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Implementation of a milking system and quality management for buffalo milk at an experimental station in Rio Grande do Sul, Brazil

Implementação de um sistema de ordenha e manejo da qualidade do leite bubalino em uma estação experimental no Rio Grande do Sul, Brasil

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Abstract
Buffalo milk production is an activity that has grown in recent years, mainly due to the physicochemical characteristics found in this type of milk, which is the second most produced in the world. The objective of this study was to implement a milking system and monitor the identity and quality standards of buffalo milk produced at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul. Samples were collected fortnightly between June and November 2021, totaling ten collections. The means obtained at the end of the monitoring were 4.84 g/100 g for fat, 4.64 g/100 g for protein, 5.06 g/100 g for lactose, 15.26 g/100 g for total solids, 10.42 g/100g for non-fat solids, 0.18 g lactic acid/100 mL of milk for acidity, 1.037 g/cm³ for density, -0.533°C for cryoscopic index, 3.5 x 10^5 cells/mL for somatic cell count, and 8.0 x10^3 CFU/ml for standard plate count. Residues of antibiotics and antiparasitics were not found. An increase in the concentration and frequency of some fatty acids was observed, after an increment in the nutritional management of the animals, in addition to predominance of palmitic acid (C16:0) and oleic acid (C18:1- cis (n9)). The results indicate a significant improvement in the quality of the milk in the assessment period, due to the corrective actions that were established, and the standard plate count showed good agricultural practices as to milk sourcing and the hygiene of the materials, being below the limits required by NI 76. The buffalo milk produced by the herd at the Experimental Station showed physicochemical and microbiological results in accordance with the legislation, being a raw material to be explored in the prospection of derivative products.
Keywords: *Bubalus bubalis*; buffalo; good agricultural practices; raw milk.

Resumo
A produção de leite bubalino é uma atividade que tem crescido nos últimos anos, principalmente devido às características físico-químicas apresentadas pelo leite, que é o segundo mais produzido no mundo. O objetivo deste trabalho foi implementar um sistema de ordenha e monitorar os padrões de identidade e qualidade do leite bubalino produzido na Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul. Amostras foram coletadas quinzenalmente entre os meses de junho e novembro de 2021, totalizando dez coletas. As médias obtidas ao final do monitoramento foram de 4,84 g/100 g para gordura, 4,64 g/100 g para proteína, 5,06 g/100g para lactose, 15,26 g/100 g sólidos totais, 10,42 g/100g para sólidos não gordurosos, 0,18 g ácido lático/100 mL de leite para acidez, 1,037 g/cm³ para densidade, -0.533°C para índice crioscópico, 3.5 x10^5 cél/mL para contagem de células somáticas e 8.0 x10^3 UFC/ml para contagem padrão em placa. Não foram identificados resíduos de antibióticos e antiparasitários. Observou-se aumento de concentração e frequência de alguns ácidos graxos, após incremento de manejo nutricional dos animais, além da predominância dos ácidos palmítico (C16:0) e oleico (C18:1- cis (n9). Os resultados indicam que houve uma melhora significativa na qualidade do leite no período avaliado, decorrente das ações corretivas que foram estabelecidas, e a contagem padrão em placa demonstrou boas práticas agropecuárias na obtenção do leite e na higienização dos materiais, estando abaixo dos limites exigidos pela IN 76. O leite de búfala produzido pelo rebanho da Estação Experimental apresentou resultados físico-químicos e microbiológicos de acordo com a legislação, sendo uma matéria-prima a ser explorada na prospecção de produtos derivados.
Palavras-chave: boas práticas agropecuárias; *Bubalus bubalis*; búfalo; leite cru.

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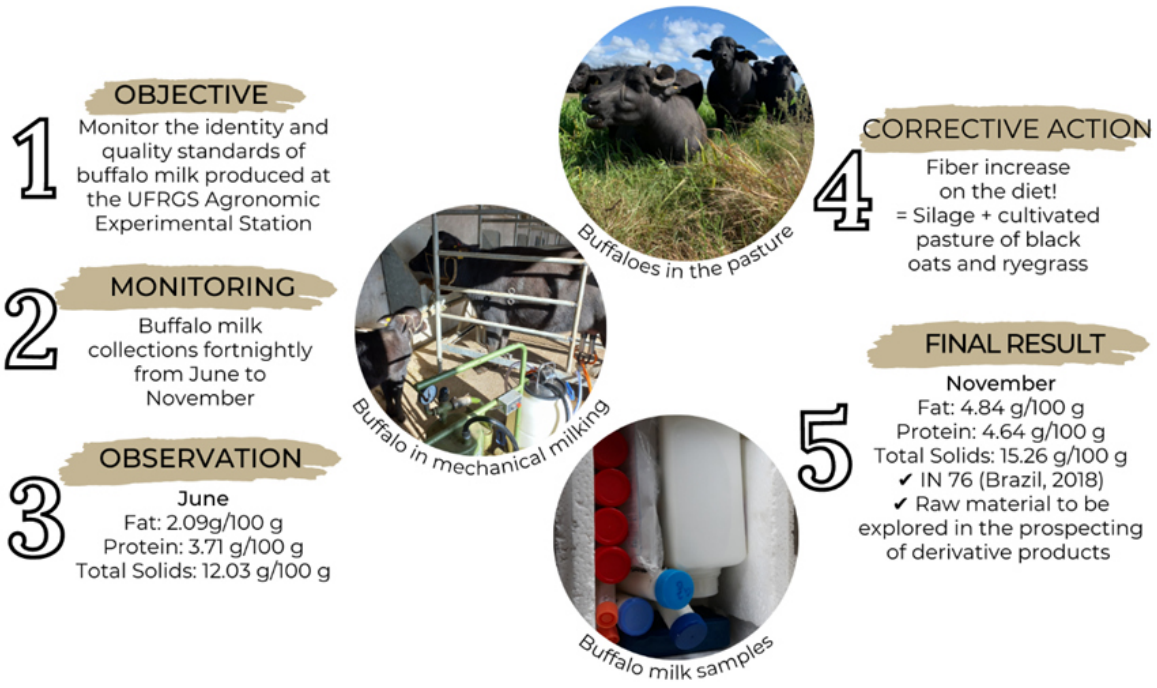

Graphical abstract -Implementation of a milking system and quality management for buffalo milk at an experimental station in Rio Grande do Sul, Brazil

1. Introduction

Buffalo milk is the second most produced in the world, second only to bovine milk, and, in 2019, accounted for approximately 15% of the world's milk production[1]. According to a collaboration between the Organization for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization of the United Nations (FAO), this production is expected to rise by 1.7% per year over the next decade to meet the increased consumption of dairy products worldwide, driven by the increase in population and income in developing countries[2].

Buffalo milk exploration is an activity that has grown in recent years, and, between 2010 and 2020, this growth stood at approximately 45%[1], mainly due to the physicochemical characteristics found in this type of milk, which has higher protein and fat contents compared to bovine milk[3, 4]. The results presented by Pignata et al.[3] indicated high levels of long-chain fatty acids, such as conjugated linoleic acid (CLA), popularly known as Omega 3, which acts against obesity, has an anti-inflammatory effect, and decreases blood triglyceride levels. According to Ménard et al.[5], buffalo milk has higher levels of saturated fatty acids, mainly palmitic acid, and trans fatty acids, such as linolenic acid and conjugated linolenic acid, compared to cow's milk.

However, the quality of raw milk may vary depending on environmental factors that influence each of its components, such as animal nutrition, lactation stage, and season of the year. Godinho et al.[4], in a study conducted with buffalo breeders in Rio Grande do Sul, observed that, in the summer, the means for protein and non-fat solids (NFS) stood at 4.27 g/100 g and 10.17 g/100 g, respectively, while in the winter, protein contents reduced to 3.9 g/100 g, and NFS contents, to 9.83g/100g. These variations were associated with lower milk production in warmer seasons, which increases the concentration of this component in milk; and the same is reflected in the density, which was higher in the summer compared to fall. Raw milk may still be exposed to microbiological contamination from the production environment, which is determined by factors such as milking management and the hygiene of utensils, as they contribute significantly to the microbiological conditions of milk as a product; for this reason, it is important for milking management to be in accordance with the Good Agricultural Practices (GAP) protocols, which, according to the guide published by the FAO & International Dairy Federation (IDF)[6], deal with the implementation of procedures at all stages of milk production on rural properties, which must ensure that milk and its derivatives are safe.

For its characteristics, mainly due to its high levels of total solids, buffalo milk represents a quality raw material for the making of cheeses and dairy products, presenting high yield in the production of some types of cheeses, such as mozzarella. However, it is a milk on which there is no legislation in force at the federal level in Brazil that meets all the parameters for standardization, as there is for bovine milk, through Normative Instruction No. 76 of November 26, 2018[7], which can be used, in view of the proximity between the two genera (*Bos* and *Bubalus*), as they are part of the same sub-family, *Bovinae*.

This study aimed to monitor the identity and quality standards of buffalo milk produced during the implementation and consolidation of a buffalo milk production system in its first year at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA-UFRGS).

2. Material and methods

2.1 Research site and experimental buffalo herd

The research was carried out at the shed of the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA-UFRGS), located at KM 146 of BR 290 in the municipality of Eldorado do Sul, RS (30º05'32" south latitude and 51º40'37" west longitude). In 2021, between April and December, the milking system was implemented. To this end, five multiparous buffalo females (*Bubalus bubalis*) with an average of 60 days of lactation, of the Murrah and Mediterranean breeds, with an average weight of 583.7 ± 47.12 kg, and age between 5 and 20 years, were subjected to milking management once a week with the calf present; this was done in a bucket-by-foot mechanical milking system with a set of teat cups. For milking management, the following procedures were performed: cleaning of the milker's hands with neutral detergent and running water, iodine-based pre-dipping (GlobalVac) and drying of the teats with paper towel, black-bottomed mug test, California Mastitis Test (CMT), mechanical milking, and iodine-based post-dipping (GlobalVac). The buffaloes were kept in native field (1.65 ha per AU), with access to mineral salt, 0.9% of live weight in corn silage supplied twice a week, and corn- and soy-based concentrate during milking (90% corn bran and 10% soybean bran). In September and October, the buffaloes had access to cultivated pasture (0.6 ha per AU) composed of black oats and ryegrass and were given twice as much silage twice a week (1.8% of live weight).

2.2 Buffalo milk collection and analysis

The buffalo milk collections, both individual and mixed, happened every two weeks in the period comprising June and November 2021. Since this is a project involving animals, it was submitted to the UFRGS's Ethics Committee for the Use of Animals (CEUA) and approved under number 40325.

2.3 Somatic cell count (SCC) and centesimal composition analysis

Individual collections of 50 ml of buffalo milk were done in a sterile flask with Bronopol preservative for SCC and centesimal composition. For the collection of individual samples, each buffalo underwent complete milking. After the milking was completed, the milk was homogenized in a pail so that it could later be collected. The samples were identified with date, collection number, and buffalo tag, refrigerated in a thermal box and taken to Univates's Accredited Milk Quality Laboratory (Cgcre - Inmetro certificate number CRL 0754). SCC was performed by flow cytometry, in accordance with ISO 13366-2[9]. The analysis of the centesimal composition, for fat (g/100 g), protein (g/100 g), lactose (g/100 g), non-fat solids (NFS) (g/100 g), and total solids (TS) (g/100 g) was performed using infrared spectrometry, in accordance with the ISO 9622 methodology[10].

2.4 Analysis of physicochemical and microbiological parameters

For these determinations, collections were taken through the sampling of 1000 ml of mixed buffalo milk in a sterile flask for physicochemical analysis, and 50 ml in a sterile flask with Azidiol preservative for microbiological analysis. The samples were identified with the date and collection number in order to be sent to Univates' Milk Quality Laboratory. For physicochemical parameters, density was checked in accordance with Method 2.12 of the Manual of Official Methods for the Analysis of Animal-Origin Food[11], and titratable acidity, in accordance with Method 2.2[12] of the same manual. The cryoscopic index (CI) was obtained by means of the ISO 5764 methodology[13]. The Standard Plate Count (SPC) microbiological analysis was performed in accordance with the ISO 4833-1 methodology[14].

2.5 Antibiotic and antiparasitic residue analysis

For the analysis of antimicrobial and antiparasitic residues, samples of 50 mL of mixed buffalo milk were collected in sterile Falcon-type flasks, identified with the collection date and collection, and frozen to be sent for analysis at the Federal Laboratory of Agricultural Defense of the Ministry of Agriculture, Livestock and Supply (LFDA/RS-MAPA). The analysis was performed by screening 46 antimicrobial and antiparasitic residues belonging to different classes, using liquid chromatography-electrospray-ionization-tandem mass spectrometry (LC-ESI-MS / MS), as previously described[15-17]. The residues checked for antibiotics and antiparasitics were: tetracycline, oxytetracycline, chlortetracycline, doxycycline, sulfadiazine,

sulfathiazole, sulfamethazine, sulfamethoxazole, sulfaquinoxaline, sulfadimethoxine, sulfadoxine, sulfapyridazine, sulfamerazine, sulfisoxazole, nalidixic acid, ozonyl, flumequine, ciprofloxacin, enrofloxacin, difloxacin, sarafloxacin, danofloxacin, norfloxacin, penicillin G, penicillin V, ampicillin, amoxicillin, oxacillin, cloxacillin, dicloxacillin, ceftiofur, cefapirin, cefoperazone, nafcillin, cefquinome, cephalonium, cephalexin, erythromycin, spiramycin, tilmicosin, azithromycin, tylosin, lincomicin, clindamycin, trimethoprim, bromhexine, chloramphenicol, thiamphenicol, florfenicol, abamectin, doramectin, eprinomectin, ivermectin, monensin, moxidectin, and albendazole.

2.6 Buffalo milk fatty acid profile

For the analysis of the free fatty acids (FFA) profile, samples of 50 mL of mixed buffalo milk were collected in a sterile Falcon-type flask, identified with the collection date and number, and frozen for analysis at the UFRGS's Institute of Food Science and Technology (ICTA). Fatty acids in the milk samples were quantified using gas chromatography with a flame ionization detector (GC-FID Model GC 2010, Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column (Rtx-Wax, Restek, 30 m × 0.25 mm × 0.25 µm), in accordance with the AOCS[18]. The samples were previously esterified to allow analysis by GC-FID. Fatty acids were identified by comparing the retention time of the components with a standard FAME mix fatty acid reference mixture (Supelco 37 Component FAME Mix; Supelco Inc., Bellefonte, PA, USA).

2.7 Statistical analysis

The data referring to milk quality monitoring, with the appropriate sample identifications, were organized in 2013 Microsoft Excel spreadsheets for the means and standard deviations to be obtained, and for tables to be prepared. The results were subjected to evaluation in the RStudio 3.4 software by analysis of variance (ANOVA), in which a completely randomized design (CRD) was used with repeated measures over time and with the buffaloes as the experimental unit. Following the ANOVA, Tukey's test was applied with a significance level of 5%, as a way of comparing the results obtained.

3. Results

3.1 Experimental buffalo herd

The milking management of the buffaloes was carried out between April and December 2021, totaling eight months of lactation. The samples, in a total of ten collections, were received in the respective laboratories within 48 hours, with an average temperature of 6.16 ± 2.30°C. As it is an experimental herd in its first year in the milking system, five multiparous buffalo females were milked during the period, identified as buffaloes A, B, C, D and E.

3.2 Somatic cell count (SCC) and centesimal composition analysis

The results for SCC, protein, fat, lactose, NFS, TS, as well as the average amount of raw buffalo milk produced by the EEA-UFRGS herd are presented in Table 1. The average SCC of the herd was 4.88 x10^5 cells/mL, varying between 6.7 x10^4 cells/mL and 9.8 x10^5 cells/mL, with the lowest value being obtained at the beginning of lactation. It was possible to observe that there was a statistically significant difference during the evaluated period for fat, protein, NFS, TS, and production, with the best means being found in October. Only lactose remained unchanged throughout lactation, with a mean of 5.13 g/100g.

The mean for fat content of the buffalo milk produced by the experimental herd was 3.71 g/100 g, and the fat content varied between 1.71 g/100 g and 5.32 g/100 g. As for protein, the mean in the evaluated period was 4.06 g/100 g, varying between 3.45 g/100 g and 4.68 g/100 g. TS ranged from 11.54 g/100g to 15.84 g/100g, with a mean of 13.72 g/100g; while for NFS, in this study, the mean was 10.00 g/100g, with values ranging from

Table 1. Monthly means referring to individual analyses of five buffaloes for somatic cell count (SCC), centesimal composition, and buffalo milk production at EEA/UFRGS in the year 2021

Analysis	June	July	August	September	October	November	Mean
SCC (x 10^3 cells/mL)	6.68 ^{f} ± 15.36	60.43 ^{b} ± 101.99	44.97 ^{d} ± 87.29	98.28 ^{a} ± 139.67	47.28 ^{c} ± 62.56	35.62 ^{e} ± 48.06	48.87 ± 29.89
Fat (g/100 g)	2.09 ^{c} ± 1.07	3.09 ^{abc} ± 1.38	3.04 ^{bc} ± 1.17	3.91 ^{abc} ± 2.59	5.32 ^{a} ± 2.75	4.84 ^{ab} ± 3.31	3.71 ± 1.24
Protein (g/100 g)	3.71 ^{bc} ± 0.33	3.51 ^{c} ± 0.42	3.82 ^{bc} ± 0.30	4.00 ^{b} ± 0.20	4.68 ^{a} ± 0.27	4.64 ^{a} ± 0.37	4.06 ± 0.49
Lactose (g/100 g)	5.42 ^{a} ± 0.15	5.00 ^{a} ± 0.54	5.23 ^{a} ± 0.16	5.06 ^{a} ± 0.35	5.04 ^{a} ± 0.15	5.06 ^{a} ± 0.24	5.13 ± 0.15
NFS (g/100 g)	9.94 ^{ab} ± 0.37	9.4 ^{b} ± 0.65	9.87 ^{ab} ± 0.25	9.87 ^{ab} ± 0.29	10.51 ^{a} ± 0.22	10.42 ^{a} ± 0.15	10.00 ± 0.41
TS (g/100 g)	12.03 ^{c} ± 1.28	12.49 ^{c} ± 1.59	12.91 ^{c} ± 1.29	13.79 ^{bc} ± 2.53	15.84 ^{a} ± 2.78	15.26 ^{ab} ± 3.42	13.72 ± 1.57
Production (kg/animal)	4.00 ^{bc} ± 0.70	3.43 ^{c} ± 0.29	3.80 ^{bc} ± 0.52	5.05 ^{ab} ± 0.67	5.97 a ± 0.72	4.94 ^{ab} ± 0.60	4.53 ± 0.95

SCC: somatic cell count. TS: total solids. NFS: non-fat solids. *Equal letters on the same line indicate absence of statistical difference ($p<0.05$).

9.09 g/100g to 10.51 g/100g. The lowest mean production per buffalo occurred in July, with 3.43 kg of milk per animal at milking, and peak production occurred in October, with 5.97 kg of milk per animal.

The means for individual centesimal composition and milk composition of the buffaloes are described in Table 2. It was possible to observe that buffaloes A and D had higher SCC and showed a significant reduction in milk components, such as fat and total solids, in addition to significantly lower production compared to the other buffaloes.

Table 2. Means obtained in the analyses of individual samples of milk from five buffaloes (A, B, C, D and E) during lactation for SCC, centesimal composition, and milk production

Analysis	A	B	C	D	E
SCC (x 104 cells/mL)	124.36 ^{a} ± 141.73	12.97 ^{b} ± 31.11	3.39 ^{b} ± 4.65	77.42 ^{ab} ± 52.78	29.76 ^{ab} ± 110.03
Fat (g/100 g)	3.15 ^{abc} ± 1.28	5.81 ^{a} ± 2.54	5.06 ^{ab} ± 1.99	1.82 ^{c} ± 1.34	2.49 ^{bc} ± 1.58
Protein (g/100 g)	3.91 ^{a} ± 0.48	4.10 ^{a} ± 0.62	4.38 ^{a} ± 0.52	3.89 ^{a} ± 0.53	3.91 ^{a} ± 0.42
Lactose (g/100 g)	5.23 ^{a} ± 0.10	4.92 ^{a} ± 0.42	5.20 ^{a} ± 0.26	5.12 ^{a} ± 0.26	5.13 ^{a} ± 0.45
NFS (g/100 g)	10.01 ^{a} ± 0.45	9.86 ^{a} ± 0.73	10.33 ^{a} ± 0.29	9.82 ^{a} ± 0.57	9.87 ^{a} ± 0.36
TS (g/100 g)	13.17 ^{ab} ± 1.64	15.68 ^{a} ± 2.92	15.40 ^{a} ± 2.17	11.65 ^{b} ± 1.33	12.37 ^{ab} ± 1.64
Production (kg milk/ animal)	3.42 ^{b} ± 0.85	5.79 ^{a} ± 1.11	5.82 ^{a} ± 1.44	3.21 ^{b} ± 1.25	3.85 ^{a} ± 1.21

SCC: somatic cell count. ST: total solids. NFS: non-fat solids. *Equal letters on the same line indicate absence of statistical difference ($p<0.05$)

3.3 Physicochemical and microbiological parameters

Table 3 describes results for the density, cryoscopic index (CI), and titratable acidity of the buffalo milk. Just as for centesimal composition, the results showed a significant difference between the months of August and September, with an increase in acidity and density.

Table 3. Monthly means referring to the physicochemical and Standard Plate Count results of the buffalo milk produced at the Experimental Station in the year 2021

Analysis	June	July	August	September	October	November
Density (g/cm³)	1,039 ^{a}	1,035 ^{d}	1,035 ^{cd}	1,037 ^{bc}	1,037 ^{ab}	1,037 ^{ab}
Acidity (g lactic acid/100 mL)	0.13 ^{b}	0.14 ^{b}	0.15 ^{ab}	0.16 ^{ab}	0.18 ^{a}	0.18 ^{a}
Cryoscopic Index (°C)	-0.527 ^{ab}	-0.524 ^{b}	-0.523 ^{b}	-0.528 ^{ab}	-0.536 ^{a}	-0.533 ^{ab}
SPC (x 103 CFU/mL)	<8 ^{b}	19 ^{b}	139.5 ^{a}	9.5 ^{b}	17 ^{b}	<8 ^{b}

SPC: standard plate count. *Equal letters on the same line indicate absence of statistical difference ($p<0.05$).

The mean obtained for density in the milk produced at the Experimental Station was 1,037 g/cm³, ranging from 1,035 g/cm³ to 1,039 g/cm³. For titratable acidity, it was 0.16 g lactic acid/100 mL, ranging from 0.13 g lactic acid/100 mL to 0.18 g lactic acid/100 mL; it was possible to observe an increase in titratable acidity when the milk has its other components increased, whereas for CI, the mean was -0.528ºC, ranging from 0.523ºC to -0.536ºC. Regarding the microbiological quality of milking, the mean for SPC was 3.35 x 10^4 CFU/mL, with the highest value obtained in August collections, 1.39 x10^5 CFU/mL.

3.4 Antibiotic and antiparasitic residue analysis

In the buffalo milk samples analyzed, none of the 46 antibiotic or antiparasitic residues searched were identified.

3.5 Buffalo milk fatty acid profile

The results presented in this study provide a nutritional assessment of the FFA profile of the buffalo milk produced at EEA-UFRGS through gas chromatography analysis. Their concentrations were expressed in mg/100 mg of fat, as reported in Table 4 on the results for Free Fatty Acids (FFA).

Considering the FFA analyzed, it was possible to observe a predominance of Palmitic acid (C16:0) and Oleic acid (C18:1- cis (n9)) in almost all samples. In these parameters, the second collection in June had the lowest FFA content (<40 mg/100 mg of fat) and the lowest frequency (16 of the 26 FFA analyzed), whereas the first collection in September was the most abundant (83.5 mg/100 mg of fat) and the most frequent (26 of the 26 FFA analyzed). There was an increase in the proportion of

polyunsaturated fatty acids and a decrease in monounsaturated fatty acids between September and October, whereas the highest amount of saturated fatty acids is found in September collections. It was observed that the second collection in June, despite being the one with the lowest frequency of FFA, had the highest proportion of saturated fatty acids (29.0 out of 39.9 mg of fatty acid per 100 mg of fat).

Table 4. Identification of the free fatty acid (FFA) profile of the buffalo milk from the Experimental Station herd in the year 2021

Fatty acids (mg FFA/100 mg fat)		June	June	July	July	August	August	September	September	October	November
Caproic acid	C10:0	0.268	0	0.312	0	0	0.333	0	0	0.236	0.122
Lauric acid	C12:0	0.802	0.475	1.136	0.89	0.710	0.759	0.849	1.295	0.946	0.67
Tridecylic acid	C13:0	0.406	3.36	1.856	2.243	4.006	0.674	2.935	2.584	3.003	1.102
Myristic acid	C14:0	4.816	4.075	5.26	4.143	4.665	4.664	3.586	6.817	4.349	5.035
Myristoleic Acid	C14:1	0.218	0.183	0.322	0.25	0.516	0.471	0.397	0.428	0.519	0.232
Pentadecanoic acid	C15:0	0.431	0.447	0.511	0.404	0.668	0.487	0.353	0.653	0.449	0.495
	C15:1	0.104	0.074	0.076	0.079	0.118	0.082	0.063	0.117	0.092	0.115
Palmitic acid	C16:0	18.031	16.302	17.294	14.718	25.653	16.662	10.107	21.525	16.857	25.134
Palmitoleic acid	C16:1	0.82	0.624	0.845	0.795	1.202	0.975	1.328	1.049	1.204	0.892
Margaric acid	C17:0	0.328	0.335	0.29	0.254	0.497	0.209	0.174	0.285	0.294	0.359
	C17:1	0.899	0.485	0.244	0.324	0.199	0.085	0.329	0.371	0.314	0.094
Stearic acid	C18:0	3.851	4.042	3.487	2.952	9.438	2.839	3.401	6.025	3.645	5.466
Oleic acid	C18:1- cis (n9)	11.844	7.846	9.659	8.545	16.134	10.462	7.089	12.61	8.688	13.455
Elaidic acid	C18:1- trans (n9)	1.029	0.768	0.866	0.719	2.770	0.115	0.951	1.768	1.211	1.287
Linoleic acid	C18:2 - cis (n6)	0.435	0.299	0.432	0.382	0.254	1.06	2.364	0.63	1.408	0.671
Linolelaidic acid	C18:2 - trans (n6)	0.107	0	0.095	0	0.721	0.359	0.217	0.242	0.335	0.129
α-Linolenic acid	C18:3 (n3)	0.243	0.089	0.244	0.17	0.332	0.229	0	0.363	0.137	0.222
γ-Linolenic acid	C18:3 (n6)	0.36	0.435	0.362	0.306	0.299	0.558	0	0	0.427	0.435
Arachidic acid	C20:0	0.051	0	0.053	0	0.170	0.094	0	0	0	0
	C20:2 (n6)	0.188	0	0.276	1.584	0.067	8.105	11.668	0.155	5.826	0.739
	C20:3 (n3)	0	0	0	0	0.076	0.458	0.488	0	0.261	0
Dihomo-γ-linolenic acid	C20:3 (n6)	0	0	0	0	0.059	0.397	0.634	0	0.495	0
Arachidonic acid	C20:4 (n6)	0	0	0	0	0	0.889	1.043	2.63	0.459	0
Heneicosilic acid	C21:0	0	0	0	0	0	0.286	0.215	0	0	0
	C22:2	0.601	0	0.741	4.726	0.141	30.915	23.726	0.38	12.496	0
Tricosanoic acid	C23:0	0	0	0	0	0.092	1.365	1.234	0	0.602	0
Total (mg FFA/100 mg fat)	45.83	39.839	44.361	43.485	56.657	69.234	83.534	73.15	59.928	64.253	

4. Discussion

Although some characteristics referring to the composition of buffalo milk produced at the UFRGS's Experimental Station have shown more variability compared to data found in the literature, it is known that many of these buffalo herds are established for a longer time and at a more advanced stage of productive selection, thus enabling the finding of more stable composition results. However, the data obtained in this study, through the implementation of the milking system and with the adoption of corrective measures, aimed at establishing the production of this buffalo herd, supporting the development of further research, as well as the production of derivatives. Additionally, Brazil does not have specific federal legislation on buffalo milk;

therefore, the provisions of Normative Instruction No. 76, November 26, 2018[7], of the Ministry of Agriculture, Livestock and Supply (MAPA), and only the State of São Paulo has specific limits for buffalo milk, set forth in Resolution SAA – 03[19].

One of the parameters for evaluating the microbiological quality of milk is SCC, which can be high in cases of mastitis, an inflammation of the mammary gland that can also bring about other changes in milk composition, such as fat, protein and lactose, leading to a reduction of these components[20]. With the exception of the months of July and September, in the other months, the results obtained for SCC were below that established by NI 76 – 5.0 x 10^5 cells/mL[7]. However, they were higher than that reported by Godinho et al.[4] for buffalo milk in Rio Grande do Sul, as well as the values observed in previous studies in Brazil[21, 22]. According to a study carried out with herds of dairy buffaloes in Italy, a SCC of 2.0 x 10^5 cells/mL should be used as a cutoff point for subclinical mastitis, as it can be associated with changes in milk composition, having a direct influence on the production of derivatives[23]. The same was observed by Medeiros et al.[24], who reported that samples that resulted positive in microbiological tests showed SCC above 2.80 x 10^5 cells/ml. It should be noted that this increase in SCC during the evaluated period was due to buffaloes A and D, which had significantly higher counts than the others and showed a significant reduction in milk components, such as fat and total solids, in addition to low production compared to buffaloes B, C and E, which were under the same conditions. Both buffaloes were at the same stage of lactation as the others (B, C and E); however, the average age of buffaloes A and D was 18 years, whereas the average age of buffaloes B, C and E was 6 years.

The fat values obtained in the first collections, ranging from 2.09 to 3.09 g/100 g, were below both the requirements in NI 76[7] for raw milk and in Resolution SAA – 03[19] for buffalo milk. The fat values were also lower than those reported in studies conducted with buffalo herds in Italy[22] and India[25]. Protein values were similar to those from the above studies. NFS content represents part of the residue obtained after the complete evaporation of water from the milk, such as proteins, lactose and minerals, and is considered as a parameter in indicating the quality of the milk; in their turn, for including fat content, TS are used as an parameter that indicates the yield in the making of cheese, yogurt and other derivatives[25]. The means of NFS and TS were very close to those in other studies carried out with buffalo milk[24, 26, 27]. For the average production, it was possible to observe a significant difference in the production levels, mainly from the month of October, in which the animals left the native field (NF) and had access to pasture cultivated with oats and ryegrass, which have better quality fiber compared to the NF, presenting peak production at six months of lactation, on average. In the months of October and November, when the highest means for milk composition were obtained, the results were similar to those reported for buffalo milk produced in the State of Rio Grande do Sul[4] and in other States of Brazil[21,27], as well as higher than those reported by Pignata et al.[3] for Bahia. In that same period, the composition of the milk was adequate to the values established by NI 76[7] for raw bovine milk, and by SAA – 03[19] for raw buffalo milk. This improvement in milk quality was a consequence of corrective actions, such as changing the main source of fiber in the buffaloes' diet, increasing the supply of silage, and establishing a milking routine, which were determined after the results of the first collections were received. Adjustments were made regarding the supply of food to the herd through fiber supplementation, with an increase in silage supply from 0.9% to 1.8% of live weight, at a higher weekly frequency, which, together with access to pasture cultivated with black oats and ryegrass from the month of September, made it possible to obtain an improvement in the quality of the milk.

In the assessments of buffalo milk at EEA-UFRGS, physicochemical parameters were evaluated with analyses covering the density, CI and acidity of the mixed milk. An increase in milk density was observed with the increase in milk fat and total solids, and it was higher than the limits set by current legislation for bovine milk, and even higher than the limit established by specific state legislation on buffalo milk[19]. However, the values obtained are in line with what is observed in the literature for buffalo milk[4], which presents this high density due to the conformation of its fat globules, which are larger than those of cow's milk, characterized by having greater density[5]. The buffalo milk produced by the experimental herd showed acidity and CI values in accordance with those established by the legislation for raw bovine milk[7], with the exception of the mean of the first month, which was below the normative instruction. It was possible to observe an increase in acidity when the milk had its other components increased, reinforcing the correlation found between acidity and NFS content in buffalo milk by Hofi et al.[29]. The CI, which is the measurement of the freezing point of milk in relation to that of water, is a test used in the industry to detect whether milk has been tampered with by water addition[30]. The mean obtained for CI of the milk produced at the Experimental Station was similar to the results found for herds in Italy[31], and is in accordance with what is required by legislation for raw milk[7].

The SPC remained below the limit of 3.0 x 105 CFU/mL set by NI 76[7] throughout the period evaluated in this study. These results show a good hygienic quality in the manipulation and cleaning of the materials, and are indicative of a positive effect of the adequacy to the

protocols of Good Agricultural Practices in milking. The maximum residue limits (MRL) for active pharmaceutical ingredients (API) of veterinary drugs in food of animal origin are established by Normative Instruction No. 51 of December 19, 2019[32], which requires a periodic search of residues in milk, conducted in laboratories of the National Network of Agricultural Laboratories, which is composed of the Federal Laboratories of Agricultural Defense (LFDAs) and of laboratories accredited by MAPA. In this study, it was possible to observe that the results were in conformity, as residues of the active principles searched were not detected.

Because the FFA profile of ruminant milk is influenced by diet, especially grasses and legumes[33, 34], it was possible to correlate the changes observed with the nutrition of the female buffaloes. As the basis of the herd's diet consists of fibers, such as pasture and silage, with an improvement in the nutritional intake of the herd from these sources, there was an increase in the content and frequency of FFA between the June and September samples, as it happened with the other composition parameters of buffalo milk. The results found for C16:0 and C18:1- cis (n9) are in agreement with those found by Ahmad et al.[35] and Gagliostro et al.(36), and the latter also observed that long-chain FFA (>= C18) are frequent in buffalo milk samples. While short-chain FFA contribute to the flavor and taste of dairy products, long-chain FFA are important factors for the technological characteristics of these products. Similar results were not found in the literature for cis-11,14-Eicosadienoic and cis-13,16-Docosadienoic acids, although the presence of these FFA has been documented in plants of the *Limnanthaceae* family; therefore, it is possible that these results originate from diet[37]. Ahmad et al.[35] also reported that the proportion of saturated fatty acids would be more than double that of unsaturated fatty acids (70.8 and 29.2%, respectively). However, in this study, a similar proportion was found only in collection 2 (72.9 and 27%). In the September collections, there was even a prevalence of polyunsaturated fatty acids. Unsaturated fatty acids are extremely relevant for human health, mainly due to their role in reducing total cholesterol and low molecular weight lipoproteins without prejudice to high density lipoproteins[38].

5. Conclusion

In this research, a buffalo milk production system was implemented at the Experimental Agronomic Station of the Federal University of Rio Grande do Sul (EEA-UFRGS). From the adoption of a milking routine and food supply, satisfactory milk quality results were obtained. The management of production data enabled an increase in milk components, such as total solids, complying with current legislation for raw bovine and buffalo milk, as well as the consulted bibliography on buffalo milk. Monitoring the identity and quality standards of buffalo milk produced at the Experimental Station of the Federal University of Rio Grande do Sul supports the possibility of establishing a herd with more effective control, with the perspective of expanding the experimental herd and standardizing the animals, in addition to enabling the production of derivatives.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

Conceptualization: V. L. Di Domenico and A. de S. da Motta; *Investigation*: V. L. Di Domenico, A. R. Paiva, L. J. and A. de S. da Motta; *Methodology*: V. L. Di Domenico and A. de S. da Motta; *Data curation*: V. L. Di Domenico, A. R. Paiva, L. Jank, C. A. Tomaszewski and A. de S. da Motta; *Supervision*: Amanda de Souza da Motta. *Writing (original draft)*: V. L. Di Domenico, A. R. Paiva and A. de S. da Motta; *Writing (revision and editing)*: Vitória Leite Di Domenico and Amanda de Souza da Motta.

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