
	A	B	C	
Caprylic (C8:0)	0.2575	0.1975	0.5550	0.3601
Capric (C10:0)	0.5225	0.5525	0.9375	0.6100
Lauric (C12:0)	1.1525	1.0850	1.3000	0.8969
Miristic (C14:0)	8.8550	9.5250	8.2750	0.7938
Palmitic (C16:0)	41.0325	38.7925	32.4925	0.0919
Palmitoleic (C16:1 cis)	3.0025	2.9300	3.0025	0.9696
Stearic (C18:0)	13.0850	14.4700	11.540	0.2074
Oleic (C18:1 ω9 cis)	30.9950	31.0800	37.8575	0.4501
Linolelaidic (C18:2 ω6 trans)	1.0975a	1.4700ab	4.1225b	0.0363
Saturated (SFA)	64.91	64.63	55.10	0.2269
Monosaturated (MUFA)	33.99	33.92	40.79	0.4035
PUFA: SFA	0.02	0.02	0.08	0.0612
PUFA: MUFA	0.03	0.04	0.11	0.0632
MUFA: SFA	0.53	0.53	0.84	0.3261
Desirable Fatty Acids (DFA)	48.18	49.86	56.45	0.3611
Index of Atherogenicity (IA)	71.46 a	56.51 ab	25.12 b	0.0148
Index of Thrombogenicity (IT)	4.23	4.51	4.91	0.6454

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ($\rho < 0.05$).

Oleic acid (C18:1 ω9cis) was the FA that contributed most to the profile of unsaturated FA (MUFA + PUFA), and palmitic acid (C16:0). to the profile of SFA in buffalo milk samples. The third FA that contributed most to the overall profile was stearic acid (C18:0), a result attributed to extensive ruminal biohydrogenation of FA C18:3, C18:2, and C18:1. This outcome seems to be positive, as part of stearic acid is transformed into oleic acid by the action of the Δ-9 desaturase enzyme producing the oleic acid (C18:1 ω9cis) present in milk (Corl et al. 2001). In ruminants, milk fatty acids arise from two sources – *de novo* synthesis in the mammary gland and the mammary uptake of preformed long-chain FA. The FA-synthesizing system (*de novo* synthesis) in the mammary gland produces even-numbered FA that are 4–16 carbons in the chain length. The other FA, which includes approximately half of the 16 carbon and all those 18 carbons or greater in length, are taken up preformed from the blood, originating from the diet or the mobilization of body reserves (Bauman, Mcguire, & Harvatine, 2011).

Human nutrition can be an important risk factor for atherosclerotic cardiovascular diseases, as it contributes to the etiology of dyslipidemia, obesity, and hypertension (Iacono, Dougherty, Puska, & Pietinen, 1989). The percentage of most FA was not different between farms (Table 2), however, there was a significantly higher index of linolelaidic acid (C18:2 ω6trans) in farm C. Related to the beneficial effects on human health, a significantly lower Index of Atherogenicity (IA) was found in farm C.

In Table 1, the basis of the feeding on the three farms was pasture, rich in different species of grasses and plants. Southern Brazil is characterized by fields with 8rich flora, resulting from the high variety of soils and the climatic characteristics of this region, which allows the coexistence of plants under the most diverse conditions (Nabinger & Dall'Agnol, 2020). This richness is represented by several botanical families, also conferring high resilience to variations in animal management, as well as to abnormal climate events resulting from climate change. Thus, the FA profile of ruminant milk is influenced by the food that animals ingest, especially green forage from fresh grasses and legumes (Kalač & Samková, 2010; Bauman et al., 2011). Silva and Nascimento Junior (2007) reported that the highest index of remaining leaf area, that is, the amount of photosynthetically active tissue that remains in the plant after grazing or cutting, is of fundamental importance for pasture management. Comparing farm C to the others, the native field and the cultivated pasture are inferred to improve the fatty acid profile, providing a lower amount of saturated acids and a higher amount of unsaturated acids, compared to the other two farms.

Despite no statistical differences were detected for the FA profile between buffalo milk samples collected in the four seasons of the year (Table 3), the season with greatest growth of pastures (summer) also had the highest percentage of linolelaidic acid (C18:2 ω6 trans). Bauman et al. (2011) suggest an increase in the activity of the enzyme Δ-9 desaturase during summer. The concentration of CLA in products derived from ruminants depends on two processes: ruminal biohydrogenation and endogenous desaturation of vaccenic acid by Δ9-desaturase in tissues; and the diet is the main factor influencing CLA levels (Oliveira et al., 2009). Moreover, the greener and fresher the pasture, the higher the concentration of precursors of conjugated linoleic acid (CLA). Green pastures have 10 times more linolenic acid than cereals (Daley, Abbott, Doyle, Nader, & Larson, 2005). There is the understanding that the pasture has greater potential to increase polyunsaturated fatty acids compared to preserved forages, silage, or hay (Modesto et al., 2009).

Table 3. Fatty acids area percentage (g 100 g^{-1}) of buffalo milk in the different seasons.

Fatty Acids	Season				p
	Spring	Summer	Fall	Winter	
Caprylic (C8:0)	0.16	0.47	0.50	0.21	0.6168
Capric (C10:0)	0.34	0.93	0.84	0.58	0.7005
Lauric(C12:0)	0.84	1.22	1.42	1.23	0.7581
Myristic (C14:0)	7.58	9.53	10.19	8.24	0.5896
Palmitic (C16:0)	36.88	37.04	38.95	36.89	0.9765
Palmitoleic (C16:1 cis)	2.84	3.76	2.67	2.42	0.2324
Stearic (C18:0)	14.30	12.39	14.70	10.74	0.1034
Oleic (C18:1 cis ω9)	35.70	31.49	29.07	36.98	0.6904
Linolelaidic (C18:2 trans ω6)	1.37	3.17	1.66	2.72	0.6919
Saturated (SFA)	60.10	61.10	66.60	57.89	0.7375
Monosaturated (MUFA)	38.54	35.26	31.75	39.40	0.6867
PUFA: SFA	0.0228	0.0527	0.0248	0.0652	0.6470
PUFA: MUFA	0.0356	0.0918	0.0534	0.0590	0.6256
MUFA: SFA	0.6433	0.5744	0.4787	0.8275	0.6606
Desirable Fatty Acids (DFA)	54.21	50.82	54.62	53.03	0.8629
Index of Atherogenicity (IA)	50.74	45.73	54.62	53.03	0.9842
Index of Thrombogenicity (IT)	3.75	4.94	4.89	4.70	0.3391

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ($\rho < 0.05$)

Importantly, polyunsaturated fatty acids are essential to human health but cannot be produced by most mammalian species (Daley et al., 2005, Koba & Yanagita, 2014). For this reason, it is necessary to have knowledge about fatty acids. In this way, these results may provide useful information about the nutrient composition of this milk and its variation according to specific factors, which may be used to improve the technological and nutritional characteristics of dairy products. In the present study, we demonstrated that buffalo milk represents a good source of DFA for humans. The higher DFA index in buffalo milk than in cow milk, according to a previous study (Ménard et al., 2010), makes it a potential substitute for cow milk, especially for consumers with cow milk allergy. Furthermore, using buffalo milk for health purposes, beyond nutrition, opens a new field for the dairy industry that can explore the control of their FA composition, mainly by managing the diet.

Conclusion

This study is the first to report the FA profile, including the desirable fatty acid (DFA) content of buffalo milk from southern Brazil. We demonstrate that the DFA content of milk tanks can be significantly increased through handling and feeding the animals. In addition, buffalo milk represented a good source of DFA for humans. Furthermore, the use of buffalo milk for health purposes in addition to nutrition opens a new field for the dairy industry.

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