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Influence of collection interval, abstraction volume and seasonality on milk quality in the region of Lavras, MG

Influência do intervalo de coleta, volume de captação e sazonalidade na qualidade do leite na região de Lavras – MG

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Abstract: The objective of this study was to determine the influence of production volume, collection interval and time of year on the composition and quality of refrigerated raw milk in the region of Lavras, MG. This study collected information from milk quality monitoring performed by dairies as required by the Federal Inspection Service (SIF). For this study, the farms were classified by milk production per farm (G): 0-200 L/day, 201-500 L/day, 501-1000 L/day, 1001-2000 L/day, and greater than 2000 L/day. The collection intervals were 24 and 48 hours (C), and seasonality (S) was treated as a function of the months of the year (November 2020 to October 2021). The following milk characteristics parameters were analyzed: fat, protein, lactose, total solids (TS), defatted dry extract (DDE), somatic cell count (SCC), urea nitrogen content (UNC), standard plate count (SPC), fat/protein ratio (F/P) and cryoscopic index. There was a seasonality influence for all the parameters studied except for UNC. The production volume per group influenced UNC, TS and SPC. Differences in UNC, protein, lactose, TS, DDE and SPC were associated with the collection interval. There was an interaction effect between seasonality and production volume (SxG) for the parameters SCC, fat and F/P. The findings of this study revealed that the supplier production volume, collection interval, and time of year contribute to variations in chemical composition and milk quality parameters.

Keywords: Milk quality, production, composition.

Resumo - O objetivo desse estudo foi verificar a influência do volume de produção, intervalo entre coletas e época do ano em relação a composição e qualidade do leite cru refrigerado na região de Lavras-MG. O estudo foi conduzido através de coleta de informações a partir do monitoramento da qualidade de leite que é realizada por um laticínio sob inspeção federal – SIF. Para o estudo as fazendas foram classificadas de acordo com as seguintes escalas de captação de leite por propriedade (G): até 200 L/dia, 201-500 L/dia, 501-1000 L/dia, 1001-2.000 L/dia e acima de 2.000 L/dia e; intervalo de coleta de 24h e 48h (C) e a sazonalidade (S) foi considerada em função dos meses do ano (novembro de 2020 a outubro de 2021). Os parâmetros analisados no leite foram: Teor de Gordura, Proteína, Lactose, Sólidos Totais (ST), Extrato Seco Desengordurado (ESD), Contagem de Célula Somática (CCS), Teor de Nitrogênio Ureico (NU), Contagem Padrão em Placa (CPP), Relação Gordura/Proteína (G/P) e Índice Crioscópico.

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Houve influência da Sazonalidade para todos os parâmetros pesquisados, exceto NU. Quanto ao volume captado por grupo, houve efeito sobre o NU, ST e para CPP. Para intervalo de coleta, houve diferença nos parâmetros de NU, Proteína, Lactose, ST, ESD e CPP. Houve interação entre a sazonalidade e volume de produção (SxG) para os parâmetros de CCS, gordura e G/P. O presente estudo revelou que o volume de produção dos fornecedores, intervalo de coleta na propriedade e época do ano contribui para ocorrência de variação nos parâmetros da composição química e qualidade do leite.

Palavras-chave: Qualidade de leite, produção, composição do leite

1. Introduction

Brazil is one of the world´s main milk-producing countries, and among Brazilian states, Minas Gerais occupies a prominent position, accounting for approximately 27% of national milk production [1]. The Lavras region in southern Minas Gerais is notable for its agricultural production, as represented by several products, especially milk and coffee. There are several dairy farms that rely predominantly on family labor in this municipality and in the region. These farms supply milk to for the production of cheese and other dairy products [2, 3].

Small producers generally rely on family labor and animals with diverse genetic backgrounds, and use less technology associated with the milking process. Thus, small dairy farms are highly dependent on the condition and quality of pastures as a food source for herds [4, 5].

In recent years, there have been updates to parameters related to the physicochemical and microbiological composition and quality of raw milk in Brazil based on normative instructions 76 and 77 [6, 7]. These parameters are important for industries that depend on yield and to consumers who expect a certain level of quality in dairy products [8]. Additionally, producers must adapt to meet these standards.

In general, the composition of milk, as well as milk quality indices, can be influenced by rainfall and seasonality [9, 10, 11, 12, 13, 14], farm milk production volume [15, 4, 5, 1, 16] and several other factors, such as the production system and genetic background of the animals [17], hygiene and refrigeration practices, water quality and type of milking system [12]. These parameters are interrelated and must be taken into consideration to maintain the quality standards required by legislation.

Research investigating the influence of various factors on milk quality and composition has yielded variable results, suggesting that these factors may differ by region within Brazil; *i.e.,* certain factors may have varying impacts on milk parameters depending on the location. Thus, t is necessary to conduct studies across different regions of Brazil, considering the diverse climatic, cultural, and social characteristics that influence milk production nationwide [5].

Therefore, the objective of this study was to verify the influence of milk production volume farms, intervals between milk collection, and the time of year on the composition and quality of refrigerated raw milk in the Lavras microregion in southern Minas Gerais, with a focus on the standards described in recently implemented legislation.

2. Materials and Methods

Information was collected during milk quality monitoring performed as part of federal dairy inspections of 144 dairy farms in the region of Lavras in southern Minas Gerais. The locations of the producers in the different cities were mapped using GPS, and the average daily production and the collection interval over a period of one year were recorded. The region of the city of Lavras where the study was conducted is located at approximately 21° 14′ 43 south latitude, 44° 59′ 59 west longitude at an elevation of approximately 919 meters [18]. The climate is characterized as highland tropical, with a short rainy season (November to March) and a long dry season (April to October) [19]. The average annual temperature from November 2020 to October 2021 was 20.4°C, ranging from a low of 15.8°C in July 2021 to a high of 23.2°C in September 2021. The mean annual rainfall in the region was approximately 1,235 mm, with the lowest rainfall occurring in July 2021 and the highest in December 2020 [20].

The farms were classified by production volume according to previous studies conducted by Borges et al. [15] and Marcondes et al. [4]: Group 1 (less than 200 L/day), Group 2 (201 to 500 L/day), Group 3 (501 to 1000 L/day), Group 4 (1001 to 2000 L/day) and Group 5 (above 2000 L/day). For the collection interval, 24 and 48 hours were used, and seasonality was determined as a function of monthly collections for the 12-month interval from November 2020 to October 2021.

The dairy farms were distributed in ten municipalities near the city of Lavras. The municipalities of origin and number of producers per municipality were as follows: Itumirim (n = 39), Lavras (n = 18), Ingaí (n = 20), Itutinga (n = 20), Bom Sucesso (n=17), Nazareno (n=10), Luminárias (n=7), Carrancas (n=6), Conceição da Barra de Minas (n=3), São Tiago (n=3), and Ijaci (n=1).

Milk samples were collected monthly from the expansion tank of each farm and analyzed according to the milk quality monitoring plan, and the parameters analyzed were those included in Normative Instructions n. 76 and n. 77 from 11/26/2018 [6, 7] for refrigerated raw milk. The parameters measured were fat, protein, lactose, total solids (TS), defatted dry extract (DDE), somatic cell count (SCC), milk urea nitrogen content (UNC), standard plate count (SPC), cryoscopic index and fat/protein ratio (F/P).

After homogenization by mechanical agitation, the samples were collected, and the samples were subsequently removed from the cooling tank with a sanitized stainless-steel ladle. The vials for SPC analysis contained the preservative azidiol in tablet form, and the bottles for the analysis of SCC, UNC, and other parameters contained the preservative bronopol in tablet form. All the samples were stored at temperatures between 2°C and 6°C until analysis.

The homogenized samples were sent for composition, SCC, and UNC analyses at the Laboratory of Lactation Physiology, Clínica do Leite, Department of Animal Science, "Luiz de Queiroz" School of Agriculture (ESALQ/USP), Piracicaba, São Paulo. The components (fat, protein, lactose, TS, and DDE) and UNC were analyzed by mid-infrared spectrometry [21].

SCC and SPC were determined by flow cytometry [22, 23]. Cryoscopy of the milk samples was performed using a digital electronic cryoscope.

The effects of production volume (G), collection interval (C), and seasonality (S) were determined using the F test (α=0.05) in the statistical program SPSS® 20.0 to identify associations between the factors studied (SxG and SxC). The variables and/or interaction variables with significant effects in the analysis of variance (F test) were submitted to the Tukey test at significance level of 5% ($p<0.05$).

3. Results and Discussion

The characteristics of the farms included in this study by milk production amount from November 2020 to October 2021 are presented in Table 1. At all the dairy farms included in this study, milking occurred twice a day, and each farm had an expansion tank for storing milk until it was collected by tank trucks. The sample of farms included in this study was representative of dairy farms in other Brazilian regions, with great heterogeneity in milk production and farmers, ranging from small farmers who use dairy production as a complementary activity to agriculture to farmers who specialize in dairy farming [15, 24, 4, 5].

Table 1 **Production parameters, storage capacity, municipalities, and number of producers by milk production and collection interval from November 2020 to October 2021**

Parameters		Milk production (L)				
		≤ 200	201 to 500	501 to 1000	1001 to 2000	> 2000
Milk collected by dairy farmer (L)	Mean	144	323	675	1437	2535
	Minimum	80	205	505	1070	2010
	Maximum	200	500	990	1938	3829
Storage capacity per dairy farmer (L)	Mean	590	904	1471	2548	3450
	Minimum	300	400	700	1500	3000
	Maximum	1600	1900	3000	4600	4500
Number of dairy farmers per municipality	Lavras	6	8	2	2	-
	Itumirim	12	14	9	3	1
	Ijaci	-	-	1	-	-
	Itutinga	3	10	6	1	-
	Ingai	2	6	7	4	1
	Luminarias	1	6	-	-	-
	Carrancas	2	1	2	1	-
	Bom Sucesso	-	2	6	7	2
	Nazareno	-	5	4	1	-
	Conceição da Barra de Minas	-	2	1	-	-
	São Tiago	-	-	2	1	-
Total number of dairy farmers		26	54	40	20	4
Number of farmers per collection interval	24 h	21	38	19	9	1
	48 h	5	16	21	11	3
Volume of milk production per group (L)		3,735	17,447	27,004	28,748	10,139
Indices (%)						
Dairy farmers by category	%	18.06	37.50	27.78	13.89	2.78
Volume of milk production	%	4.29	20.04	31.01	33.02	11.64
Farmers who collect every 24 hours	%	23.86	43.18	21.59	10.23	1.14
Farmers who collect every 48 hours	%	8.93	28.57	37.50	19.64	5.36
Milk collected every 24 hours	%	6.82	26.88	28.58	29.05	8.67
Milk collected every 48 hours	%	1.68	12.98	33.52	37.11	14.71

The properties farthest from the dairy farm were in the municipality of São Tiago and had collection volumes ranging from 501 to 2000 liters (Table 1). This higher production volume makes milk collection feasible for these farms; otherwise, the cost of transportation would be economically unfeasible. The municipalities of Itumirim and Itutinga had the largest number of producers with milk production volumes of up to 500 liters. These properties are near the collection company in the city of Lavras, which enables daily collection. The municipality of Bom Sucesso had the highest concentration of producers with production volumes greater than 1000 liters.

The milk volume per day per farm was similar to production volumes previously observed throughout Brazil, with milk production concentrated among small producers [25, 26]. The milk storage capacity of the producers ranged from 1.36 to 4.10 times their daily milk production. This parameter relevant for farms, as small producers must have sufficient capacity to store milk in refrigeration tanks to comply with the Ministry of Agriculture, Livestock and Food Supply [6, 7] to ensure the microbiological quality of milk and to prevent bacterial development and quality loss in dairy products [27].

In terms of production volume, the current study showed a stratified production structure, i.e., a small proportion of producers accounting for a large proportion of milk production, with most farms producing a small volume, is a characteristic that is also present nationally and in other Brazilian states [28, 4].

The months in which the samples were collected affected all the parameters studied except for UNC (p>0.05). The production volume per group influenced UNC, TS and SPC; and the interval between collections (24 and 48 hours) caused variation in the UNC, protein content, lactose content, TS, DDE and SPC. There was an association of seasonality and production volume with SCC, fat content, and F/P (Table 2).

Table 2 **Results of the analysis of variance for milk composition parameters as a function of month, collection volume, collection interval and their interactions.**

Parameters	Collection interval (C)		Means ±SEM	P value				
	24 h	48 h		Seasonality (S)*	Milk Production (G)**	Collection interval (C)	SxG	SxC
Cryoscopic index (ºh)	538.60±0.17	539.37±0.20	538.87±0.13	<0.001	0.164	0.293	0.518	0.266
Urea nitrogen content (mg/dL)	13.02±0.09	13.85±0.11	13.31±0.07	0.056	0.004	0.024	0.562	0.570
Fat (%)	3.73±0.01	3.79±0.01	3.75±0.01	<0.001	0.080	0.115	0.038	0.429
Protein (%)	3.23±0.01	3.27±0.01	3.24±0.004	<0.001	0.087	0.005	0.343	0.701
Ratio Fat/Protein (F/P)	1.15±0.00	1.16±0.00	1.16±0.002	<0.001	0.241	0.994	0.001	0.639
Lactose (%)	4.46±0.00	4.49±0.01	4.47±0.003	<0.001	0.100	0.015	0.684	0.436
Total Solids (%)	12.35±0.02	12.47±0.02	12.40±0.01	<0.001	0.034	0.007	0.357	0.297
DDE (%)	8.63±0.01	8.69±0.01	8.65±0.01	<0.001	0.068	0.002	0.538	0.370
SPC (CFU/ml)	93.01±10.60	64.74±8.87	83.19±7.54	0.024	0.029	0.017	0.980	0.485
SCC (cell/ml)	536.41±13.22	525.67±18.31	532.84+10.72	<0.001	0.130	0.070	0.023	0.721

Parameters	P Value - S x G											
	2020		2021									
	Nov	Dez	Jan	Fev	Mar	Abr	Mai	Jun	Jul	Ago	Set	Out
SCC (cell/ml)	0.150	0.09	0.606	**0.040**	0.456	**0.044**	**<0.001**	0.126	**0.001**	0.08	**0.004**	0.126
Fat (%)	**0.027**	0.260	0.200	0.287	0.228	0.221	0.821	0.176	0.083	**0.042**	**0.045**	0.078
F/P	0.084	0.900	**0.026**	**0.044**	0.409	0.636	0.934	0.640	0.136	0.096	0.254	0.165

**12 months (November 2020 to October 2021). **Production volume: 200 L; from 201 to 500 L; from 501 to 1000 L; from 1001 to 2000 L and greater than 2000 L. SCC, somatic cell count; SPC, standard plate count; DDE, - defatted dry extract.*

The UNC was influenced by milk production volume, with the lowest values for farms that produced a volume less than 200 liters (11.87 mg/dL of milk) (Figure 1). For the other collection volumes, the average UNC was 13.81 md/dL. Results like those in the current study were observed by Meyer et al. [29], who reported a mean UNC of 13.30 mg/dL in milk. The UNC content in milk is directly related to the amount of protein in the diets of the animals [30, 31]. Thus, the lower production volumes observed herein were associated with greater dependence on pastures, which are the main source of cattle feed and which can vary in quality, with low-quality pastures leading to nutritional deficits, such as low protein values, in animals [4].

Regarding the interval between collections, milk collected every 48 hours had higher UNC than milk collected every 24 hours. According to Leão et al. [32], when diets are rich in protein that can be degraded in the rumen, urea nitrogen peaks approximately one to two hours after feeding. For diets with higher proportions of undegraded protein in the rumen, urea nitrogen peaks occur six to eight hours after feeding. Thus, UNC could be greater in milk expressed in the morning than in milk expressed in the afternoon, which could explain this difference, given that collections occur in the morning, and every 48 hours, there would be a greater volume of milk expressed.

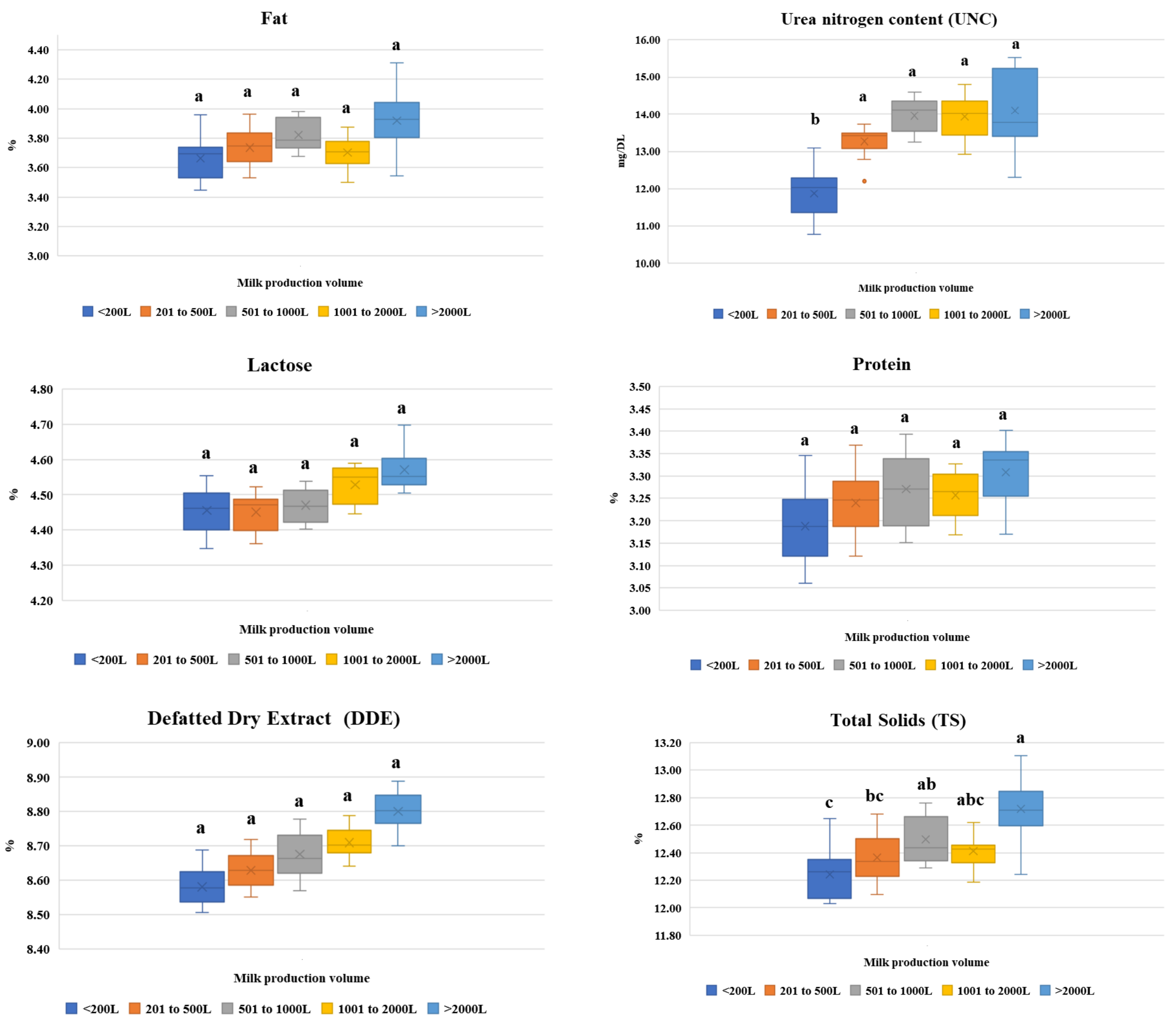

Figure 1 Chemical composition parameters of milk as a function of production volume from November 2020 to October 2021.

The UNC is strongly correlated with the UNC in the plasma or blood, and values considered normal for milk, according to Rosa et al. (33), should be between 11 and 16 mg/dL. In the present study, the UNC in milk ranged from 10.77 to 15.53 mg/dL. Because urea is a neutral molecule, that easily diffuses through membranes, the UNC has been used as an indicator for monitoring nutrition, mainly to determine the adequacy of the protein-energy ratio in the diet of lactating cows (29, 34). High UNC (above 18 mg/dL) may indicate an excess of crude protein in the diet, a low ruminal fermentation rate, or an increased protein:energy ratio (35), while low UNC indicate the opposite.

Compared with milk samples collected every 24 hours, those collected every 48 hours had higher DDE values and lactose and protein concentrations (Table 2). This difference may have been influenced by the farm groups, as farms with higher production volumes (above 500 liters) predominantly had milk collected at 48 hours. The means from these parameters tended to increase numerically as a function of milk volume (Figure 1). Although there were differences in the values for these parameters, they were less than 1% and thus were not directly associated with collection time. Thus, the results do not support changing in the collection interval to improve these milk composition parameters.

The milk from dairy farms with production volumes greater than 2000 liters had higher TS values, with the lowest TS values found for dairy farms with production volumes up to 200 liters (Figure 1). Although the production system in the region is mainly composed of smaller dairy herds with crossbred animals and lower production per cow, there are larger herds of animals with better fitness and higher milk production per cow (5). The TS production showed small variations between the production strata, especially for farms that produced between 201 and 2000 liters of milk. These variations in the TS content for the different production volumes per farm may be related to differences between breeds, as small producers have more crossbred and less specialized herds, and larger producers invest in genetics and animal selection to improve milk composition indices, promoting changes in the genetic pattern and fitness of the animals (26, 17, 36).

The evaluation of bacteria present in the milk based on the SPC showed that the farms with production volumes up to 500 liters had higher counts (mean, 100.09 × 10^3 CFU/mL) than did the farms with production volumes greater than 1001 liters (mean, 36.71 × 10^3 CFU/mL) (Figure 2B). In addition, for farms with production volumes less than 200 liters, there was greater variation in the mean SPC, with values ranging from 39.71 to 207.18 × 10^3 CFU/mL; for farms with production volumes greater than 2000 liters, this variation was lower, with averages ranging from 5.25 to 38.25 × 10^3 CFU/mL. The bacterial count rates were greater for small producers than for medium and large producers due to less hygienic control during milk production (4). Contamination sources and microorganism development during the milking process are the main factors contributing to increases in this parameter (9, 37, 5).

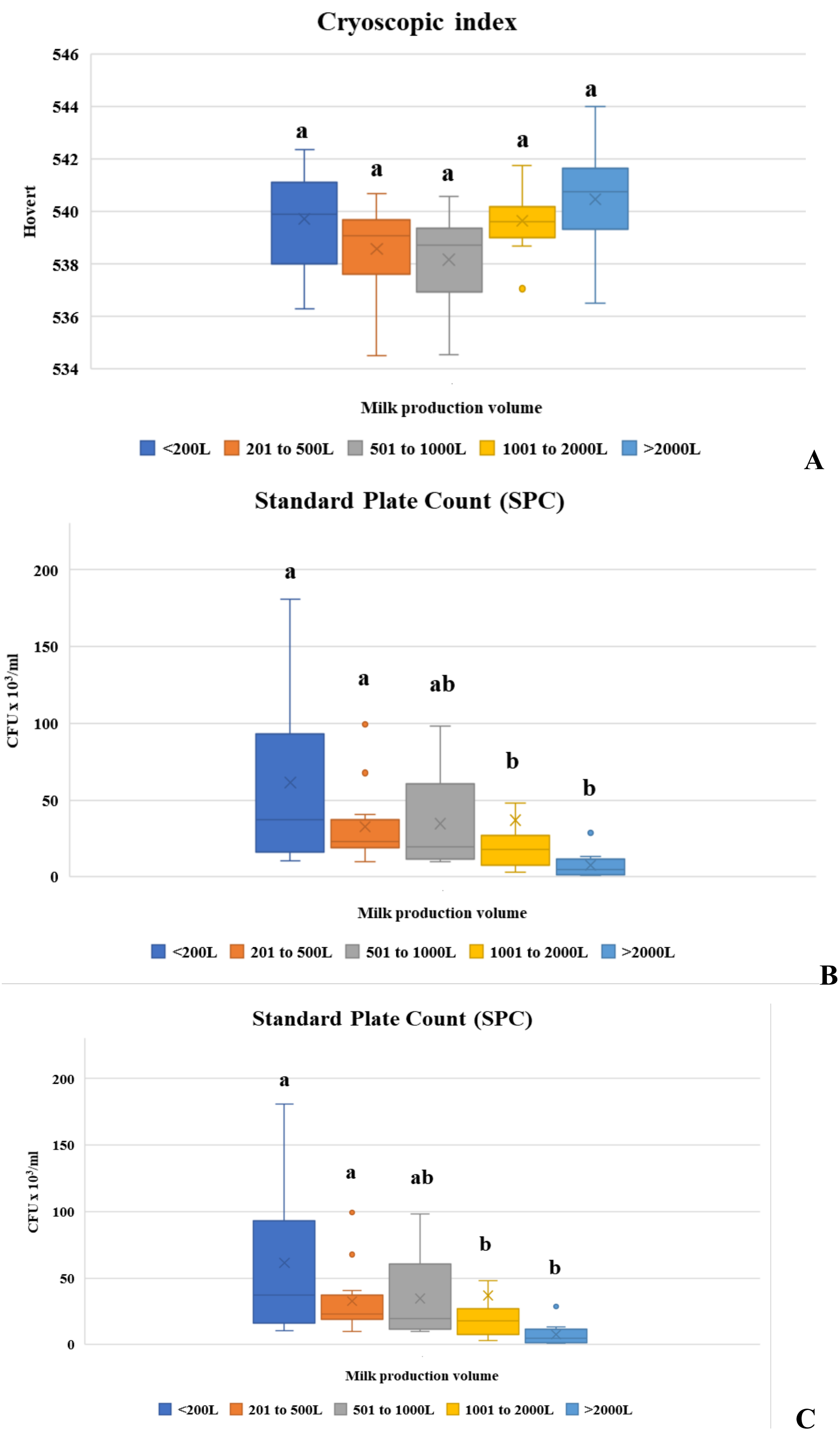

Figure 2 **Physical, chemical, and microbiological parameters of milk as a function of production volume from November 2020 to October 2021.**

Regarding the interval between collections, milk collected every 24 hours had a greater SPC value than milk collected every 48 hours. This result, similar to the trends observed for other variables, may be due to the influence of farms that produced up to 500 liters, which also had higher SPC values (Figure 2). According to the Normative Instruction n. 76 [6] for stored milk on farms, SPC values are generally expected to be below the limit of 300 × 10^3 CFU/mL as a function of production volume. This parameter is crucial for producers because if the geometric mean exceeds this standard for three consecutive months, the farm must temporarily stop milk collection[7]. This may provide an explanation for the results herein being below the microbiological contamination limit recommended by Brazilian legislation. This finding demonstrates that producers are committed to controlling bacterial growth to ensure continued milk production through the adoption of hygiene practices.

There were no differences in SCC among the groups according to milk production volume ($p = 0.129$), with an overall mean of 532.84 × 10^3 cells/mL of milk, indicating that improvements in this parameter are needed at all production levels (Table 2). However, when analyzed by production volume, farms with a volume between 1001 and 2000 liters and a volume greater than 2000 liters met the standard established in the legislation (a maximum of 400 × 10^3 cells/mL of milk), with a mean of 338.81 and 375.65 × 10^3 cells/mL of milk, respectively (Figure 2). Marcondes et al. [4] also reported lower SCC concentrations in milk from farms with production volumes greater than 1000 liters. SCC is directly related to udder health and milk quality and therefore should be monitored constantly because, in addition to animal health [38], it is related to low dairy product yield [39, 9, 8].

The analysis of the influence of seasonality on milk composition showed that lactose content varied from 4.39% to 4.54% throughout the year, with the highest concentrations occurring in November 2020, December 2020, and October 2021 and the lowest in April and May 2021 (Figure 3). Although lactose content in milk is negatively correlated with inflammation in the mammary gland during clinical and subclinical mastitis due to damage in mammary epithelium, other factors, such as dietary composition are closely linked to milk yield and production because the diet provides precursors for the synthesis of milk components [40, 41]. In this study, the lower lactose levels in autumn (March to June) can be associated to a low-energy ratio in the diet, as there is a lack of high-quality forage available during this period. Conversely, in spring (October to December) in southeastern Brazil where Lavras region is located, forage availability and quality improve, positively influencing lactose synthesis.

The highest TS values were observed in May and July 2021, and the lowest TS values were observed in November 2020, December 2020 and October 2021 (Figure 3). Seasonality is one of the main factors that influences milk composition, especially protein, fat and, consequently, TS content. Studies conducted in different regions have shown an increase in these parameters from March to June in São Paulo [13], for the month of June in different cities of the states Mato Grosso do Sul, Paraná and Rio Grande do Sul [42] and for autumn (April to June) and winter (July to September) in the northern region of Rio Grande do Sul [14]. A similar trend was observed in the current study for the months of May and July 2021, with higher TS values in these months. These months occur in the dry season in the region, during which there is a significant reduction in rainfall in addition to a reduction in temperature, which promotes a decrease in forage quality with a consequent increase in the fiber content of diets. These factors contribute to a reduction in the volume of milk produced by animals and influence milk composition, i.e., an increase in fat content [43]. Thus, the effect of seasonality on the chemical composition of milk is related to variations in the availability and quality of food in addition to environmental factors that contribute to the occurrence of thermal stress, especially in the summer. These factors primarily affect small farms whose production depends on climatic conditions and pastures [4, 14].

The increase in the content of solids in raw milk is of extreme importance for the dairy industry because this parameter is related to an increase in dairy product yield and thus is crucial to meeting consumer needs. Industries therefore stipulate criteria related to this factor for bonuses and remuneration for milk suppliers to increase competition in the market [14, 5]. These criteria are associated with greater concern for producers improving their products to increase their profitability through bonuses.

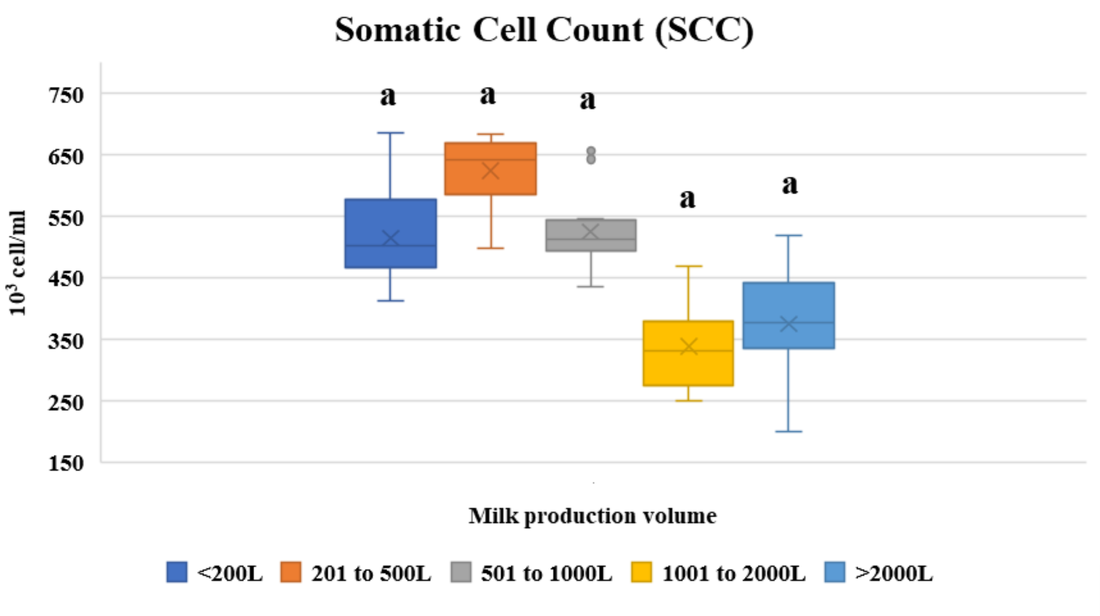

Figure 3 **Variation in lactose, protein, total solids, and defatted dry extract (DDE) in milk by month of the year (November 2020 to October 2021).**

DDE comprises the solid components of milk, excluding fat, and according to the Normative Instruction 76, the minimum required DDE value for raw milk is 8.4% [6]. In this study, the means exceeded 8.4%, with variation across months of the year and higher percentages in May and July 2021 (Figure 3). Large variations in DDE are linked to changes in protein and/or lactose content throughout the year. These parameters vary due to the feeding management of the animals and increasing nutrient availability for lactating animals [33]. During the autumn and winter months in Brazil (March to August), most farmers increase the concentrate content in cow's diet to compensate for the lower quality of pasture due to reduced rainfall during this period [16]. Increasing the concentrate content in the diet promotes the production of propionic acid in the rumen, which can spare amino acids for milk protein production in the mammary gland, thereby increasing the protein content in the milk [44]. Conversely, in spring and summer, the quality of forage improves, and farmers increase the forage content in the diet of cows, which contributes to the synthesis of lactose and increases its percentage in the milk. Thus, dairy farmers should consider adjusting the animal´s diet throughout the year to avoid deficits that can influence milk parameters ensure adequate milk yield in compliance with legislation and industry standards.

There was a difference in fat content among milk production volumes in November 2020, and January, August, and September 2021 (Figure 4). The variation in milk fat content was greater for farms with milk production greater than 2000 liters. This variation may be due to dietary adaptations aimed at improving animal production indices and milk composition parameters associated with bonuses. Nutrition and genetics are important factors for determining the fat composition of milk [4].

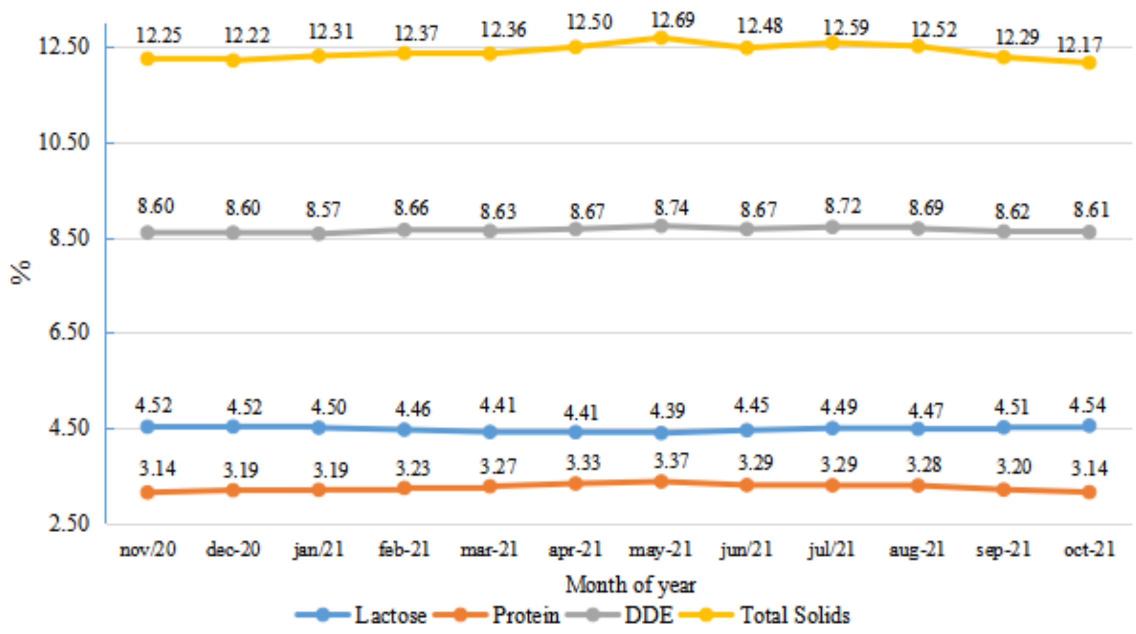

Figure 4 **Milk fat content as a function of production volume (≤200 L; 201 to 500 L; 501 to 1000 L; 1001 to 2000 L; and >2000 L) and month of the year (November 2020 to October 2021).**

There was a difference in the Fat/Protein (F/P) ratio as a function of milk production volume for the months of January and February 2021. The F/P value was highest in January for the farms with milk production greater than 2000 liters; in February, lower rates were observed for farms with milk production between 1001 and 2000 liters (Figure 5).

The relationship between milk fat and protein content is frequently used to evaluate the nutritional status of a herd and to detect metabolic disorders. Variations in these results indicate an imbalance of ingredients in the diet, where low F/P values indicate an excess of nonfibrous carbohydrates in the diet (high concentrate: forage ratio) and an increased risk of acidosis. Increases in the F/P ratio may be due to low protein content resulting from protein or energy deficiency in the diet or from a high-fat content, which consequently increases the risk of obesity and ketosis in a herd [45]. In the analyzed milk samples, there was variation in the F/P ratio across months of the years, with higher values in May and July 2021 and lower values in December 2020 and October 2021. However, this difference did not exceed 5%, indicating that animals from different dairy farms tend to have a balanced diet. Similar results to those found in the current study were reported by Borges et al. [15] in the Central and West Minas mesoregions in Minas Gerais (mean: 1.20).

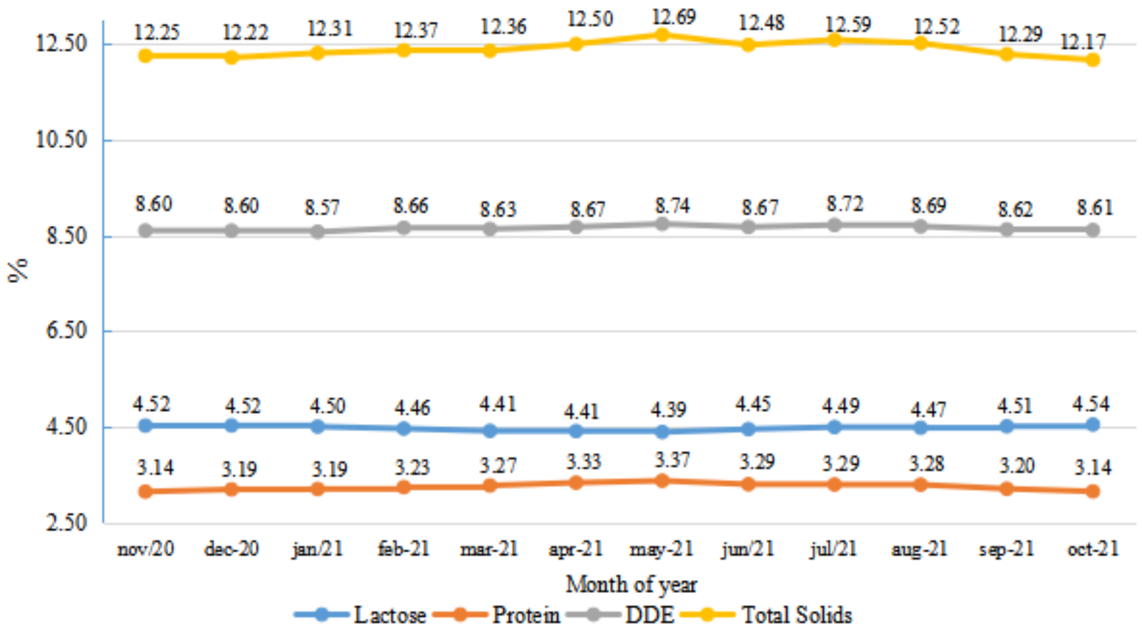

Figure 5 **Fat/protein (F/P) ratio of milk as a function of production volume (≤200 L; 201 to 500 L; 501 to 1000 L; 1001 to 2000 L; and >2000 L) and month of the year (November 2020 to October 2021).**

These variations in F/P for different production volumes and seasons may be related to the differences in the genetic characteristics of the animals and their production system. Smaller dairy farmers typically have more crossbreed herds and primarily feed them pasture in extensive production system. In contrast, larger producers invest in high-yielding cows and raise them on silage in confined system [36]. Consequently, small farmers tend to have more robust animals suited to local climatic conditions, with lower milk production volumes and metabolic demands compared to those of larger farms. As milk production aptitude in cows increases, there is a greater need for a balanced diet and adequate thermal conditions to prevent metabolic stress, which can affect energy balance and the metabolic processes of gluconeogenesis and lipolysis in cows under heat stress [46]. The significant variation in the F/P ratio observed in the group of milk production over 2000 liters in this study may be related to this factor, highlighting the importance of monitoring the conditions under which animals are raised, in addition to this parameter.

The cryoscopic indices varied by month of the year, with the lowest values occurring in January and April 2021 and the highest occurring in September 2021 (Figure 6). Considering the parameters stipulated in the legislation [6, 7], the results were considered normal for refrigerated raw milk, i.e., from -0.530°H to -0.555°H. Although a difference was found, it was not considered relevant because the cryoscopic index is used to detect milk fraud, i.e., the addition of water. Although the addition of water to milk is a common adulteration practice, the freezing point of milk can also be influenced by breed, successive lactation periods, lactation stage, geographic region, and seasonality [47, 36].

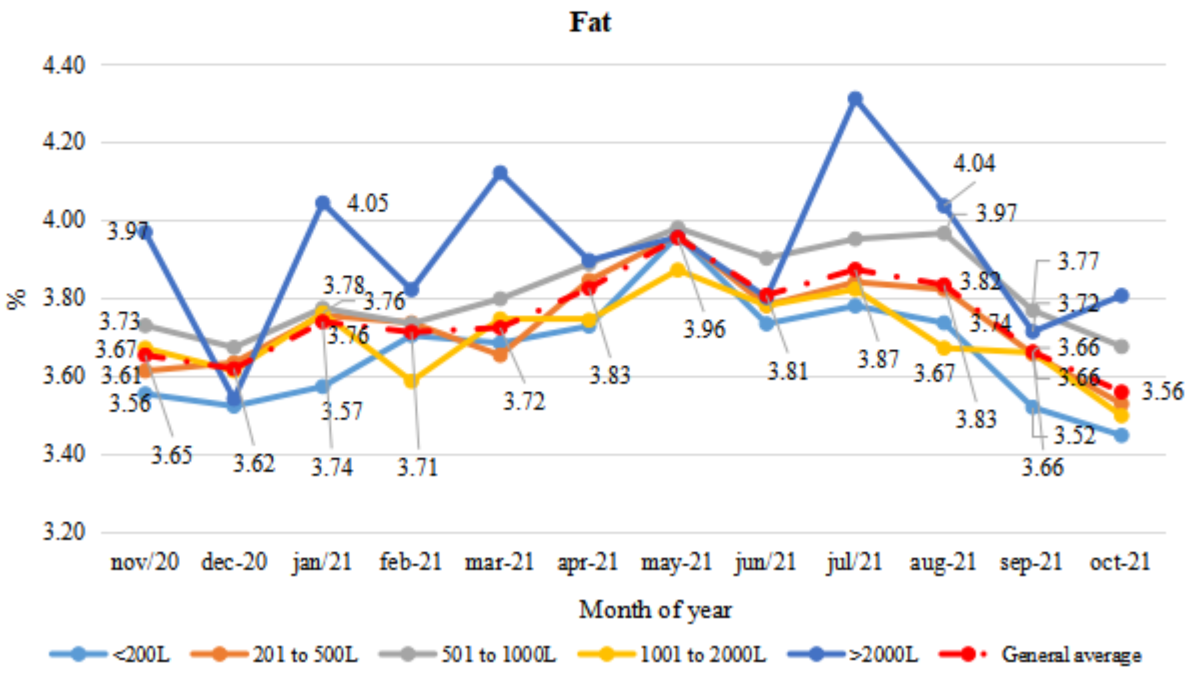

Figure 6 **Variation in cryoscopy index and standard plate count (SPC) results for milk by month of the year (November 2020 to October 2021).**

The SPC results varied across the months of the year, with the highest values occurring in December 2020, March 2021, and October 2021. Differences in the SPC may be related to problems with facility or equipment or utensil hygiene and, to a greater extent, the influence of water, e.g., the seasonality of rainfall. During the study period, higher rainfall rates were observed for the region in the months [48], potentially contributing significantly to the increased challenges in maintaining sanitary practices. The effect of seasonality has also been reported by other authors, who found greater contamination in periods of greater rainfall [16]. Although the averages herein were below the limits established by legislation, the results could be improved by training producers on the need to adopt better hygienic milking practices throughout the year and reducing the influence of external factors that serve as the main points of contamination throughout the year.

There was a difference in the SCC of the samples collected in the different months of the year by milk production volume for February, April, May, July, and September 2021 (Figure 7). A higher average SCC wase observed for properties with lower production volumes (up to 1000 liters). This result may indicate a greater occurrence of subclinical mastitis in animals at farms with these production volumes as well as possible deficiencies in milking, storage and cleaning management, which also contribute to increases in the SCC; thus, better technical protocols are needed [15].

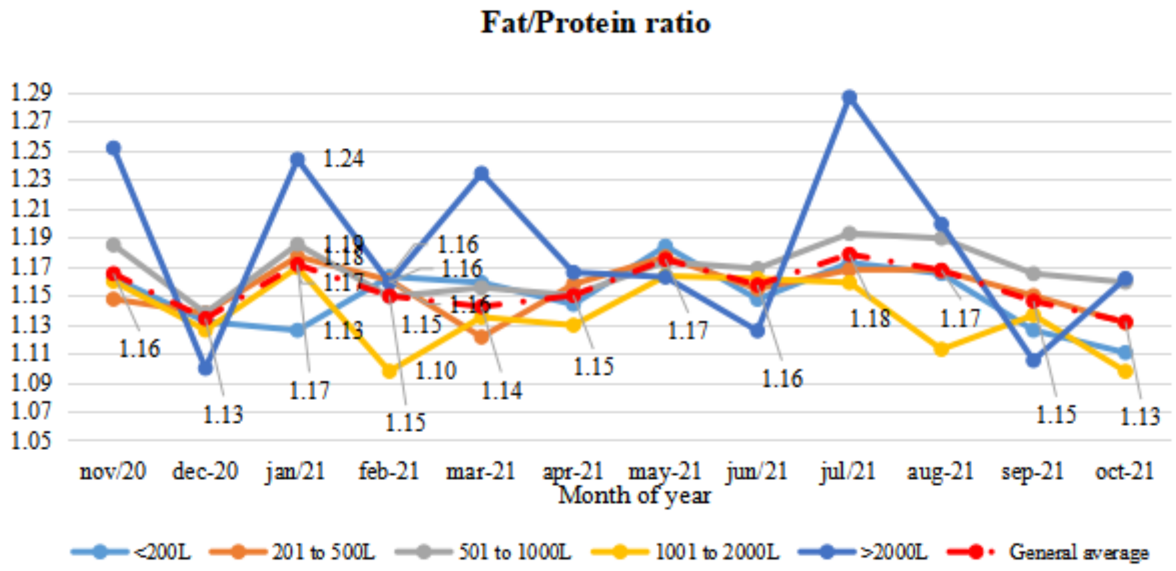

Figure 7 **Somatic cell count (SCC) in milk as a function of production volume (≤200 L; 201 to 500 L; 501 to 1000 L; 1001 to 2000 L; and >2000 L) and month of the year (November 2020 to October 2021).**

There was a tend of increase in the SCC between November 2020 and March 2021, with a decrease and stabilization occurring between April 2021 and July 2021 and a decrease occurring between August 2021 and October 2021 (Figure 7). The SCC results differed by production volume. A similar trend associated with seasonality was reported by Magalhães et al. [47] for herds in the state of São Paulo, with lower SCC in the winter (June to August) and higher SCCs in the summer (January to March). These results are related to the increase in summer stress due to higher temperatures and humidity, which may increase susceptibility to infections as well as increase the number of pathogens to which cows are exposed. The incidence of mastitis is greater at times of higher rainfall and temperature due to the accumulation of organic matter in the environment, thus favoring the proliferation of infectious microorganisms and resulting in an increased SCC in milk stored in expansion tanks [12, 16].

The values for all the milk composition parameters, except for the SCC, were within the standard values for quality stipulated in Normative Instruction 76 [6] (Figure 7). The studied factors affect milk quality, and although the values of most of the parameters were within the standards stipulated by Brazilian legislation, the finding that the SCC did not meet the standards reinforces the need for greater attention to the control of subclinical mastitis in herds. Seasonality across months of the year and the volume of milk collected at the dairy farms were the factors with the greatest influences on the physicochemical and microbiological parameters of the milk.

4. Conclusion

Except for the UNC content, all the milk parameters analyzed varied by the month of the year. The fat content, fat/protein ratio and SCC varied by production volume and month of the year, with a trend toward a decrease in parameters associated with milk quality and composition at farms with lower production volumes. To ensure compliance with the standards for parameters related to the composition and quality of milk stipulated in Brazilian legislation, greater attention and training in good agricultural practices are still needed to improve the SCC, especially at farms with a production volume of less than 1000 liters.

Conflict of interest declaration

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author contributions

Conceptualization: F. P. de Carvalho and P. B. Faria. Data curation: F. P. de Carvalho. Formal analysis: F. R. P. Bruhn. Methodology: P. B. Faria and F. R. P. Bruhn. Supervision: P. B. Faria. Investigation: F. P. de Carvalho. Writing (original draft): F. P. de Carvalho. Writing (proofreading and editing): F. P. de Carvalho and P. B. Faria

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