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MICROBIOLOGY

Retrospective evalution of non-fatty solids in samples of raw milk in the state of Rio Grande do Sul according to season, Brazil

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ABSTRACT: *The present study described the chemical composition and somatic cell score (SCS) of samples of refrigerated raw milk collected from commercial farms in the state of Rio Grande do Sul in order to better understand the behavior of constituents present in non-fatty solids (NFS) in milk according to the season of the year. Means were used to describe statistical data. To estimate the probability of NFS levels meeting IN 76 (BRAZIL, 2018), binary logistic regression was used. It was reported that 18.2% (233.817) of analytical results showed NFS below 8.4%, representing the minimum required by IN 76. The highest average NFS level observed in the five-year period was registered in the micro-region of Passo Fundo (8.70%) in winter. The microregion with the lowest results was Porto Alegre (8.53%); however, it still demonstrated levels within the limits established by IN 76. The study indicates that milk constituents show differences between seasons. In autumn and winter, the constituents remained equal to or higher than those required by current legislation, while spring and summer were the periods with the lowest NFS values. The SCS was also influenced by the seasons, with the highest rates in spring, summer, and autumn.*
Key words: *environmental factors, microregion, milk quality, lactose, protein.*

Estudo retrospectivo dos sólidos não gordurosos em amostras de leite cru no estado do Rio Grande do Sul de acordo com as estações do ano

RESUMO: *O presente estudo teve como objetivo descrever os resultados de composição química e escore de células somáticas (ECS) de amostras de leite cru refrigerado coletado em fazendas comerciais no estado do Rio Grande do Sul, para melhor entendimento do comportamento dos constituintes presentes nos sólidos não gordurosos (SNG) no leite de acordo com as estações do ano. As médias foram estudadas para descrever as estatísticas dos dados. Para estimar a probabilidade de os teores de SNG atenderem à IN 76 de 2018, foi utilizada a regressão logística binária. Foi constatado que 18,2% (233.817) dos resultados analíticos apresentaram SNG abaixo de 8,4%, que representa o mínimo exigido pela IN 76 (BRASIL, 2018). A maior média de SNG observada no período de cinco anos foi registrada na microrregião de Passo Fundo (8,70%), no inverno. A microrregião com menores resultados foi a de Porto Alegre (8,53%), no entanto com teores dentro do estabelecido pela IN 76/2018. O estudo demonstrou que os constituintes do leite apresentaram diferenças entre as estações do ano. O outono e inverno foram os períodos em que os constituintes se mantiveram iguais ou superiores aos exigidos pela legislação vigente, enquanto que a primavera e o verão foram os períodos com os menores valores de SNG. O ECS também foi influenciado pelas estações do ano. Na primavera, verão e outono ocorreram os maiores índices.*
Palavras-chave: *fatores ambientais, microrregião, qualidade do leite, lactose, proteína.*

INTRODUCTION

Brazil is the fifth largest milk-producing country (22 thousand tons), followed by Pakistan, China, and India (FAO, 2021; IEA, 2021). Milk production has great social and economic importance for the country, as it allows producers to remain in rural areas, ensuring income and good living conditions. The state of Rio Grande do Sul is the third most productive of milk producers in Brazil, after Minas Gerais and Paraná, with productivity of 4.129 liters/year and approximately 65.202 producers linked to industries that process milk (EMATER/ASCAR, 2021; IBGE, 2021; RIES, 2021). Among the main challenges for producers is to ensure the quality of the milk produced at all times of the year,

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even in times of food shortages and unfavorable environmental and climatic conditions, such as in the hottest seasons. Milk quality is of great importance for food safety and is directly associated with industrial yield, evaluated through its physical-chemical and microbiological attributes (ARRUDA JUNIOR et al., 2019). Among the main chemical components of milk are non-fat solids (NFS) made of protein, lactose, and minerals; levels of these vary according to changes in diet, genetics, health, days in lactation, environmental temperature, and handling (ARRUDA JUNIOR et al., 2019). The abrupt reduction in the content of milk components, such as total solids, is a known problem, easily identified in the laboratory, and may be related to adulteration and fraud (DIAS & ANTES, 2014).

The industry and sanitary inspection bodies report a decrease in the concentration of NFS at certain times of the year and regions in the state of Rio Grande do Sul, especially in the warmest months, due to intrinsic and extrinsic factors regarding the animals. This decrease causes losses to producers due to the lower remuneration received, and to the industry due to decreased yield of dairy products. Thus, the reduction in lactose, which makes up 50% of NFS, can cause difficulties in the coagulation and fermentation of dairy products. The decrease in protein may compromise the coagulation of dairy products, resulting in a bitter taste and lower cheese yield (ALHUSSIEN & DANG, 2018; COSTA et al., 2019).

The present described the chemical composition and SCS results of refrigerated raw milk samples collected from commercial farms in the state of Rio Grande do Sul to better understand the behavior of the constituents present in non-fat solids milk, according to the seasons. It is expected that the results will contribute to a better understanding of the causes that interfere with the quality of milk in order to avoid milk condemnation and assessment due to non-compliance with Normative Instruction 76 of the Ministry of Agriculture, Livestock, and Supply (BRASIL, 2018).

MATERIALS AND METHODS

Use of data from different dairy facilities was authorized by the Union of Dairy and Derived Products Industry of the state of Rio Grande do Sul (Sindilat, RS). Information from five years, 2014 to 2018, was used, totaling 1.281.234 quality records of refrigerated raw milk, collected in expansion tanks on commercial farms and analyzed at the Service of Analysis of Dairy Herds (SADH) of the University of Passo Fundo.

Milk composition was analyzed by Fourier transform near-infrared (FTNI) technology and somatic cell count (SCC) by flow cytometry using equipment with Delta Equipment System. The desired time interval was edited in the original database and stored in an Excel® computer file, in which each row represented a monthly tank sample and the columns contained the following groups of information: a) sample identification: city, region of location, and sample identification; b) monthly meteorological data classified according to the season of the year: total precipitation and average temperature; c) analytical results: fat content (g/100g), protein (g/100g), lactose (g/100g), total solids (ST) (g/100g), non-fat solids (NFS) (g/100g), and somatic cell count (SCC). The SCC was the only component that did not show normality, so it was transformed into log x 1000 for structuring the base and removing outliers, and the results were expressed as somatic cell score (SCS), according to the methodology proposed by BONDAN et al., (2018).

Meteorological data were obtained from the National Institute of Meteorology (INMET) through historical data from the Meteorological Database for Teaching and Research containing information regarding daily measurements, in accordance with the international technical standards of the World Meteorological Organization. For this study, monthly data from six weather stations were used: São Luiz Gonzaga, Cruz Alta, Passo Fundo, Santa Maria, Caxias do Sul, and Porto Alegre from 2014 to 2018. The variables used were total precipitation and compensated average temperature.

Division of regions for evaluating meteorological variables (Table 1) was performed based on the classification of regions according to the Rio Grande do Sul Meteorological Bulletin for rainfall as described by FEPAGRO (2014). The variables total precipitation and average temperature compensated were inserted in the analytical models (Table 1).

For statistical analysis and database consistency, records were considered in annual classes. Moderated outliers were excluded from the original file using the methodology proposed by FAVERO & BELFIORE (2017). Thus, the following intervals were considered for the five-year period: fat $\geq$ 2.70 and $\leq$ 5.14; protein: $\geq$ 2.63 and $\leq$ 3.84; lactose: $\geq$ 3.01 and $\leq$ 4.80; total solids: $\geq$ 10.71 and $\leq$ 14.29; CCS: log x 1000 $\geq$ 0.0 and $\leq$ 3.61; NFS: $\geq$ 7.81 and $\leq$ 9.46. Information related to the desired time interval was edited from the original database and stored in an Excel computer file. Each line represented a monthly

Table 1 - Meteorological station, micro-region of studies, latitude, and longitude of the respective studied units.

Weather station	Microregion	Latitude	Longitude
São Luiz Gonzaga	São Luiz Gonzaga	-28.40	-55.01
Cruz Alta	Cruz Alta	-28.63	-53.60
Passo Fundo	Passo Fundo	-28.21	-52.40
Santa Maria	Santa Maria	-29.70	-53.70
Caxias do Sul	Caxias do Sul	-29.16	-51.20
Porto Alegre	Porto Alegre	-30.05	-51.16

Source: INMET (2019) adapted by author.

tank sample, and the columns contained three groups of information with herd identification and analysis date of the sample. The division of the months according to the seasons of the year was carried out in such a way that Spring consisted of the months of October, November and December; Summer: January, February, and March; Autumn: April, May, and June; Winter: July, August, and September. For structuring the database, Excel® 2016 applications were used, and for the analysis, the R language version 3.6.1 (R CORE TEAM, 2019).

To analyze the effects, the following statistical tests were used: a) summary measures were applied, such as a number of observations, mean, standard deviation, standard errors, minimum and maximum values, percentiles, and frequency tables to present the proportions of the categories of treatments studied and describe the statistics of the data; b) to compare a quantitative variable with another categorical one generated from two independent groups, the Student's t-test was used; c) for comparing more than two independent groups, ANOVA was used. For multiple comparisons, Tukey's post-hoc test was used; d) for comparing two categorical variables, the chi-square or Fisher's exact tests were used; e) to estimate the probability of occurrence of the phenomena under study in relation to the dependent variables of these phenomena, depending on the explanatory variables inserted in the respective models, binary logistic regression was used according to the equation:

$$Z_i = \alpha + \beta_1 . X_{1i} + \beta_2 . X_{2i} + ... + \beta_k . X_{ki}$$

The general expression of the estimated probability of occurrence of an event was calculated as follows according to the methodology proposed by FAVERO & BELFIORE (2017):

$$p_i = \frac{1}{1 + e^{-(\alpha + \beta_1 . X_{1i} + \beta_2 . X_{2i} + ... + \beta_k . X_{ki})}}$$

Evaluation of normality was performed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and the Levene test was applied to assess the homogeneity between the variances. The significance level used in the tests to reject H0, when the null hypothesis is true, was 0.05 (FAVERO E BELFIORE, 2017).

RESULTS AND DISCUSSION

Over the five years of the study, it wasreported that 18.2% (233,817) of the analytical results showed NFS below 8.4%, representing the minimum required by Normative Instruction 76 (BRASIL, 2018).

The highest average NFS observed in the period of five years was registered in the micro-region of Passo Fundo (8.70%), in winter. It was observed for this microregion (Figure 1) that the average NFS oscillation was, in ascending order, 0.7% in autumn, 0.11% in summer, and 0.15% in spring when compared to the season with the highest value (winter). In all microregions studied, spring was the season with the lowest SNF values. When analyzing the quality of milk in different growing seasons for the regions of Vila Maria (a micro-region belonging to Passo Fundo), SANTOS et al. (2013) noticed that NFS content was affected by the season of the year, recording an average SNF of 8.57% in winter and 8.42% in summer, values below those recorded in this study. When studying the production and milk quality of Holstein cows according to season and calving order, SOUZA et al. (2012) reported that the feeding management that the animals receive in

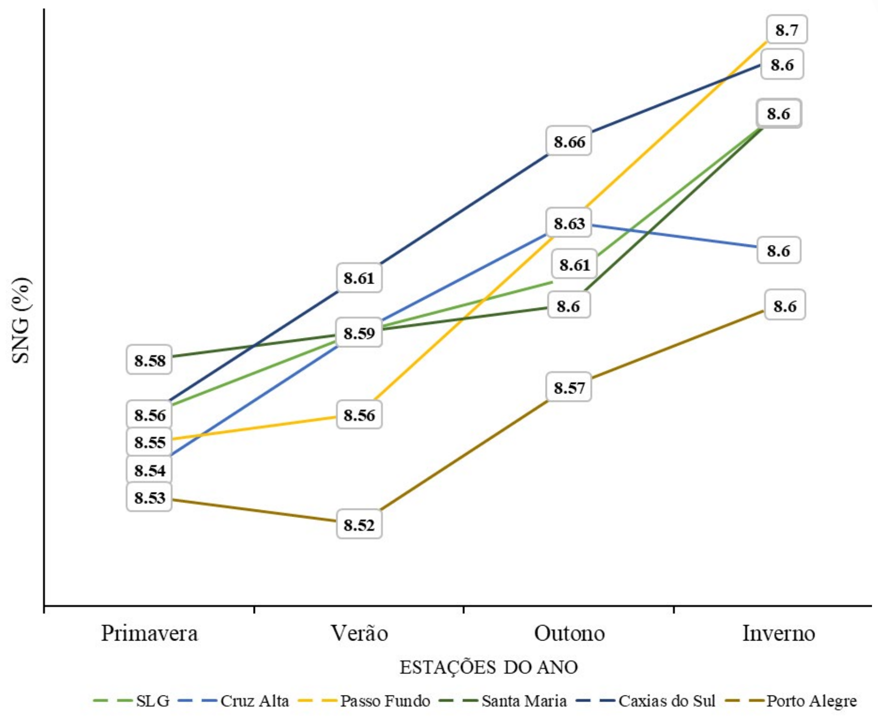

Figure 1 - Mean values of non-fat solids, according to microregion, of 1.281.234 samples of raw milk analyzed in the period 2014 - 2018 by SARLE.

summer (pasture with voluminous supplementation of corn silage and concentrate) negatively affects milk production of animals that calve in the spring. The authors also state that animals that calve in the spring and reach peak lactation at this time have the lowest milk production due to the heat stress to which they are subjected. The microregion with the lowest results in all seasons of the year was Porto Alegre; however, its values were still within those established by IN 76/2018.

Table 2 presents effects of the season on milk components. Positive residue analysis indicates the possibility of the event occurring in that place according to the Chi-square test (FAVERO & BELFIORE, 2017).

The highest probability of NFS levels being equal to or above 8.40% occurred during autumn and winter (Table 2). Concurrent with these results, and because they directly influence the levels of SNF (ARRUDA JUNIOR et al., 2019), it was observed that the highest probability of protein levels being within the standards established by current legislation (above 2 .9%) was observed in autumn and winter (96.4%) (Table 2). In the winter months, higher values of protein, lactose, and fat are observed. This fact is probably due to the feeding of the herds with cold-season grasses, showing the importance of forages in the composition of milk. Cold-season grasses have less neutral detergent fiber (NDF), and this positively influences milk digestibility, productivity, and quality (FONTANELI et al. 2012; NORO et al., 2006). It is assumed that the potential for altering the protein content of milk through nutrition is not very large, around 0.10 to 0.20 percentage points, since the main need of ruminants is for amino acids and not crude protein (MALACCO et al., 2015; PERES, 2001). Amino acids are important as the forming elements of proteins; that is, the lack of a single amino acid inhibits the formation of an entire protein molecule and impairs the efficiency of the mammary gland in the formation of a certain amount of protein (PERES, 2001). Amino acid supplementation in spring and summer is used as an alternative to overcome the nutrient deficit at these times. However, the cultivation and storage of foods with high biological value may be the most economically viable alternative. Foods

Table 2 - Association between the levels of non-fat solids (NFS) protein, lactose and somatic cell score (SCS) in different seasons, considering results that do and do not comply with those established by IN 76/2018.

Seasons		----------------------------NFS---------------------------		---------------------------Protein--------------------------	
		< 8.4 g/100 g	≥ 8.4 g/100 g	< 2.9 g/100 g	≥ 2.9 g/100 g
Spring	N	+	-	+	-
Summer	N	+	-	+	-
Autumn	N	-	+	-	+
Winter	N	-	+	-	+
Total	N	233817 (18.2%)	1047417 (81.8%)	46361 (3.6%)	1234873 (96.4%)
		--------------------------Lactose-------------------------		-----------------------------SCS----------------------------	
---------Seasons--------		< 4.3 g/100 g	≥ 4.3 g/100 g	< 500 x 10³ cells/mL	≥ 500 x 10³ cells/mL
Spring	N	-	+	-	+
Summer	N	+	-	-	+
Autumn	N	+	-	-	+
Winter	N	-	+	+	-
Total	N	314519 (24.5%)	966715 (75.5%)	537711 (42%)	743523 (58%)

**Residual analysis: (+) Positive significant association; (-) Negative significant association.

such as silage and hay are used mainly during spring forage emptiness, when winter pastures begin to age and summer pastures are still being prepared.

Lactose levels were most likely to be above 4.30% in spring and winter, a level that captures 75.50% of the samples (966.715). The decrease in lactose levels in summer and autumn can be attributed to the energy present in the diet in these seasons. It is known that changes in energy supply affect milk production and composition and can be modified according to the productive potential of each animal, stage of lactation, and feed supply, or when there is a deficiency in digestible carbohydrates from the diet (ARRUDA JUNIOR et al., 2019; HECK et al., 2009; GABBI et al., 2018). In addition, the number of lactations is a factor of great importance in the composition of lactose, since there is a linear decrease as the number of lactations increases. Furthermore, high SCS are negatively correlated with lactose (BONDAN et al., 2018). All these factors must be analyzed when we seek to understand the reasons that lactose levels are likely to be above the minimum established by IN 76 only in spring and winter.

For SCS levels, spring, summer, and autumn were more likely to be equal to or above the standards established in the legislation (<500.000 cells/mL). Summer is the season with the highest number of somatic cells, which may be indicative of health problems in the herd (BONDAN et al., 2018). MACHADO et al., (2000) report that the SCC of samples from the refrigeration tank indicates the occurrence of mastitis and that tanks with higher SCS averages present greater oscillation in the milk constituents. TORRES et al., (2016) state that a somatic cell count above 600.000 cells/mL results in a 28% probability of mastitis in the herd.

CONCLUSION

Through description of the levels of non-fat solids (NFS) in milk produced in the state of Rio Grande do Sul, it was shown that they are within the parameters established by the legislation only in the autumn and winter seasons. The lowest NFS values were described in spring in all studied microregions. The somatic cell score (SCS) was high in the spring, summer, and autumn seasons.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest. Funding sponsors had no role in the study design, or in the collection, analysis, or interpretation of data.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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