DOI: 10.5433/1679-0359.2019v40n1p203

Variation in the content of defatted dry extract in cooling tanks milk samples of dairy farms

Variávéis relacionadas ao teor de extrato seco desengordurado em amostras de leite de tanques de resfriamento de estabelecimentos rurais

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Abstract

The defatted dry extract (DDE) comprises of the solid constituents of the milk, except for the fat, and must have a minimum established content of 8.4% for raw milk in Brazil. The objective was to evaluate the effects of commercialized milk volume, somatic cell count (SCC) and total bacterial count (TBC) on the DDE content of dairy farm milk samples. The sampling included data from monthly milk analyzes of dairy farms, which supply four dairy industries located in the state of Santa Catarina, Paraná and Rio Grande do Sul, and comprised of 106.470 observations sampled in 2015.The data were submitted to variance and regression analysis. To evaluate the influence of the volume of milk commercialized by the farmers, the SCC and TBC levels, and the levels of DDE, the values of these explanatory variables were stratified in quintiles. The mean DDE was 8.55%, with 25.6% of the samples being below the established minimum. In regard to the volume of milk marketed by the farmers, seasonal variations were observed in all quintiles of dairy farms analyzed, but with lower levels of DDE in milk samples from small farms throughout the year. Samples of milk with high TBC and, especially high SCC, presented lower percentage of DDE during the year. It is concluded that the DDE content of milk is related to the season of the year, the production volume per dairy, SCC and TBC.
Key words: Lactose. Legislation. Milk composition. Protein.

Resumo

O extrato seco desengordurado (ESD) compreende os constituintes sólidos do leite, excetuando-se a gordura, devendo apresentar teor mínimo estabelecido de 8,4% para leite cru no Brasil. Objetivou-se avaliar efeitos do volume de leite comercializado, contagem de células somáticas (CCS) e contagem bacteriana total (CBT) sobre o teor de ESD em amostras de leite de estabelecimentos rurais. A amostragem contemplou dados de análises mensais de leite de estabelecimentos rurais, fornecedores para quatro laticínios localizados nos estados de Santa Catarina, Paraná e Rio Grande do Sul, sendo composta por 106.470 observações amostradas em 2015. Os dados foram submetidos à análise de variância e de regressão. Para avaliar a influência do volume de leite comercializado pelos produtores, nível de CCS

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Received: Mar. 01, 2018 - Approved: Sept. 17, 2018

e nível da CBT, sobre os teores de ESD, os valores destas variáveis explanatórias foram estratificados em quintis. A média do ESD foi de 8,55%, sendo que 25,6% das amostras estavam abaixo do mínimo estabelecido. Quanto ao volume de leite comercializado pelos produtores, observou-se variação sazonal em todos os quintis de estabelecimentos rurais analisados, porém com teores mais baixos de ESD em amostras de leite de pequenos produtores ao longo de todo o ano. Amostras de leite com elevada CBT e, especialmente alta CCS, apresentaram menores percentuais de ESD durante o ano. Conclui-se que o teor de ESD do leite está relacionado com a época do ano, o volume de produção por propriedade leiteira, com a CCS e com a CBT.
Palavras-chave: Lactose. Legislação. Composição do leite. Proteína.

Introduction

Milk quality is of great importance for the industrial yield, as well as for public health, being able to be evaluated through the physical, chemical and microbiological attributes. In this sense, the chemical composition should be analyzed in dairy industries in compliance with the Ministry of Agriculture, Livestock and Food Supply (MAPA). Dairy producers and industries across the country have been challenged to reach the limits set by milk quality standards, especially since the publication of Normative Instruction 51/2002 by MAPA (BRASIL, 2002). Among the milk composition indicators, the defatted dry extract (DDE), which is comprised of the solid constituents of milk, except for fat, has caused concern for industries and farmers due to the occurrence of milk samples below the established legal minimum. In Brazil, the DDE content of milk must be to at least 8.4% (BRASIL, 2011; 2017). However, other minimum DDE standards, other than that of Brazil, have been used, as in the case of the 8.5% regulated in European Union countries (MENEGHINI, 2011) and the 8.2% DDE content used in Argentina (ARGENTINA, 2014). The occurrence of non-conforming samples for DDE is substantial, and Montanhini et al. (2013) observed that 24.6% of the samples from cooling tank milk did not conform, in a study with a database in the state of Paraná.

Under Brazilian conditions, there is usually high heterogeneity, not only in terms of the genetic groups, but also for production systems and the level of feed given to the animals, which can lead to considerable variations in the milk composition and may be associated with the fact that different levels of production also determine different milk composition. The fluctuation of the availability and quality of roughages foods that occurs in most dairy farms, with less food available in the autumn contributing to the reduction of lactose at this time of year (ALESSIO et al., 2016), and may be related to lower levels of DDE. Cows subjected to stressful heat conditions may also produce milk with lower lactose contents (WHEELOCK et al., 2010; TIAN et al., 2015) and milk protein (BERNABUCCI et al., 2002), leading to a reduction in DDE content in the summer. Factors inherent to animals may also be related to the DDE content of milk, with a decrease in DDE as the cow ages. A relatively high DDE content was observed during the first month of lactation, falling in the second month and then increasing in late lactation, as a result of the variation in the protein content (HARRIS JÚNIOR; BACHMAN, 2003). In the South of Brazil, small dairy farms are predominate (WINCK; THALER NETO, 2012; GABBI et al., 2013; LANGE et al., 2016; WERNCKE et al., 2016) and the greatest differentiation between small dairy farms is related to how the cows are fed. (GABBI et al., 2013). Thus, considerable variations in milk composition can be expected in these dairy farms.

Conditions such as clinical and subclinical mastitis may lead to a decline in the percentage of DDE, mainly related to a reduction of the lactose content (ALESSIO et al., 2016). The reduction of the lactose content as a function of subclinical mastitis, indicated by high SCC, occurs due to factors such as changes in mammary gland homeostasis (PESSOA et al., 2012) and an increase in lactose plasma concentration due to the reduction of cohesion between the tight junctions of secretory cells (STUMPF et al., 2013). Similarly, the microbiological quality of milk may also be related to the concentration of milk DDE, since lactose, as the main carbohydrate in milk, is an important source of food for the microorganisms (BLUM et al., 2008), may be reducing the lactose content. The greatest variations in milk DDE levels are linked to changes in both protein and/or lactose content, such changes may occur, for example, when handling the diet of the animals in the different seasons of the year, aiming to increase the availability of nutrients offered to lactating animals (ROSA et al., 2012), so the inadequate feeding given to cows is one of the main causes of problems related to milk quality Gonzalez et al. (2004). Therefore, some dairy farms with adequate sanitary and hygiene conditions and with low SCC and TBC, still showed a reduction in the DDE content due to inefficient nutritional conditions.

Despite the importance of the DDE content, there are a lack of studies that evaluate the relation between the different indicators of milk composition and environmental variables with the DDE content, and there is a lack of information of how to improve the DDE, and to help farmers and technicians in making decisions on non-compliance with the DDE content. A MAPA Normative Instruction 62/2011 (BRASIL, 2011) establishes that at least once a month milk samples must be collected in the cooling tanks at the dairy farms and analyzed in laboratories that are part of the Brazilian Network of Milk Quality Control (RBQL), for milk composition, SCC and TBC, this system of continuous analysis of large numbers of samples has formed databases that can help as a source of information to assist in the elucidation of the factors involved in the variation in milk quality. In this sense, the aim was to evaluate the effects of marketed milk volume, season of the year, somatic cell count (SCC) and total bacterial count (TBC) on DDE content of cooling tank milk samples of dairy farms.

Material and Methods

The research was carried out from a database composed of monthly milk samples from cooling tanks of individual dairy farmers supplying milk to four dairy industries under Federal Inspection Service (SIF), located in the State of Santa Catarina. Approximately 94% of these rural establishments were located in the state of Santa Catarina and the remaining located in border regions, in the neighboring states of Paraná and Rio Grande do Sul. After the standardization of the data, 106.470 observations were recorded, from samples taken in the period from January to December, 2015. The database was composed of information related to dairy industry, the month the sample was taken, milk composition, total bacterial count (TBC), somatic cell count (SCC) and the volume of milk marketed by the farm in the respective month.

Milk analyzes were carried out in laboratories participating of the Brazilian Network of Milk Quality Control (RBQL). The samples destined for the TBC were conserved with Azidiol® as bacteriostatic agent and the samples destined for analysis of composition and SCC had Bronopol® as a preservative agent. The SCC was analyzed in an electronic counter by the Flow Cytometry method according to International Dairy Federation (2006). The milk composition (fat, protein, lactose and ESD contents) was analyzed using the infrared absorption spectrophotometry technique, according to International Dairy Federation (2000). The TBC analyzes were performed using Flow Cytometry methodology, according to International Dairy Federation (2004).

For the statistical analysis, data were subjected to a previous cleaning to remove outliers. The residual obtained from the model (real value - estimated value) for the DDE variable was taken as the basis, and data below and above 3.5 standard deviations of the mean of the residue were removed, following the logic described by Motulsky and Brown (2006). Non-conforming samples were considered to be those with a DDE content lower than 8.4%, protein less than 2.9% (BRASIL, 2011; 2017) or lactose less than 4.3% (BRASIL, 2017).

Data were submitted to ANOVA using the MIXED procedure of the statistical package SAS®, being previously tested for normality of residues by the Kolmogorov-Smirnov test. To evaluate the influence of the explanatory variables the of volume of milk marketed, the SCC level and TBC level on the DDE levels, their values were stratified into quintiles and separate statistical analyzes were carried out to evaluate the effect of each of these variables. The statistical model was constituted by the effects of the month of the year, the dairy industry, the interaction month vs. year, the class (quintile) of market milk volume, SCC or TBC and their interaction with the month. The averages were compared by the Tukey test with 5% significance. At the same time, the effects of the months of the year on the variables lactose and protein, commercialized milk volume, SCC and TBC were analyzed. The relationship between the explanatory variables volume of marketed milk, SCC or TBC with DDE content was also evaluated by regression analysis using the MIXED procedure using a statistical model composed by quintile of milk volume, SCC or TBC as continuous variables, as well as the classes month of the year, dairy industry and interaction month vs. year. The regression according to the quintiles of the explanatory variables was performed due to the fact that the data are unevenly distributed, with a higher concentration of data in the lower values of milk volume, SCC and TBC, which does not allow analysis of unprocessed data.

Results and Discussion

The average DDE of 8.55% (Table 1) was above the minimum of 8.4% established in Decree Nº 9.013/2017, which provides for the industrial and sanitary inspection of animal products in the country (BRASIL, 2017) and in Normative Instruction 62/2011 of MAPA (BRASIL, 2011). However, variability occurs in the data (standard deviation = 0.24), so that 25.2% of the samples analyzed were below 8.4%. In a database analysis performed in the state of Paraná, Montanhini et al. (2013), a slightly higher average value (8.63%), with 24.6% of non-compliance was observed.

Table 1. Mean values and standard deviations of the analyzed variables.

VARIABLES	MEAN	STANDARD DEVIATION	MINIMUN	MAXIMUM
Volume of marketed milk (kg/month)	8.016.92	8.876.26	901.00	276.264.00
Fat (%)	3.80	0.37	2.00	6.98
Protein (%)	3.17	0.18	2.01	4.28
Lactose (%)	4.38	0.15	3.15	4.97
Defatted Dry Extract (%)	8.55	0.24	7.57	9.58
Somatic cell counts x 1.000 (cell./mL)	637.51	454.50	1.00	9.500.00
Total bacterial Count x 1.000 (UFC/mL)	455.96	869.40	2.00	9.955.00

Non-conformances also occur for the major constituents of DDE (protein and especially lactose content). For the lactose content 24.6% of the samples were below 4.3%, the limit established by decree Nº 9.013/2017 (BRASIL, 2017) and for the protein content 4.7% of the samples were below 2.9 % (BRASIL, 2011; 2017). If we consider non-conformances for DDE, protein and lactose together, 36.4% of the samples showed non-compliance for at least one of these components.

The average volume of milk commercialized (Table 1) shows the profile of dairy farms in the South of Brazil, with the extract formed by the first two quintiles (40% of the samples) selling up to 4,314 liters/month (Table 2), corresponding to approximately 140 liters of milk per day. Dairy farms in this region are mainly composed of small rural settlements, as demonstrated by works in Santa Catarina (WINCK; THALER NETO, 2012; WERNCKE et al., 2016), in Rio Grande do Sul (GABBI et al., 2013) and Paraná (LANGE et al., 2016).

Table 2. Mean percentages of protein, lactose and Defatted Dry Extract (DDE), Somatic Cell Count (SCC) and Total Bacterial Count (TBC), from the small (1st quintile) to the largest farmers (5th quintile), as a function of monthly milk volume marketed, SCC and TBC.

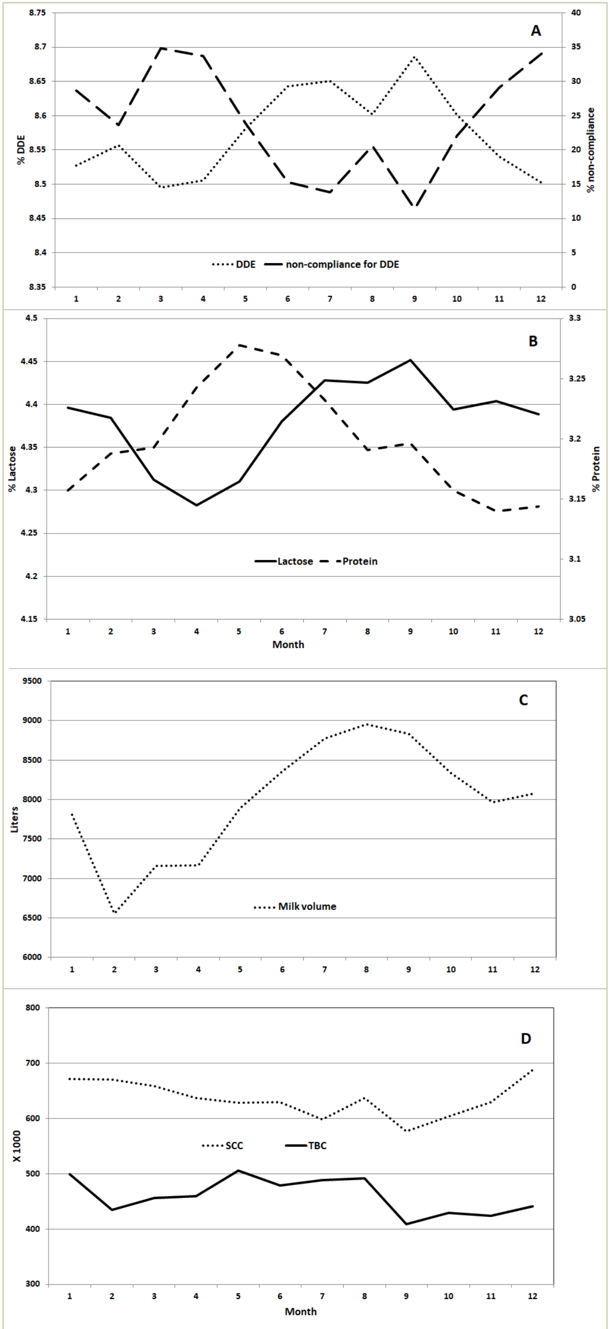
QUINTILE	PROTEIN	LACTOSE	DDE	SCC	TBC
Monthly milk volume marketed (x 1.000 Kg)*					
1° (< 2.599)	3.20a	4.33e	8.53e	661.7	713.2
2° (2.600 a 4.314)	3.20a	4.35d	8.55d	643.9	521.4
3° (4.315 a 6.816)	3.20a	4.38c	8.58c	638.1	420.8
4° (6.817 a 11.442)	3.20a	4.40b	8.60b	628.3	363.4
5° (> 11.443)	3.18b	4.43a	8.61a	621.3	281.8
Somatic Cell Count (SCC x 1.000 cell./mL)*					
1° (< 308)	3.17e	4.45a	8.62a	213.6	333.3
2° (309 a 454)	3.19d	4.41b	8.59b	382.1	367.8
3° (455 a 624)	3.20c	4.38c	8.58c	535.7	384.5
4° (625 a 890)	3.21b	4.35d	8.56d	742.6	440.8
5° (> 891)	3.23a	4.29e	8.52e	1.322.0	775.5
Total Bacterial Count (TBC x 1.000 UFC/mL) *					
1° (< 35)	3.19e	4.43a	8.62a	453.4	19.3
2° (36 a 91)	3.20d	4.40b	8.59b	545.0	59.9
3° (92 a 225)	3.20c	4.38c	8.58c	608.5	148.2
4° (226 a 614)	3.21b	4.36d	8.56d	704.1	380.6
5° (> 615)	3.21a	4.32e	8.54e	884.3	1.696.1

* Values followed by different letters in the same column differ from each other ($P < 0.05$).

The ESD levels (Figure 1A), as well as their main constituents (lactose and protein) (Figure 1B) presented intense variation throughout the year, with lower levels of DDE in summer and fall, with high percentages of non-compliance in some months in these seasons (Figure 1A). On the other hand, the variations for SCC and TBC throughout the year are less pronounced (Figure 1D). According to Heck et al. (2009) the lactose content in milk fluctuates seasonally, so the variation may originate from the diet given to the cows. During the autumn, low levels of DDE are related to the reduction in lactose content (Figure 1B), which had already been demonstrated in dairy farms in Santa Catarina by Alessio et al. (2016). This reduction in the lactose content, and consequently the DDE content, coincides with the lower volume of milk marketed by dairy farmers at this time (Figure 1C).

The lower levels of lactose can be attributed to nutritional deficiency in lactating cows, an aspect that usually occurs at this time of year, especially due to a reduction in the quality and quantity of roughage available. The low quality and availability of roughage can affect food intake, nutrient metabolism and thus the availability of glucose, which is the substrate for lactose synthesis (ALLEN; LEMOSQUET et al., 2009; NRC, 2001; PIANTONI, 2014), thus, reducing production and lactose content. So, the imbalance in the diet that dairy herds receive, especially energy deficiency, can reduce the lactose content and, consequently, the DDE content of milk, since lactose is the main osmoregulator influencing the quantity of milk produced (SANTOS; FONSECA, 2007).

Figure 1. Mean of the minimum squares for Defatted Dry Extract (DDE), percentage of non-compliance for DDE (A), lactose and protein (B), milk volume marketed by farmer (C), Somatic Cell Count (SCC) and Total Bacterial Count (TBC) (D) throughout the year.



The main precursor for the synthesis of lactose is glucose, derived especially from the production of propionic acid, produced in the rumen by the metabolism of fermentable carbohydrates. Two molecules of glucose are required to form a molecule of lactose in the epithelial cells of the mammary gland, where a unit of glucose is converted into galactose and lactose-synthetase catalyzes the reaction of glucose and galactose to form the lactose in the device of Golgi (AKERS, 2002; GONZÁLEZ, 2001). Therefore, the amount of glucose available in the bloodstream is one of the limiting factors in milk synthesis (HARRIS JÚNIOR; BACHMAN, 2003), and about 79% of the circulating glucose is used for the synthesis of lactose in the mammary gland (GONZÁLEZ, 2001; KADZERE et al., 2002; QIAO et al., 2005; RIGOUT et al., 2002).

As lactose can not diffuse out of the secretory vesicles of the Golgi apparatus, a considerable volume of water is drained into the vesicles to balance the osmotic pressure. Thus, the activity of the lactose-synthetase enzyme is necessary for the synthesis of lactose and also for the attraction of water in the mammary secretory vesicles, and is therefore essential in the maintenance of lactation and milk secretion. Lactose is responsible for 50% of milk osmotic pressure, and its content has an inverse relationship with potassium and sodium concentrations in milk (SANTOS et al., 2012). However, milk, in relation to blood plasma, presents an isosmotic relationship favoring the aspect that when the lactose is synthesized the water diffuses in the light of the alveolar lumen, aiming to maintain the isotonic content in relation to the cytoplasm of the alveolar cell. The accumulation of fluid ions in the Golgi vesicles increases the osmotic pressure, also stimulating the attraction of water (GONZÁLEZ, 2001; SANTOS et al., 2012).

Thus, the volume of secreted milk is directly related to the amount of lactose synthesized. Therefore, milk production is strongly related to the food intake available to cows. Non-structural carbohydrates such as starch raise the production of the main precursor of lactose in milk, propionic acid via hepatic gluconeogenesis. So, changes in lactose levels in milk may be indicative of a dietary digestible carbohydrate deficiency to meet the requirements of lactating animals (MÜHLBACH, 2010; NRC, 2001).

The low levels of lactose in autumn are partially offset by the increase in protein levels (Figure 1B), as the lower milk/cow/day production is related to an increase in protein and fat content (ALESSIO et al., 2016). In contrast, the higher levels of lactose in milk sold in winter and spring may be related to the higher quantity and quality of pastures available to lactating cows in southern Brazil, mainly oats (*Avena spp*.) and ryegrass (*Lolium multiflorum*) (NORO et al., 2006). Studies by Borges et al. (2009), Martins et al. (2006) and Gonzalez et al. (2004), in the state of Rio Grande do Sul, involving research on milk production systems, also observed an intense variation in DDE levels throughout the year. Borges et al. (2009) reported a lower volume of milk produced and marketed per farmer during the fall. Martins et al. (2006) observed lower levels of DDE in autumn due to the low lactose content, when pastures had a lower crude protein content and a higher fiber content, with a consequent reduction in the daily production of milk per cow. Gonzalez et al. (2004) observed lower values of DDE in late summer and early fall, mainly related to the reduction in milk protein levels, coinciding with the season of lower quality of forage available.

During the summer, a reduction in DDE content was observed as a consequence of the decrease in protein and lactose contents (Figure 1B). This fact may be related to the stressful heat conditions to which the cows are subjected. Wheelock et al. (2010) observed a daily reduction of 200 to 400g in the lactose production of dairy cows under heat stress, with glucose being consumed at a higher rate in cows under these conditions. There is an increase in glycolysis and anaerobic respiration to maintain energy balance during situations of heat stress (TIAN et al., 2015) and the decrease in glycemia may explain the reduction of lactose content (SHWARTZ et al., 2009). Muscle catabolism also increases during heat stress, with a higher concentration in plasma urea nitrogen, favoring a greater redistribution of protein nitrogen to urea (WHEELOCK et al., 2010), and decreasing the ability of protein synthesis of mammary cells, reducing the casein content (BERNABUCCI et al., 2002). In addition, there is also a reduction in feed intake by dairy cows under heat stress (RHOADS et al., 2009), which may reduce lactose and protein levels.

Milk samples from dairy farms with a lower production scale have lower levels of DDE throughout the year (Table 2 and Figures 2A and 3A). It was observed that the seasonal variations occurred in all samples, regardless of the volume of milk produced, however, the DDE content increases linearly with the increase in the volume of milk commercialized. Gabbi et al. (2013), analyzing data from dairy herds in the states of Rio Grande do Sul and Santa Catarina, it was observed that the greatest differentiation among dairy farms was due to the different ways that the dairy cows were fed. Small farms usually have a lower capacity to maintain adequate feeding conditions throughout the year, with less forage conservation capacity and a predominantly lower level of technology.

The commercialized milk volume is also related ($P < 0.0001$) with lactose and protein contents. However, the variability observed in the levels of DDE as a function of the volume of milk marketed is especially due to the lactose content, with a lower difference in protein content, contrary to the DDE and lactose contents, and the samples of milk from the last quintile producers (who sell the largest volume of milk/month) presented a lower protein content (Table 2). This can be related to the fact that intensive production systems usually result in a higher average daily milk yield/ per cow (WERNCKE et al., 2016), and cows with higher milk yields tend to have lower milk protein levels (ALESSIO et al., 2016). In the rumen fermentative processes, propionic acid is produced, which has as its site of absorption and metabolism in glucose the liver, therefore, as discussed above, glucose plays an important role in the synthesis of lactose (ALLEN; PIANTONI, 2014; LEMOSQUET et al., 2009; POLLOTT, 2004; NRC, 2001). Milk protein levels are determined by synthesis of the mammary gland epithelium through the use of amino acids extracted from the bloodstream and absorbed in the intestine, the main source of which is the ruminal microbial protein (PATTON et al., 2014; NRC, 2001).

The SCC levels were related to the DDE content homogeneously throughout the year (Figure 2B), and samples with high SCC had lower levels of DDE (Table 2), with a linear decrease in DDE content with an increase in SCC (Figure 3B). It was observed that for the 5th quintile (above 891 thousand somatic cells/ml), in some months of the year, the mean levels of DDE were below the established minimum limit (Figure 2B), corroborating with the results obtained by Montanhini et al. (2013). This effect is due to the reduction of the lactose content resulting from the increase in SCC, since the protein content increased slightly with the higher SCC (Table 2). The reduction of lactose contents due to the increase in SCC was demonstrated by Alessio et al. (2016). The reduction of lactose content due to subclinical mastitis, indicated by high SCC, is due to factors such as changes in mammary gland homeostasis (PESSOA et al., 2012) and by increased vascular permeability, resulting in lactose leaving the mammary alveolus to the bloodstream due to less cohesion between the tight junctions of the secretory cells (STUMPF et al., 2013).

Figure 2. Mean of the minimum squares for Defatted Dry Extract content (DDE) as a function of the monthly volume of milk marketed (A), Somatic Cell Count (SCC) (B) or Total Bacterial Count (TBC) from the small (1st quintile) to the largest farmers (5th quintile), according to the months of the year.

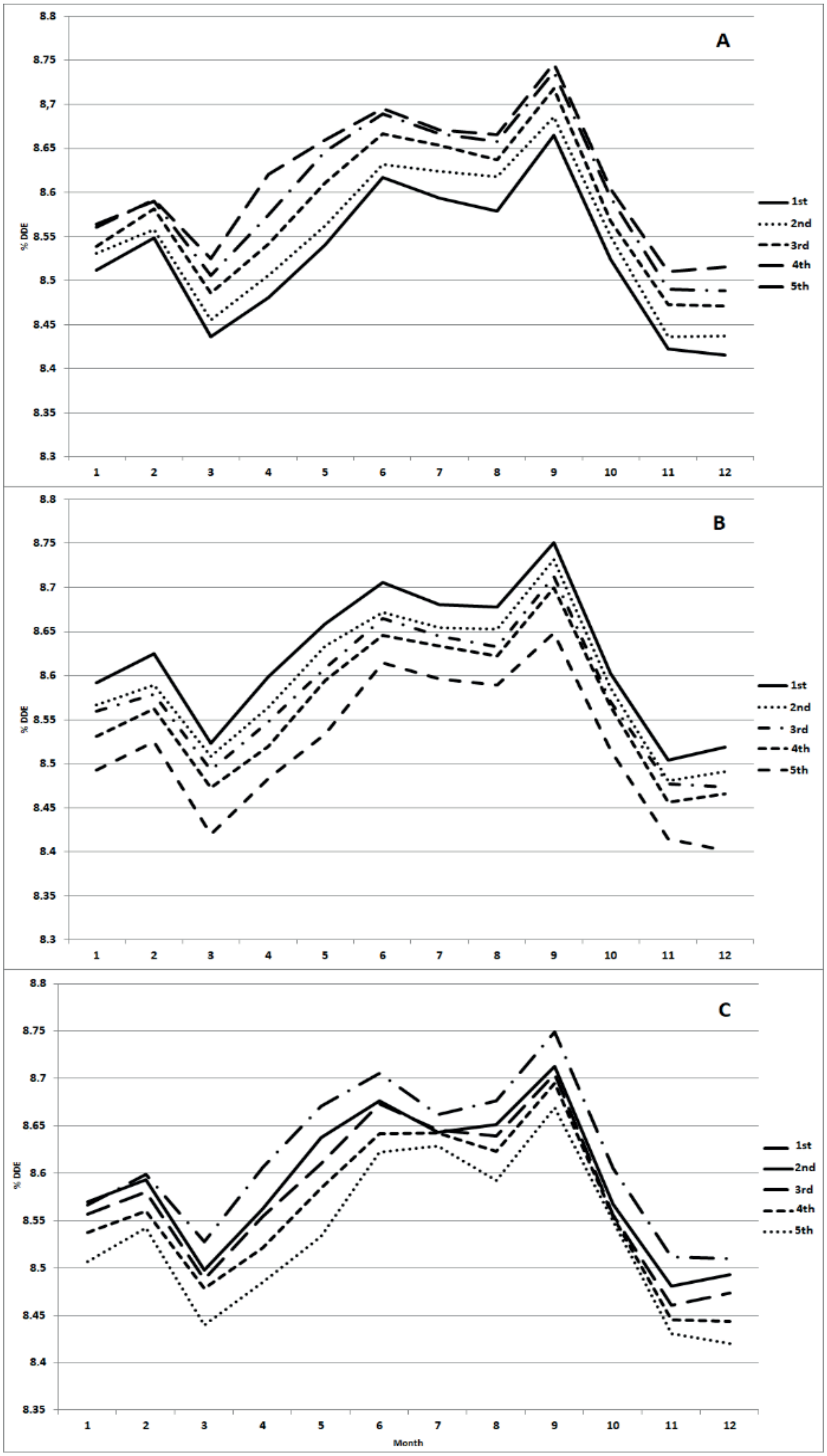
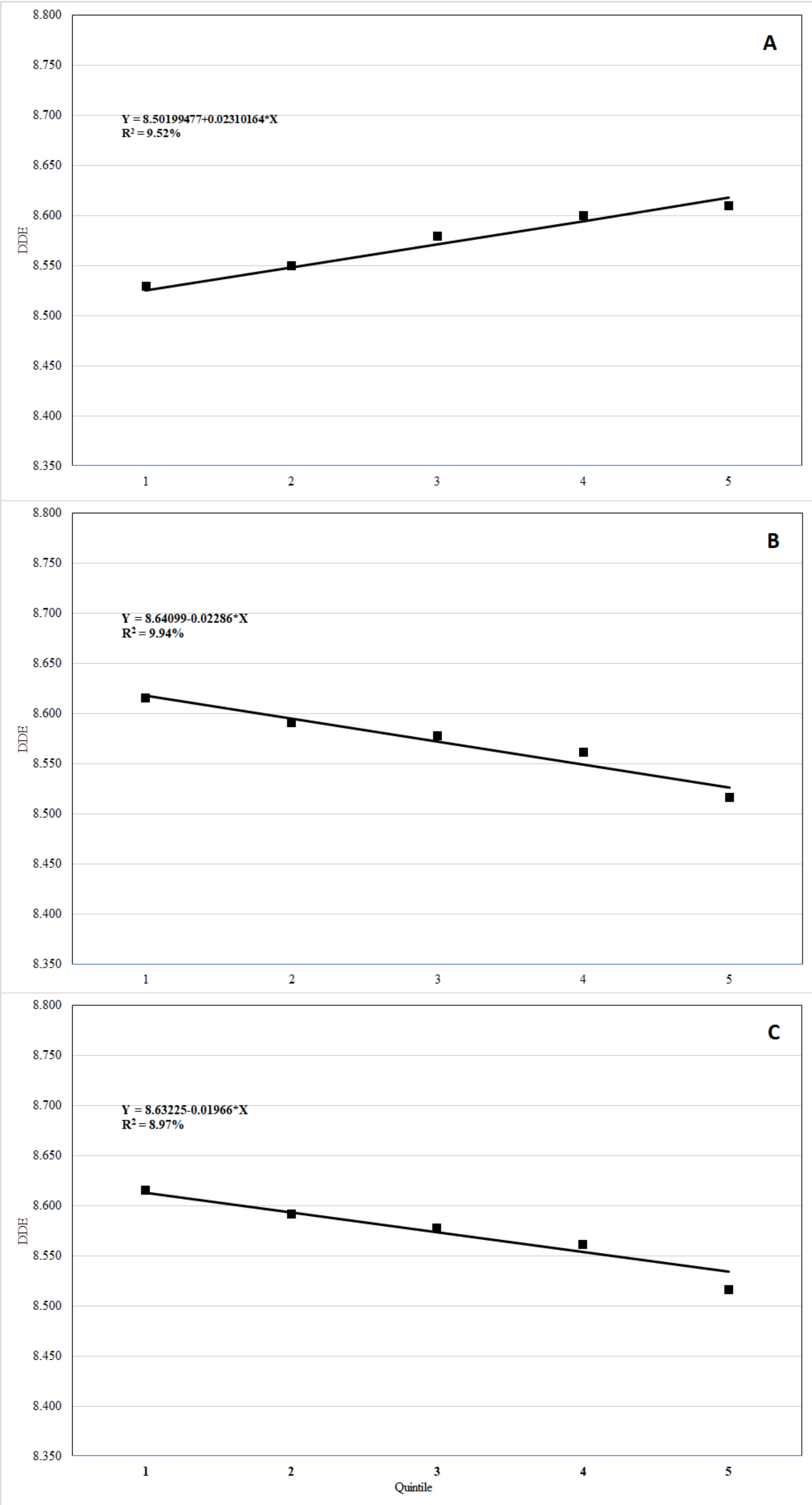


Figure 3. Linear regression and average of the minimum squares for the Defatted Dry Extract content (DDE) as a function of the monthly volume of milk marketed (A), Somatic Cell Count (SCC) (B) or Total Bacterial Count (TBC) (C) as a function of the quintile.



The TBC is related to the DDE content (Figure 2C) similar to the effect of the high SCC, with lower DDE values being verified in the samples with high TBC, with a linear decrease in the DDE content with the increase of the quintiles for TBC (Figure 3B). The negative effect of TBC on DDE is also due to the variation in lactose content, as is the effect of SCC on DDE (Table 2). Forsback et al. (2011) also demonstrated a reduction in the lactose content with the increase of TBC. Lactose, as the main carbohydrate in milk, is an important source of food for microorganisms (BLUM et al., 2008), and increased microbial contamination reduces the lactose content. Samples of milk from larger-scale producers had lower TBC (Table 2).

Conclusions

There is a reduction in the milk contents of defatted dried extract (DDE) in autumn, due to a lower lactose content and, in the summer, due to lower levels of lactose and protein, with a high percentage of milk samples from cooling tanks with DDE below the established minimum of 8.4% at these times of the year.

Seasonal variation occurs similarly in dairy farms that market small or large milk volumes, but DDE levels are lower in small dairy farms throughout the year.

High total bacterial count (TBC) and, mainly, high somatic cell counts (SCC) are important causes of the reduction of milk DDE.

Acknowledgements

To the Union of Dairy Industries of Santa Catarina (SINDILEITE) and to the dairy industries for allowing the use of the data for the accomplishment of this study.

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