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## Abstract

The objective of this study was to evaluate the seasonal influence on the chemical composition, somatic cell count (SCC), and total bacterial count (TBC) of raw bulk-tank milk in northeastern Brazilian states. Data were obtained from milk samples from tanks collected monthly by industries registered with the Federal Inspection Service. According to normative instruction #62 (IN-62), two validity periods were considered. The highest recorded averages for chemical composition were between May and July. The mean fat content varied from 3.51% to 3.69%, and the protein content ranged from 3.07% to 3.17%. The averages of SCC ranged from 4.66 to 4.90 × 1,000 cells/ml, with the highest being recorded in July. At the same time, the TBC ranged from 2.34 to 2.53 cfu/ml. The highest TBC was recorded in March. The mean values of fat, protein, defatted dry extract, SCCs, and TBC were influenced by the months of the year. The means for these variables decreased in periods when Brazilian legislation was more severe. However, the SCC and TBC averages found in this study were still high, considering the quality of raw milk production. SCC and TBC presence still did not comply with the limits established by the legislation.

**Keywords:** Cow milk. Milk composition. Somatic cells count. Total bacterial count.

## 1. Introduction

The physical-chemical characteristics of milk and its composition can vary due to factors such as lactation stage, feeding, animal health status, and genetics. Additionally, seasonal variations influence milk features (Larsen et al. 2010; Bernabucci et al. 2015). Dairy production occurs heterogeneously in all Brazilian regions, especially regarding production systems. The potentials of different animal breeds, with different management, technological levels, and productivity, are explored in milk production (Eurich et al. 2016; Araújo et al. 2018). Grazing is mainly adopted by Brazilian milk production systems regarding animal feed. This type of management represents a necessary means for the nutritional supply of animals to provide constant milk production (Costa et al. 2008).

The National Program for the Improvement of Milk Quality (PNMQL) emerged in the 1990s and is characterized by measures to improve the quality of milk produced in Brazil (Brazil 2002). In 2011, new deadlines and quality parameters for Brazilian milk producers were provided by normative instruction #62

(IN-62) (Brazil 2011). The previous normative instruction #51 (IN-51) was amended by IN-62, and the limits set by IN-62 must be respected by Brazilian milk producers.

Nonetheless, there are weaknesses in the Brazilian raw milk production chain. This fact was made evident as the IN-62 again had its terms extended using a decree for two more years, which was valid from 01/07/2018 for the south, southeast, and central-west regions and 01/07/2019 for the north and northeast regions. Nutritional indicators, such as protein, fat, total, and defatted dry extract, somatic cell count (SCC)—an indicator for controlling mastitis in herds, and total bacterial count (TBC)—an indicator of the hygiene conditions in obtaining milk, are among the primary analyses required by legislation.

Dairy industries registered with the Federal Inspection Service are supervised by MAPA, which aims to guarantee products with sanitary and technological certification for Brazilian consumers, respecting current national and international laws (Dipoa 2017). Additionally, only the reports issued by RBQL laboratories are official for this practice. The Ministry supervises the industries, which, in turn, monitor their milk producers.

An overview of the raw milk quality in some Brazilian regions has been shown by surveys conducted by these laboratories. Machado and Cassoli (2016a), in a diagnosis performed in some states from the south, southeast, and central-west regions, considered the current limit of  $300 \times 1,000$  cfu/ml. This would represent 65% of the producers following the ordinance. However, when the 2018 limit was projected, which was  $100 \times 1,000$  cfu/ml, this percentage declined to 40%. In continuing the diagnosis for SCC, Machado and Cassoli (2016b) found that 62% of Brazilian producers would comply with the current limit of  $500 \times 1,000$  cells/ml. Still, when projected to  $400 \times 1,000$  cells/ml, which would come into force in 2018, only 49% of producers would comply with this rule. Rodrigues et al. (2017) observed that around 40% to 50% of dairy herds in southeastern Brazil would not reach SCC regulatory limits of  $400 \times 1,000$  cells/ml over the next few years. In the northeast region, mean values from all states for samples collected from July 2009 to June 2010 ranged from 472 to  $837 \times 1,000$  cells/ml for SCC and 997 to 1619 million cfu/ml for TBC (Ribeiro Neto et al. 2012).

It is demonstrated by these diagnoses that producing good-quality milk is a considerable challenge in the Brazilian production chain as a whole. Therefore, diagnosing the situation for monitoring is crucial for making more effective decisions to comply with the legislative targets and improve milk quality parameters. This study aims to evaluate the seasonal influence of the raw bulk-tank milk quality produced in the northeast Brazilian region regarding chemical composition, SCC, and TBC requirements.

## 2. Material and Methods

The data were obtained from milk samples from tanks collected by 41 industries distributed across the nine states of northeastern Brazil, which were analyzed monthly for three years (January 2013 to December 2015), following sample collection standards and analysis methodology adopted by the *PROGENE/UFRPE* laboratory.

Bronopol (2-bromo-2-propoxy-1,3-diol) preservative was used to maintain the integrity of the samples from collection until analysis of samples for analyzing the chemical composition and SCC. In contrast, the preservative azidiol (0.1% sodium azide and chloramphenicol) was used for the samples intended for TBC analysis.

Chemical composition, fat, protein, lactose, and total solid content were determined using Bentley 2000 equipment. Defatted dry extract (DDE) was calculated using the difference between total solids and fat. SCC and TBC were calculated using Somacount 300 and Bactocount IBC equipment, respectively (Bentley Instruments, Inc.). A total of 76,230 samples were collected for fat, protein, DDE, SCC, and TBC.

The data normality test (Shapiro-Wilk) was performed for the SCC and TBC variables (Takahashi et al. 2012). Therefore, data transformations were performed to log scale according to the SCC equations ( $10^3$  cells/ml), where  $SCCS = \log_2 (SCC/100) + 3$ , and for the TBC data ( $10^3$  colony forming units/ml), the transformation used was  $TBCS = \log_{10} (TBC + 0.5)$ .

Some restrictions were applied to the data through frequency analyses to avoid defects in the milk sampling process (Ribeiro Neto et al. 2012). Samples were considered in the following ranges of values for the composition components according to the constituent: fat, from 2.00% to 7.97%, and protein, from

2.25% to 5.18%. Samples above 13,000 were considered for SCC and TBC analyses because transformations on a logarithmic scale below this value would result in negative values.

The data were separated into two distinct periods for the conformity assessment of samples, considering the same period of validity as presented in IN-62 for the northeastern region. Period one: samples collected from January/2013 to June/2015; Period two: samples collected from July/2015 to December/2015.

Descriptive statistical analyses were performed to identify some position and dispersion measures of the data. Absolute frequency, relative frequency, and percentage relative frequency analyses were performed for each category (SCC and TBC) to verify the conformity of the milk samples with IN-62. The analysis of variance and the Tukey test were performed to confirm whether the month factor had significant effects on the variables. The Student's t-test was applied to independent paired samples, considering the two validity legislation periods. The confidence level of 95% ( $p < 0.05$ ) was considered for the comparison tests of means. The statistical program used was SPSS Statistics.

### 3. Results

Average values above the minimum values of 3.0%, 2.9%, and 8.4% were verified for fat, protein, and DDE, respectively. SCC was lower than  $500 \times 1,000$  cells/ml of milk (Table 1). TBC presented a mean well above the recommended limit of  $300 \times 1,000$  cfu/ml of milk (Table 1).

**Table 1.** Mean values, median and coefficient of variation (CV) for chemical composition, somatic cell counts and total bacterial counts in raw bulk-tank milk produced in the northeastern region of Brazil between January to December.

Component	n	Mean	Median	CV (%)
Fat (%)	76230	3.60	3.58	13.47
Protein (%)	76230	3.13	3.12	6.34
DDE* (%)	76230	8.57	8.58	3.39
SCC**	73454	482	362.00	69.01
TBC***	66667	831	276.00	97.11

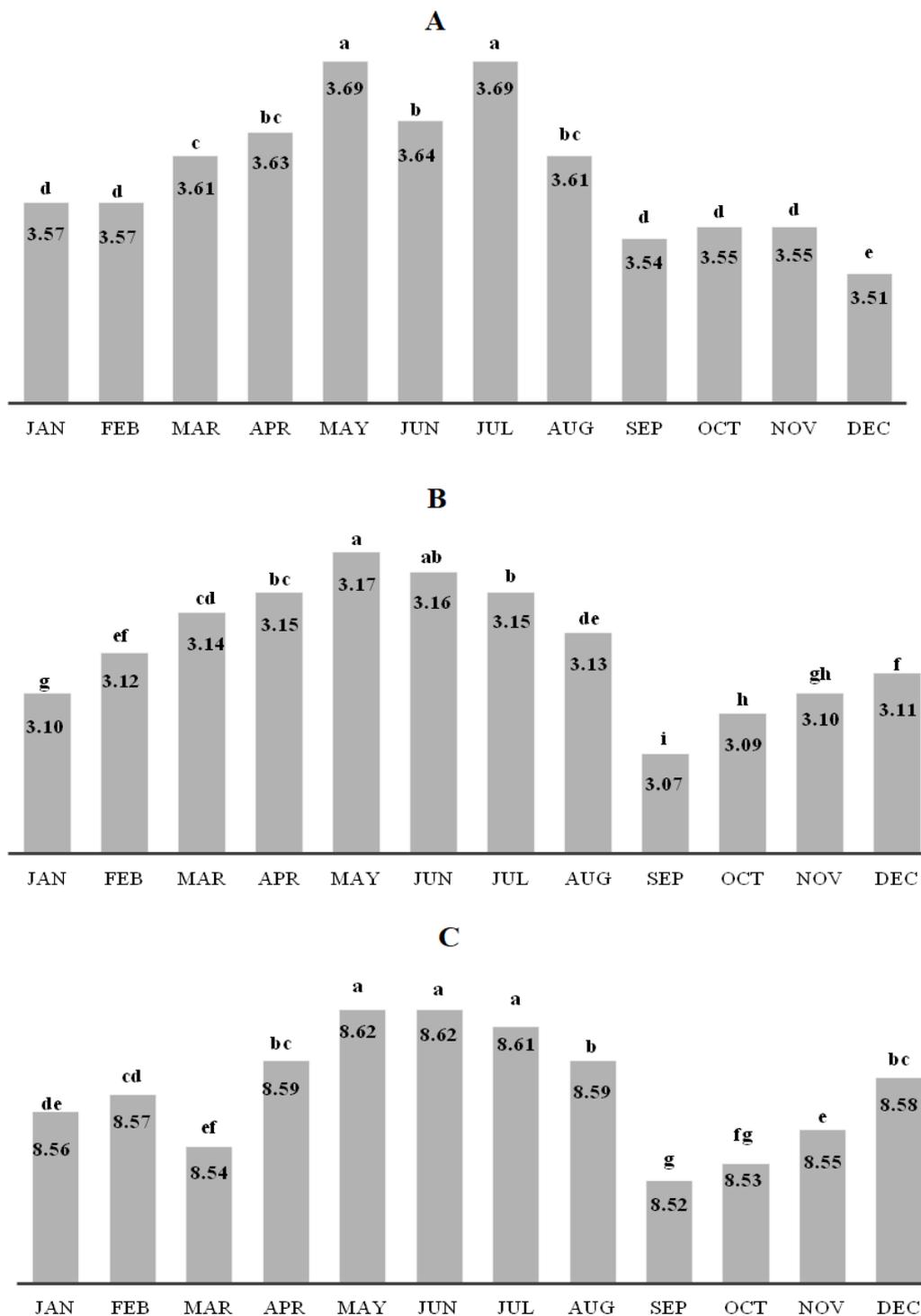
\*Defatted Dry Extract; \*\*SCC - x thousand cells per mL of milk; \*\*\*TBC - x thousand CFU per mL of milk.

The months of the year significantly influenced ( $p < 0.05$ ) the average chemical composition (Figure 1), SCC (Figure 2), and TBC (Figure 3). The highest averages for all variables of raw milk in cooling tanks were concentrated between March and July. The highest averages for fat, protein, and dried extract defatted (DED) were 3.69%, 3.17%, and 8.62%, respectively. The lowest averages were in the period of lowest rainfall, at 5.51%, 3.07%, and 8.52% for fat, protein, and DED, respectively.

The highest averages of the SCC and TBC scores were recorded in July (4.90) and March (2.53) ( $p < 0.05$ ), respectively (Figures 2 and 3). At the same time, the lowest averages for both variables were in December. There was a decrease for all variables in period two (Table 2) compared to the averages for the two periods of legislation validity with different recommended limits. The reductions were from 3.61% to 3.53%, 3.13% to 3.10%, and 8.58% to 8.53% for fat, protein, and DDE, respectively. For SCCS and TBCS, the reductions were from 4.76% to 4.70% and 2.47% to 2.33%, respectively ( $p < 0.001$ ).

As these were critical in the production process, the percentages of nonconforming samples were stratified for both periods. The limit of  $600 \times 1,000$  cells/ml was established for SCC in period 1, and from a total of 63,638 analyzed samples, 14,679 (23.05%) were above this standard. Moreover, when this limit became more rigorous ( $500 \times 1,000$  cells/ml in period two), the 9,816 analyzed samples presented 3,178 samples (32.38%) above this limit (Figure 4).

These percentages were even higher for the TBC variable, given the importance of hygiene in obtaining raw materials. In period one, the  $600 \times 1,000$  cfu/ml limit was established, and 20,136 samples (34.85%) from 57,779 were above this standard. In period two, this limit decreased to  $300 \times 1,000$  cfu/ml, in which 3,561 (40.07%) of the 8,888 analyzed samples were above this specification (Figure 4).

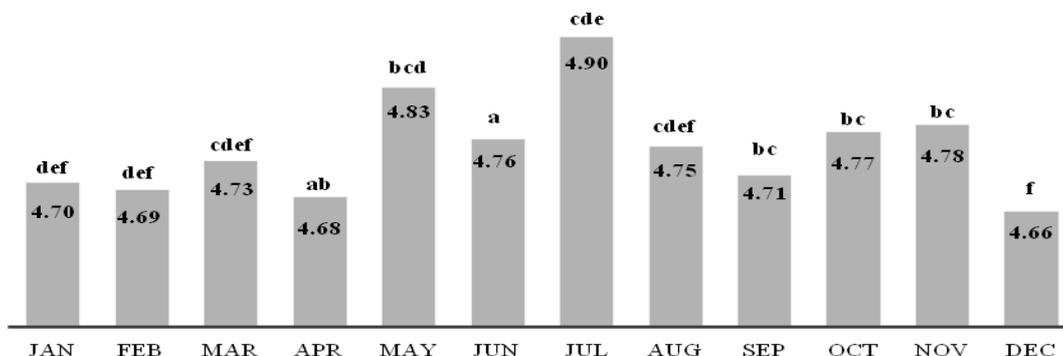


**Figure 1.** A - Adjusted means of fat, B - protein and C - dried extract defatted contents according to the milk collection month. Different letters in the column indicate differences by the Tukey test ( $P < 0.05$ ).

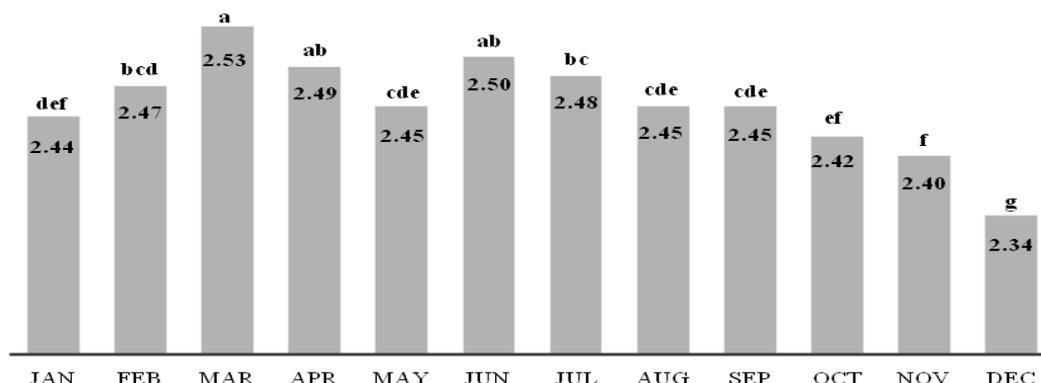
**Table 2.** Comparison of averages for the chemical composition, somatic cell counts and total bacterial counts in raw milk produced in the northeastern region of Brazil considering two valid periods of Normative Instruction no.62.

Component	Period 1*			Period 2**			P-Value***
	N	Mean	SD	n	Mean	SD	
Fat (%)	64557	3.61	0.48	11673	3.53	0.50	<0.001
Protein (%)	64557	3.13	0.20	11673	3.10	0.19	<0.001
DDE (%)	64557	8.58	0.29	11673	8.53	0.29	<0.001
SCCS	63638	4.76	1.27	9816	4.70	1.39	<0.001
TBCS	57779	2.47	0.68	8888	2.33	0.67	<0.001

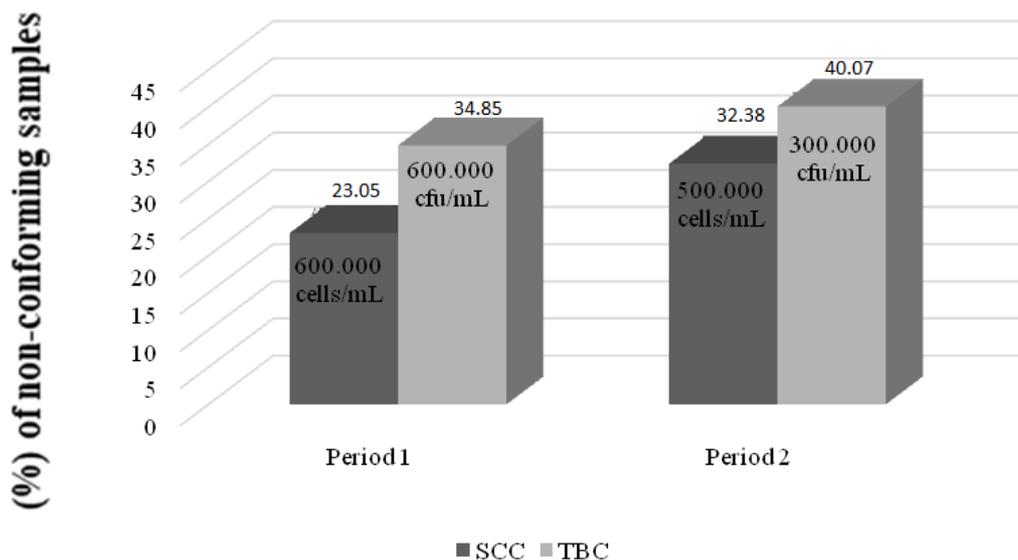
SCCS (Somatic cell count score); TBCS (Total bacterial count score). \* From 01/01/2013 until 06/30/2015; \*\* From July 1, 2015 to December 31, 2015; \*\*\* Student's t-test at significance level of 5%.



**Figure 2.** Means adjusted for the somatic cell count scores according to the milk collection month. Different letters in the column indicate differences by the Tukey test (P<0.05).



**Figure 3.** Means adjusted for the total bacterial counts according to the milk collection month. Different letters in the column indicate differences by the Tukey test (P<0.05).



**Figure 4.** Percentage of non-compliant samples for somatic cell count and total bacterial count of raw milk produced in the northeastern region of Brazil considering two valid periods of Normative Instruction no.62.

**4. Discussion**

The minimum values of 3.0%, 2.9%, and 8.4% for fat, protein, and DDE indicate that raw bovine milk produced in the northeast Brazilian regions has adequate nutritional quality. Knowledge of milk composition is essential for determining its quality. It is increasingly used to detect failures in the nutritional management practices of cows, also serving as a reference for valorizing the raw material in payment programs by quality (Silva et al. 2016).

It was demonstrated by SCC lower than  $500 \times 1,000$  cells/ml of milk (Table 1) that the raw milk produced by the suppliers of these industries in the northeastern Brazilian region meets the legislation requirements set by IN-62 for SCC. However, levels close to  $200 \times 1,000$  cells/ml for SCC are recommended for quality (Quintão et al. 2017). High values reflect animal inflammation and the inadequate management of milking and environmental hygiene properties (Nasr and El-Tarabany 2017). Directive 92/46 of the European Communities Council, from April 1992, stated that milk with SCC above  $400 \times 1,000$  cells/ml could not be used in liquid milk, not even for human consumption, starting in 1998 (Rodrigues et al. 2017). Another major issue related to maintaining mastitis control was the economic losses related to the disease in dairy herds (Ruegg 2017).

TBC above the recommended limit of  $300 \times 1,000$  cfu/ml of milk indicates that urgent and more effective measures should be taken to improve this indicator. This metric reflects a lack of hygiene in obtaining milk, storage, and transport.

Rainfall substantially influences chemical composition, SCC, and TBC (Picinin et al. 2019; Lima et al. 2019). Based on this assertion, it is possible to understand such behavior regarding fat, protein, and DDE percentages. The highest averages for all variables were concentrated between March and July, considered the highest rainfall period, while the lowest averages occurred between September and December (Figure 1).

Along with the increased rainfall volume, there was a considerable supply of fodder in the animal feed. This increase in the quantity and quality of the diet provided to the animals favors an improvement in the chemical (nutritional) composition of milk. Milk constituents are altered by the animal diet and modified according to climatic factors (Andrade et al. 2014).

Specifically, we can infer that the increase in fat percentages (Figure 1A) was related to more considerable fodder availability in the northeast region. Consequently, the animals consumed large amounts of fiber in their diet.

Among the factors affecting fat content in milk, fodder availability may be one of the main factors associated with these dynamics. The opposite scenario is also true since there is a great supply of concentrated foods in times of low rainfall and forage. Thus, the higher the nonstructural carbohydrates content of food, the greater the potential to decrease the milk fat content. Among the factors affecting milk protein content, energy intake is the primary nutritional factor related to milk protein content and production. Increased energy intake through carbohydrate sources increases milk protein production and percentage (Ferland et al. 2018). This may also be associated with the increase in protein percentages (Figure 1B) in the high rainfall months and food supplies in the northeast region during these periods. The same behavior could also be observed for the DDE percentages (Figure 1C).

Considering the hygienic-sanitary aspect, periods of high rainfall often compromise the application of acceptable agricultural practices for milk production. This explains the highest averages for the SCC and TBC scores in periods with the most significant rainfall (July and March) and the lowest averages in December. According to Cunha et al. (2016), in times of high temperature and humidity, animals have less capacity to respond to diseases due to stress. In these moments, microorganisms present more significant proliferation, and there is a greater accumulation of organic matter in the environment of animals. Such conditions make hygiene more difficult during the milking process.

These failures in the production process favor an increase in SCC and TBC levels, which is undesirable. Thus, strategies in productive processes in these critical periods are necessary since this is a known condition and has been repeated over the years. Another exciting point regards the performance of the industry with its milk suppliers in developing corrective actions that encourage them during times of great adversity.

Tank SCC is an essential resource for monitoring milk quality. Also, mammary gland health in herds is crucial since the occurrence of subclinical mastitis and possible economic losses resulting from it are indicated by it (Cassoli et al. 2016; Savic et al. 2017).

SCC is essential for estimating teat health and milk quality in dairy herds worldwide (Alhssien and Dang 2018). Moreover, SCC may be influenced by productivity, health, calving, lactation stage, breed, environmental conditions, inadequate management practices, and stress conditions.

In a study developed by Quintão et al. (2017), high SCC levels in tank milk were caused by high precipitation and increases in temperatures caused by seasonality. Mechanical milking, low milk production, productivity, inadequate milking procedures, equipment hygiene, and water quality are factors cited by the authors that influenced this process. Additionally, they emphasized that training for milkers is necessary for producing low SCC in milk. A basic procedure, but of fundamental importance, it was also related to acceptable agricultural practices for milk production with low TBC levels.

It was demonstrated by the results observed in Table 2 for the SCC and TBC variables with the low reduction from periods one and two that milk producers in the northeastern region of Brazil have difficulties adapting to hygienic-sanitary aspects. More stringent limits were employed as of 2019 for the northeast Brazilian region, with a maximum limit for SCC of  $400 \times 1,000$  cells/ml and TBC of  $100 \times 1,000$  cfu/ml. This is not an isolated situation, but it only recurs in the northeast. The situation extends to all Brazilian territories. Nonconformity percentages to the limits recommended by Brazilian legislation in the south, southeast, and central-west regions have been reported in the literature (Machado and Cassoli 2016a).

One of the identified solutions that impacts reducing these indicators (SCC and TBC) is stimulation through subsidies to producers and focusing government actions that ensure training. This way, producers can reach the determined levels for producing quality raw milk (Marcondes et al. 2014).

It is imperative to produce quality raw milk even if the liquid milk is pasteurized to reduce its microbial load since the postpasteurization deterioration of milk by microbes is still a reality. In a study developed by Marcondes et al. (2014), postpasteurization contamination with psychotolerant gram-negative or psychotolerant spiroform bacteria that were able to survive pasteurization were present as spores in raw milk and subsequently grew at refrigeration temperatures. On a scale of priorities, TBC is an indicator that deserves more decisive action in corrective measures since the averages presented for milk from cooling tanks in the northeastern Brazilian states are outside the recommended standards for milk considered to be quality.

On a scale of priorities, TBC is an indicator that deserves stronger actions in corrective measures, since the averages presented for milk from cooling tanks in the northeastern Brazil states are outside the recommended standards for milk considered to be quality.

## 5. Conclusions

The mean values of fat, protein, DDE, SCC, and TBC were influenced by the months of the year and had a decrease in their means for stricter periods of Brazilian legislation. However, the averages for SCC and TBC found in this study were still high, considering the quality of raw milk production.

**Authors' Contributions:** ARAÚJO, V.M.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; BARBOSA, S.B.P.: conception and design and critical review of important intellectual content; RANGEL, A.H.N.: analysis and interpretation of data and critical review of important intellectual content; BORBA, L.H.F.: analysis and interpretation of data; OLIVEIRA, J.P.F.: critical review of important intellectual content; BATISTA, A.M.V.: conception and design and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Ethics Approval:** Not applicable.

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