







Chilled raw milk quality: a case study in Zona da Mata region, Minas Gerais State, Brazil

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ABSTRACT: *This paper aimed to analyze the evolution of the quality of raw milk produced by producers in the Zona da Mata region, in the state of Minas Gerais, between 2012 and 2018. For this purpose, we used the linear mixed-effects model to analyze the monthly evolution of the results of milk composition indicators (fat, protein, and defatted dry extract – DDE), somatic cell count – SCC, and total bacterial count – TBC, from the official monthly registry of 94 milk producers, suppliers of a region's dairy. Results indicate a continuous reduction in the milk composition indicators between 2014 and 2018. For the SCC and TBC indicators, we identified only one-off reductions. The supply of a larger volume of milk was associated with increased TBC. Seasonality influenced all quality indicators analyzed. According to these results, we concluded that the analyzed quality of chilled raw milk offered by producers was proven worse from 2012 to 2018, despite the actions implemented by the National Milk Quality Improvement Program – PNMQL.*

Key words: dairy, legislation, National Milk Quality Improvement Program.

Qualidade do leite cru refrigerado: um estudo de caso na região da Zona da Mata de Minas Gerais

RESUMO: *Este artigo objetivou analisar a evolução da qualidade do leite cru refrigerado produzido por produtores da região da Zona da Mata, do Estado de Minas Gerais, entre os anos de 2012 e 2018. Para tal, foi utilizado o modelo linear de efeitos mistos, a fim de analisar a evolução mensal dos resultados de indicadores de composição do leite (gordura, proteína e extrato seco desengordurado – ESD), contagem de células somáticas – CCS, e contagem bacteriana total – CBT, provenientes de análises oficiais mensais de 94 produtores de leite, fornecedores de um laticínio da região. Os resultados indicaram uma redução contínua dos indicadores de composição do leite entre os anos de 2014 e 2018. Para os indicadores de CCS e CBT foram observadas apenas reduções pontuais. O fornecimento de um maior volume de leite foi associado ao aumento da CBT. A sazonalidade influenciou todos os indicadores de qualidade analisados. De posse desses resultados, pode-se afirmar que, apesar das ações implementadas pelo Programa Nacional de Melhoria de Qualidade do Leite – PNMQL, a qualidade do leite cru refrigerado, proveniente dos produtores avaliados, piorou ao longo do período em análise.*

Palavras-chave: laticínio, legislação, Programa Nacional de Melhoria de Qualidade do Leite.

INTRODUCTION

Since price deregulation and trade liberalization in the 1990s, there has not been a period with so many changes and influence on national dairy farming as in recent decades. From the elaboration of the National Milk Quality Improvement Program – PNMQL (in Portuguese) in 1996 to the present day, quality standards have been established for refrigerated raw milk (BRASIL, 2002), the use of refrigeration tanks by direct expansion in rural properties (BRASIL, 2011b), official analysis of

total bacterial counts for refrigerated raw milk stored in dairy products (BRASIL, 2018c), among several other actions.

Until now, the recently published regulatory framework has contributed strongly to the regulation of animal handling and milking activities, storage and transport of milk, its receipt and storage in industry, etc. (BRASIL, 2002; 2011b; 2018b; 2018c). However, changes in somatic cell count – SCC and total bacterial count – TBC indicators (maximum 1.0×10^5 TBC/mL for individual tank TBC, 3.0×10^5 cfu/mL for tank TBC, and 4.0×10^5 cells/mL for SCC) did

not occur according to the terms initially provided for by Normative Instruction No. 51 (BRASIL, 2002). The implementation of stricter standards for SCC and TBC has been extended many times (BRASIL, 2011a; 2011b; 2016; 2018a), and the current standards (BRASIL, 2018b) set limits higher than those initially provided by Normative Instruction No. 51 (BRASIL, 2002).

Nevertheless, two decades after the implementation of PNMQL, it cannot be said that the chilled raw milk that reaches the industry is of higher quality. RIBAS et al. (2016), in a study with milk samples from 10 regions of Paraná State, identified a reduction in the TBC indicator between January 2012 and May 2014. However, in this and several other studies, researchers reported a high percentage of samples in non-compliance with current SCC and TBC standards (BORGES et al., 2013; MILANI et al., 2016; RIBAS et al., 2016).

Coupled with the scenario of uncertainty regarding the implementation agenda of the new standards, studies evaluating the quality of refrigerated raw milk in Brazil over the years are still scarce. However, these studies are extremely important for the sector, as they allow, among other things, the evolution of the quality of milk produced in different regions of the country to be evaluated, the factors that may be associated with the production of quality milk to be identified, and future reviews of the milk legal framework to be supported. Along these lines, this article aimed to determine whether the monthly quality of the results of composition indicators (fat, protein, and defatted dry extract – DDE), SCC, and TBC of chilled raw milk produced by producers in the Zona da Mata region, in the state of Minas Gerais, evolved between 2012 and 2018.

MATERIALS AND METHODS

Data

The data come from official monthly analyses of 94 dairy farmers (individual and collective), suppliers of a dairy located in the Zona da Mata region, state of Minas Gerais. The data cover a period of 78 months from July 2012 to December 2018.

The variables considered in the study were monthly production volume (Volume), year (Year; ranging from 2012 to 2018), and seasonality (Seasonality; as a function of month), referring to analyses of fat (Fat), protein (Protein), degreased dry extract (DDE), SCC and TBC, in addition to the temperature of the milk sample analyzed (T_SCC for SCC and composition analyzes and T_TBC for TBC analysis) and the number of days elapsed between

milk sample collection and analysis (Age). Values of (i) SCC zero or greater than $9,999 \times 10^3$ cells/mL; (ii) TBC equal to zero or greater than $9,999 \times 10^3$ cfu/mL; (iii) Fat less than 0.5% or greater than 7.0%; (iv) Protein less than 1.0% or greater than 5.0% (OSTRENSKY, 1999); (v) T_SCC (or T_TBC) greater than 10 °C; and (vi) Age greater than 7 days (CASSOLI, 2005) were removed from the analysis. We also removed the outliers referring to the monthly volume of milk supplied by producer, due to inconsistent data. In this case, we considered outliers those values lower than the expression: first quartile minus 1.5 times the interquartile distance, and those higher than the expression: the third quartile plus 1.5 times the interquartile distance.

For months with more than one official analysis record per producer, we calculated the arithmetic mean for composition (Fat, Protein, and DDE), temperature (T_SCC and T_TBC), and Age analyzes, and geometric mean for SCC and TBC analyzes. In these cases, the use of geometric mean was due to the lognormal behavior of these data (SHOOK, 1982). Also noteworthy it was the use of geometric mean for these variables when there were results from more than one monthly analysis, according to Normative Instruction No. 76 (BRASIL, 2018b).

Statistical analysis

Initially, we calculated the descriptive statistics of the data. Then, we used the linear mixed-effects model to identify the effect of the variables Producer, Volume, Year (2013, 2014, 2015, 2016, 2017, and 2018) and Seasonality (Station I and Station II), under the indicators of quality of chilled raw milk. In longitudinal studies, which considered repeated measures over time, the use of a linear mixed-effects model is more appropriate than Analysis of Variance – ANOVA. Using ANOVA for longitudinal studies ignores the correlation between observations and treats them as uncorrelated (FAUSTO et al., 2008).

The linear mixed-effects model includes fixed and random effects and is commonly used in longitudinal studies in the dairy field (NIGHTINGALE et al., 2008; BOTARO et al., 2013; FLORES-MIYAMOTO et al., 2014; O'CONNELL et al., 2015; MILANI et al., 2016). In this, fixed effects are directly estimated, being analogous to the coefficients of a standard regression, while random effects are summarized according to their estimated variances and covariance (STATA CORP, 2013). The linear mixed-effects model can be represented by the following equation:

$$y = X.\beta + Z.u + \epsilon$$

where y is the known vector of observations for a particular quality indicator of chilled raw milk; β is the unknown vector of fixed effects; u is the unknown vector of random effects; ϵ is the waste vector; X and Z are known matrices, related to observations y for β and u , respectively. It is assumed that Z has a normal distribution with mean zero and variance and covariance G , regardless of ϵ , which has a normal distribution with mean zero and variance σ^2 .

In this study, we analyzed the variables Year, Volume, and Seasonality as fixed effects, while the control variable Producer was analyzed as a random effect. For the Year variable, we broke down the analysis year by year by using dummy variables for each of the years. The variables Fat, Protein, DDE, SCC, and TBC were analyzed as dependent terms, each being in a distinct model. The model used in this study is represented in the equation below:

$$\text{Indicator} = \text{Intercept} + \text{Seasonality} + \text{Year} + \text{Volume} + \text{Producer (random)} + \text{Error}$$

where Seasonality is a regression term for the sine (Season I) and cosine (Season II) functions, according to NIGHTINGALE et al. (2008), which thus describe the monthly variation of the evaluated quality indicator:

$$\text{Season I} = \sin\left(\frac{2\pi \cdot \text{Month}}{12}\right) \quad \text{Season II} = \cos\left(\frac{2\pi \cdot \text{Month}}{12}\right)$$

Since the values of SCC and TBC are typically lognormal (SHOOK, 1982), they were log transformed (base 10) before statistical analyses (Log_SCC and Log_TBC, respectively). The reference unit considered for the Volume variable was 1,000 L of milk. We estimated the fixed effects and variance components of the linear mixed-effects model by the Restricted Maximum Likelihood method – REML (PATTERSON and THOMPSON, 1971). This method provided non-biased estimates of variance, removing the addiction that exists in the estimation of the mean (RESENDE et al., 1996).

RESULTS AND DISCUSSION

Descriptive statistics

The average number of analytical records per producer was 53, ranging from 30 to 75. The average number of analytical records per year was 706, ranging from 319 to 916. Table 1 illustrates a summary of the descriptive statistics for raw milk variables of the 94 producers analyzed. The illustrated data indicated that the average values for Fat, Protein, and DDE are in accordance with the legal standards in force for the region—minimum 3.0 g/100 mL for fat content, 2.9 g/100 mL for protein content, and

8.4 g/100 mL for DDE (BRASIL, 2011b; BRASIL, 2018b)—while the average values for SCC and TBC are above them for most of the period up to June 30, 2014—maximum of 6.0×10^5 cfu/mL for TBC and 6.0×10^5 cells/mL for SCC (BRASIL, 2011b)—and from July 1, 2014—maximum of 3.0×10^5 cfu/mL for TBC and 5.0×10^5 cells/mL for SCC (BRASIL, 2011b; BRASIL, 2018b).

The minimum values observed for Fat, Protein, and DDE, as well as the maximum values observed for SCC and TBC, indicated the existence of cases that do not comply with the legislation. The mean values of T_SCC, T_TBC, and Age are in accordance with the suggested temperature and sample age limit for the analysis of chilled raw milk (CASSOLI, 2005). The monthly volume of milk supplied by producers was the variable with the highest standard deviation, with an average volume of 2,520 L. The evolution of the historical average of the quality indicators studied for chilled raw milk is illustrated in figure 1.

From the monthly history of milk quality indicators over the period under study (Figure 1), it is possible to verify the existence of large oscillations, which represents a problem for the dairy industry. The content of milk composition indicators is closely related to the yield of various dairy products (MAGANHA, 2006), while the SCC and TBC indicators are not only related to the yield of various dairy products (HAND et al., 2012) but also to its validity (BARBANO et al., 2006; FLORES-MIYAMOTO et al., 2014; CASTRO et al., 2016) and their sensory characteristics (MA et al., 2000; BARBANO et al., 2006). Thus, the observed oscillations make it difficult to standardize production processes and the quality of the products produced.

Among the indicators of milk composition, it is possible to identify the presence of cyclic oscillations, with a slight downward trend over the years. In this case, the oscillations may be associated with the influence of the animal's diet on the quality of milk produced (JENKINS and McGUIRE, 2006). When it comes to grazing animals, climate change can influence the availability and quality of their food (MILANI et al., 2016).

The SCC and TBC indicators, in turn, did not show such a well-defined behavior. However, it was possible to verify the presence of larger oscillations of the TBC indicator, when compared with the SCC indicator (Figure 1), which was corroborated by the coefficient of variation of the logarithm of these indicators (Table 1). This result may be associated with the fact that SCC is directly associated with

Table 1 – Descriptive statistics of the raw milk variables of 94 producers analyzed.

Variable	Number of cases	Minimum	Mean	Maximum	Standard deviation	Coefficient of variation (%)
Fat (g/ 100 mL)	4,915	0.50	3.49	7.00	0.67	19.3
Protein (g/ 100 mL)	4,924	1.56	3.22	4.27	0.25	7.8
DDE (g/ 100 mL)	4,924	4.47	8.61	10.03	0.37	4.3
Log_SCC (cells/mL)	4,913	3.30	5.45	6.70	0.49	8.9
Log_TBC (cfu/mL)	4,797	2.82	5.49	6.70	0.57	10.4
Volume (10 ³ L)	4,530	0.03	2.52	80.4	5.08	201.3
Age (days)	4,732	2	3.85	7	1.79	46.6
T_SCC (°C)	4,318	0.8	5.1	10	2.87	56.3
T_TBC (°C)	4,147	0.8	5.1	10	2.88	56.1

the health status of the cow's mammary gland (MACHADO et al., 2000), while TBC is associated, among other factors, with hygienic-sanitary milking conditions (PANTOJA et al., 2009). Therefore, the latter tends to be more impacted by climate change, since, in rainy periods, the maintenance of hygienic milking conditions tends to be impaired (BUENO et al., 2008; CASTRO et al., 2016). In a study conducted with data from 16,491 milk samples analyzed at the Milk Quality Laboratory of the Food Research Center of the Veterinary School of the Universidade Federal de Goiás, between October 2002 and September 2003, BUENO et al. (2008) reported higher values for the logarithm of the TBC indicator in the rainy season when compared with the dry season.

In any case, the evolution trend of the quality indicators of chilled raw milk is not always clear, and the oscillations may be associated with a set of factors, such as seasonal variations, producer entry or exit, change in technical assistance policy by the dairy, etc. (NIGHTINGALE et al., 2008; BOTARO et al., 2013; FLORES-MIYAMOTO et al., 2014). Therefore, the use of statistical models, such as linear mixed-effects models, is necessary to measure the specific contribution of each variable under the evolution observed for the indicators of milk quality.

Evolution of chilled raw milk quality

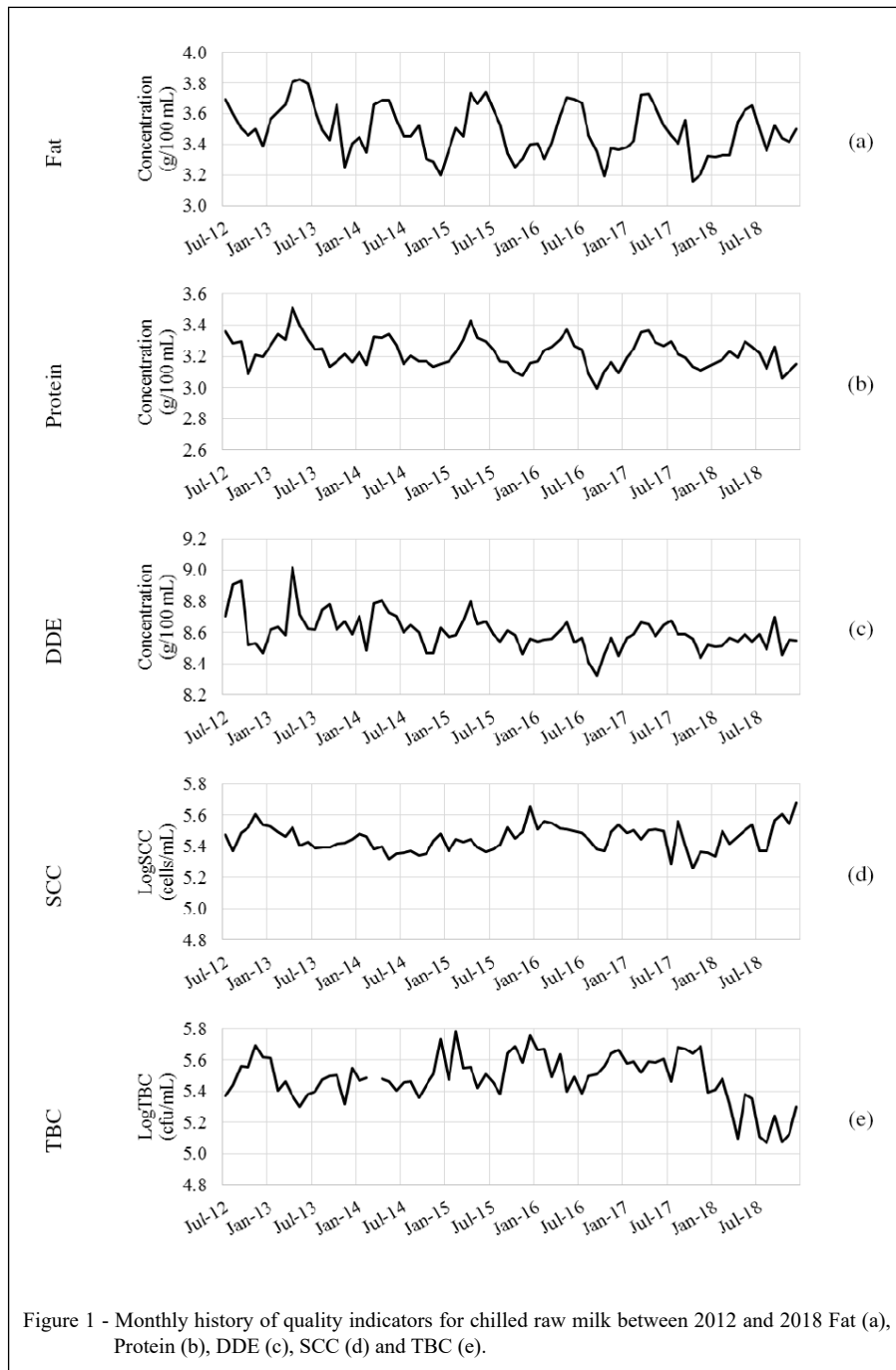
Table 2 presents the statistics for the mixed-effects linear models generated for the quality indicators of chilled raw milk. The average number of observations recorded per producer was 53, ranging from 30 to 75, while the average number of observations recorded per model was 4,488, ranging from 4,400 to 4,514. All models were significant ($P < 0.0001$) according to the Wald test and significantly higher than the reference linear regression model

($P < 0.0001$) according to the likelihood ratio test. The Year and Seasonality variables were significant ($P < 0.05$) in all models, while the Volume variable was significant only for the TBC indicator model.

The years between 2014 and 2018 were associated with the reduction in the indicators of milk composition (Table 2). Even so, the monthly averages for Fat, Protein, and DDE levels were always higher than the minimum values established for these indicators (3.0 g/100 mL, 2.9 g/100 mL, and 8.4 g/100 mL, respectively). The only exception was made in September 2016, when the monthly average DDE was 8.3 g/100 mL, lower than the minimum value established by current legislation (BRASIL, 2011b; BRASIL, 2018b). It is still necessary to emphasize the positive correlation between the milk solids content and the productivity that the dairy will have when converting this raw material into dairy derivatives (MAGANHA, 2006). Thus, the observed reduction in milk solids content between 2014 and 2018 is a bad result, especially in light of the PNMQL objectives.

Most of Brazil's milk production comes from small producers (IBGE, 2019). These, in general, do not have technical assistance (OLIVAL et al., 2004; OLIVEIRA et al., 2011), are unaware of ways to prevent milk contamination (OLIVAL et al., 2004), are poorly capitalized (OLIVEIRA et al., 2011), have a low profit margin under the price of a liter of milk (BÁNKUTI et al., 2008; LOPES et al., 2012; 2015), and are not financially rewarded for milk quality (VALEEVA et al., 2007). These are, therefore, obstacles to improving milk quality and as such need to be targeted by PNMQL actions.

It should be noted that the herd profile also has an influence on milk solid content (MILANI et al., 2016). Thus, these results may be associated with a change in the herd profile over the period



analyzed. However, this inference cannot be ratified due to the unavailability of monthly production data for each of the producers.

For the SCC indicator, there was only a reduction of 0.08 \log_{10} (cells/mL milk) in 2014 (Table 2). This result may reflect the improvement of management practices by producers, since SCC

is directly associated with the health status of the cow's mammary gland, which is the main indicator of mastitis occurrence (MACHADO et al., 2000). According to REZENDE et al. (2012), SCC values greater than 2.0×10^5 cells/mL indicate the occurrence of mastitis. However, it should be remembered that this year was marked by the implementation of a SCC

standard for chilled raw milk, which is stricter than that in force at the time (BRASIL, 2011b). Therefore, the result may also be associated with dairy farmers' interest in meeting the new established standard.

For the TBC indicator, a reduction of 0.12 \log_{10} and 0.23 \log_{10} (cfu/mL milk) was observed in 2013 and 2018, respectively, and an increase of 0.10 \log_{10} (cfu/mL milk), in 2017 (Table 2). The improvement observed for the TBC indicator is indicative of improved management practices (VELTHUIS and VAN ASSELDONK, 2011), milking hygienic-sanitary conditions (PANTOJA et al., 2009) and/or milk storage conditions (BYLUND, 1995) by the producers. It should be noted that the years 2012 and 2014 were marked by the implementation of stricter TBC standards (BRASIL, 2011b) than that in force at the time (BRASIL, 2002; 2011a) for chilled raw milk. Thus, at least for 2013, the result can also be associated with the impact of the implementation of the new TBC standards on producers.

Regarding the impacts of legislation on milk quality indicators, it should be noted that the establishment of stricter standards should be carried out in conjunction with other actions in order to inform, guide, and provide conditions for producers to achieve them. For example, there is an increase in the provision of technical assistance to producers and transporters of milk by the government, industries, or producer associations and an intensification of supervision and an increase in credit supply by the government. Increased milk production in Europe in the 1970s, for example, was stimulated by a subsidy policy (BERTOLDI, 2012); while the world's largest milk producer, the United States, has its production subsidized by a minimum price policy (CARVALHO, 2008).

Volume influenced the TBC indicator over the period analyzed. The results indicate that a 1,000 L milk size increase in a producer's monthly output is associated with a 0.01 \log_{10} (cfu/mL milk) increase in TBC (Table 2). This result, in turn, indicates that the larger the volume of milk supplied by the producer, i.e. its size, the higher the bacterial counts of milk supplied by the producer. In a study with quality data of chilled raw milk from a cooperative located in southern Brazil, between August 2005 and April 2008, BOTARO et al. (2013) identified a significant effect ($P < 0.05$) of volume on the variables SCC, TBC, and protein content. The authors also identified that higher TBC values were associated with producers who provided the largest volume of milk. However, it is noteworthy that, in theory, producers who provide larger volumes of milk tend to be more automated than those who provide smaller volumes (RODRIGUES et al., 2005; O'CONNELL et al., 2015). Therefore, larger producers could be expected to have lower values for TBC, as the less automated milking process tends to make milk susceptible to a greater amount of contaminants.

All quality indicators of chilled raw milk were influenced by Seasonality (Table 2). The maximum values of the indicators of milk composition were observed in the months of April, the end of the rainy season, while the minimum values of these indicators were observed in the months of November (Figures 1a, 1b, 1c), just after the dry season in the region. For the SCC and TBC indicators, the maximum values were observed in January during the summer, the period with the highest temperatures, while the minimum values were observed in July

Table 2 - Coefficients of linear mixed effects models, referring to the variables analyzed.

-----Variable-----	Fat (g/100 mL)	Protein (g/100 mL)	DDE (g/100 mL)	Log_SCC (cells/mL)	Log_TBC (cfu/mL)	
Year	2013	-0.0166	-0.0225	-0.0417	-0.0598	-0.1166*
	2014	-0.1405*	-0.0900*	-0.1221*	-0.0775*	-0.0555
	2015	-0.1219*	-0.0898*	-0.1584*	-0.0311	0.0556
	2016	-0.1407*	-0.1172*	-0.2480*	0.0191	0.0533
	2017	-0.1320*	-0.0702*	-0.1764*	0.0034	0.1001*
	2018	-0.1389*	-0.0988*	-0.1875*	0.0605	-0.2316*
Volume	-	-0.0057	-0.0015	0.0034	-0.0004	0.0092*
Seasonality	Season I	0.0774*	0.0824*	0.0479*	0.0284*	0.0265*
	Season II	-0.1597*	-0.0728*	-0.0592*	0.0275*	0.0639*

* $P < 0.05$.

(Figures 1d, 1e), during the winter, the period with the lowest temperatures.

The rainy season, concentrated in the summer, makes it difficult to maintain hygienic conditions during milking (BUENO et al., 2008; CASTRO et al., 2016) and, consequently, contributes to the milk being susceptible to a larger number of microorganisms. In addition, according to FLORES-MIYAMOTO et al. (2014), the average bacterial count of milk tends to increase in summer, since temperatures are higher, hindering the process of preservation of the product. Other common sources of contamination of refrigerated raw milk are the presence of lesions on the animal's skin and teat (CASTRO et al., 2016), failures in teat hygiene procedures before milking (ELMOSLEMANY et al., 2010; OLIVEIRA et al., 2011), conditions of the environment in which the animals were raised, the milking equipment, employees, tanks used for transportation (CASTRO et al., 2016) and milk storage.

With regard to SCC, it is known that periods of heat stress, most common in summer, tend to expose animals to a higher number of pathogens. This greater exposure to periods of thermal stress, in turn, increases susceptibility to infections and, consequently, the occurrence of mastitis (SMITH et al., 1985), which increases the milk SCC.

Regarding fat, protein, and DDE contents, the results (Table 2) are associated with the influence of the production system (MILANI et al., 2016) and diet (JENKINS and McGUIRE, 2006) on the milk composition indicators. The dairy herd of the region under study is raised on pasture, which makes the quality of its food very dependent on the available pastures and, consequently, on the weather seasons.

In turn, the results regarding the seasonality effect on quality indicators of chilled raw milk (Table 2) corroborate those found in several other studies (NIGHTINGALE et al., 2008; BOTARO et al., 2013; FLORES-MIYAMOTO et al., 2014; MILANI et al., 2016; RIBAS et al., 2016). MILANI et al. (2016) identified a significant effect of seasonality on fat and protein content, while NIGHTINGALE et al. (2008) and MILANI et al. (2016) identified a significant seasonality effect under the SCC indicator, and BOTARO et al. (2013), FLORES-MIYAMOTO et al. (2014), MILANI et al. (2016), and RIBAS et al. (2016) identified a significant seasonality effect on the TBC indicator of chilled raw milk.

Finally, it can be inferred that the quality of chilled raw milk from the evaluated producers

deteriorated between 2012 and 2018. Fat, protein and defatted dry extract levels decreased continuously between 2014 and 2018. Somatic cell count and total bacterial count indicators showed only point reductions over the analyzed period. Thus, regarding the group of producers studied, it is not possible to state that there was an improvement in milk quality. Therefore, further longitudinal studies with milk producers from other regions of the country are needed to (i) map the regions that contain the producers with the greatest difficulties in meeting the evolution of quality standards of chilled raw milk and (ii) enable the adoption of specific milk quality improvement strategies in each of these regions.

In a study of 517 dairy herds from different regions of Brazil, between January 2011 and May 2015, BUSANELLO et al. (2017) found a high percentage of cases of subclinical mastitis (mean prevalence of 46.4%) in all regions studied. The authors also identified an increasing tendency of these cases over the years.

CONCLUSION

Despite the actions implemented by PNMQL, the quality of chilled raw milk from the evaluated producers deteriorated over the period under review. Fat, protein and defatted dry extract contents decreased continuously between 2014 and 2018. The indicators of somatic cell count and total bacterial count showed only occasional reductions over the analyzed period.

Therefore, effective actions by dairy farmers and producers are needed to change this reality. Just the implementation of regulations or stricter indices for chilled raw milk quality parameters are not enough. These actions need to involve more effective technical assistance, establishment of payment for quality with bonuses, and even automation of producers.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict 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, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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