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RELATIONSHIP BETWEEN SEASONAL VARIATION IN THE COMPOSITION OF BULK TANK MILK AND PAYMENT BASED ON MILK QUALITY

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ABSTRACT

Payment programs based on milk quality (PPBMQ) are important in the dairy sector as they enable farmers to improve profitability upon reaching payment based on milk quality (PBMQ). We used data submitted to a PPBMQ from a dairy farm referring to a four-year period (January 2013 − December 2016). Correlation, multiple regression, and principal component analysis were performed. We found significant correlations between PBMQ and fat (r = 0.32), protein (r = 0.51), and total bacterial count (TBC) (r = -0.66), as well as an effect of all studied variables on PBMQ using multiple regression analysis (with somatic cell count [SCC] also affecting PBMQ). Thus, protein and fat positively and SCC and TBC negatively affected PBMQ value. Principal component analysis revealed an inverse relationship between summer and winter months. In summer months, the PBMQ was affected by the increase of TBC and SCC and decrease protein, whereas in winter months, protein increase and TBC and SCC decrease were relevant. A varied behaviour was detected for the remaining months. Milk components (fat, protein, SCC, and TBC) significantly affected the final value the PBMQ paid to the farmer. Moreover, there was seasonal effect on PBMQ, with PBMQ being higher in winter months and lower in summer months. Variation in milk composition and payment due to the seasonality should be considered by farmers to reach higher values of bonuses, and by the dairy sector to plane adequate payment throughout the year.

Key words: dairy science; milk production; multivariate analysis; principal component analysis

INTRODUCTION

In Brazil, farming dairy cattle is one of the main economic activities, reaching a total of 35 billion of litres of milk produced in 2015 (EMBRAPA, 2017). However, the milk produced is of lower quality than that in the other countries (England, Germany, Italy and Canada), mainly with respect to the total bacterial count (TBC) and somatic cell count (SCC) (Cassoli *et al.*, 2016; Cassoli and Machado, 2016). Together with TBC and SCC, milk compounds (fat, protein, and lactose) are very important to dairy companies and industries because all these five variables directly affect the yield of milk products (More, 2009; Geary *et al.*, 2014; Meneghini *et al.*, 2016; Murphy *et al.*, 2016).

The use of payment programs based on milk quality (PPBMQ) constitutes one of the approaches used by dairy companies and industries to sensitise and incentivise the dairy farmers to improve the milk quality. Such programs seek to improve the milk quality via a monetary incentive paid by litre of milk (Busanello *et al.*, 2017a) and are based on the levels of certain milk compounds, mainly SCC, TBC, protein,

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and fat. These programs commonly use payment systems with bonuses, penalties, or a mixed system (bonuses and penalties) (Huijps *et al.*, 2010). However, programs with an approach based only on penalties appear to be the most effective in sensitizing the dairy farmers to improve milk quality (Valeeva *et al.*, 2007).

Studies with PBMQ, as the above-mentioned, used only univariate approaches. Although some of them (for example, Busanello *et al.*, 2017a) used multiple regression analysis, that approach many times is associated as a multivariate one, but being a misunderstanding because only one response variable is used (Rencher, 2002). Therefore, in the present study, we used univariate analyses (correlation and multiple regression) in addition to a multivariate analysis (principal component analysis [PCA]). In particular, PCA enables the creation of linear combinations between the original variables while maintaining their multiple inter-relationships, as well as enabling the characterization of observations derived from resultant new variables, which are termed principal components (Manly, 2004). Specifically, we aimed to study the relationship between variables of milk composition (fat and protein) and quality (SCC and TBC) with PBMQ paid to the farmer, as well as to characterize the months of the year in regard to these milk variables and PBMQ through PCA using data from a commercial dairy farm that was submitted to a single PPBMQ.

MATERIAL AND METHODS

We used the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (Sargeant and O'Connor, 2014) statement as a guideline for this research, in which it was designed as an observational retrospective longitudinal study. The data regarding milk composition and quality represent the period of January 2013 until December 2016 and were provided by the Unidade Educativa de Bovinocultura de Leite (UEBL) at the Escola Estadual Técnica Celeste Gobbato (EETCG), which is a technical school of farming/agriculture. This school is located in Palmeira das Missões County (latitude: 27° 53' 58'', longitude: 53° 18' 49'', and altitude of 639 metres), in the Northwest Region of the Rio Grande do Sul state, Brazil.

The UEBL worked with an integrated crop-livestock system, in an area with 27 hectares that were designated for milk production and herd handling. The herd was composed of an average of 25 lactating dairy cows (of different ages, number of lactations, and days in milk), and all cows were of the Holstein breed. Moreover, an average of 40 additional animals was present in other categories (calves, heifers, and dry cows). The UEBL had the following facilities: milking parlour, feeding shed, and shed for calves and heifers. The milking parlour was a herringbone type with piped milking equipment with four closed-circuit claws and a room for the milk cooler. Feeds offered in the feeding shed were corn silage (*Zea mays*), ryegrass hay (*Lolium multiflorum*), and concentrated feed. Each cow received a quantity of concentrated feed according to its milk production.

The milking was performed twice daily at 5:30 and 16:00 hours, and was performed by the students of the technical school. Pre-dipping, withdrawal of the first three milk jets in the background of a black mug, use of individual paper towels to dry the teats, and post-dipping were implemented. Every 15 days, the California Mastitis Test was performed to detect possible cases of subclinical mastitis.

After milking, the milking equipment was washed out with sanitizer for 5 minutes, and then rinsed with water at 40 °C. In the sequence, a chlorinated detergent alkaline solution (pH > 11) was used to wash out the milking equipment with water at 70 to 75 °C for 10 minutes. Finally, an acid detergent was used (pH < 3) with water at 30 to 35 °C for 5 minutes and a final rinse was made after that.

Cows were maintained in a semi-confinement system where they had access to pasture after milking in the morning (until 11:00 hours) and at 13:30 until 16:00 hours. Moreover, the cows also had access to pastures after the 16:00 hours milking in the summer. In the winter, ryegrass (*Lolium multiflorum*) and double-purpose wheat (BRS Tarumã; *Triticum aestivum*) pastures were used, whereas in the summer, sorghum (*Sorghum bicolor*) and Tifton 85 (*Cynodon spp.*) were used. The pasture area was divided into paddocks and water was available when the cows were in the feeding shed and waiting room.

Cows were handled according to the guidelines of the Program of Good Practices in the Farm as outlined by the dairy company that purchased the milk from the EETCG. In particular, all the cows were identified with earrings, care was taken in the production and storage of the feeds offered to the animals, and an exclusive area for cows was provided during the pre-partum. Cows were also identified for milking. Cows with a blue collar were in a transition period, those with a yellow collar were producing colostrum, and cows with a red collar were medicated (for example, with antibiotics), indicating that their milk should be discarded.

For milk analysis, two to four bulk tank milk samples were collected by the dairy company that purchased the milk from the EETCG (one sample per week or each 15 days). Milk samples were sent to Serviço de Análise de Rebanhos Leiteiros (SARLE) at the University of Passo Fundo, which is certified by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) of Brazil, where total dry extract (TDE), defatted dry extract (DDE), lactose, protein, and fat were analysed using near-infrared spectroscopy (Bentley 2000, Bentley Instruments, Chaska, MN, USA) according to the ISO 9622. The SCC and TBC were also analysed but using flow cytometry (Somacount 300, Bentley Instruments) according to the ISO 13366-2. The methods cited above are described by INMETRO IEC 17025:2002 considering that the dairy company remunerates according to milk composition and quality.

The dairy company that purchased the milk from EETCG applied the PPBMQ with regard to milk composition (fat and protein) and milk quality (SCC and TBC). The value paid was calculated considering PPBMQ from a payment table provided by the dairy company, where the values of PBMQ were actualized in June 2017 and such values were used as a reference to calculate the PBMQ (Table 1). The PPBMQ was based on a mixed system (bonuses and penalties) for all the variables (fat, protein, SCC, and TBC). Lactose was not included in the payment system and, because of it, this variable was not used in the statistical analysis of our study.

Table 1. Payment by milk quality table with bonus and penalty values based on milk composition and quality*

Variable	Classes	Payment (in R$)[1]	Payment (in milk-equivalent litres)[1]
Fat Content (g.kg^{-1} in milk)[2]	20.00 – 29.90	-0.029	-0.023
	30.00 – 32.90	0.000	0.000
	33.00 – 36.30	0.013	0.010
	36.40 – 39.70	0.028	0.022
	39.80 – 50.00	0.034	0.027
Protein Content (g.kg^{-1} in milk)[2]	20.00 – 24.90	-0.079	-0.062
	25.00 – 28.90	-0.028	-0.022
	29.00 – 30.90	0.000	0.000
	31.00 – 33.80	0.030	0.024
	33.90 – 36.90	0.083	0.066
	> 37.00	0.100	0.079
Somatic Cell Count (× 1000 cells.mL^{-1})	1.00 – 200.00	0.060	0.047
	201.00 – 400.00	0.040	0.032
	401.00 – 500.00	-0.010	-0.008
	> 501.00	-0.030	-0.024
Total Bacterial Count (× 1000 cfu.mL^{-1})	1.00 – 500.00	0.040	0.034
	51.00 – 100.00	0.030	0.024
	101.00 – 200.00	0.000	0.000
	201.00 – 300.00	-0.020	-0.016
	> 301.00	-0.040	-0.032

*Milk composition – fat and protein content, Milk quality – somatic cell count and total bacterial count, [1]In Payment '-' indicates penalty, [2]Average values, original table with the authors.

The values of PBMQ are presented in Reais (R$; Brazilian currency) and in milk-equivalents, a measure that reflects the financial value equal to one litre of milk, which was also used by Martins *et al.* (2003) and Busanello *et al.* (2017a). Milk-equivalents were provided with the intent that readers could extrapolate our results to other currencies and facilitate the understanding of PBMQ data for other countries. Thus, PBMQ values were calculated in milk-equivalents by dividing them by R$ 1.2669, the average price of a litre of milk in Brazil considering the year of 2017 (Center for Advanced Studies in Applied Economics – CEPEA; College of Agriculture "Luiz de Queiroz"/University of São Paulo – ESALQ/USP). The value of R$ 1.2669 represents US$ 0.3969 and € 0.3475, considering the average value of one Real for the dollar and euro for 2017 of US$ 3.1290 and € 3.6462, respectively.

For the statistical analyses, first, an exploratory data analysis was performed using boxplots to find possible outliers and uncommon values for the variables of fat, protein, SCC, TBC, and PBMQ (in R$ and milk-equivalents). Values of minimum, maximum, median, interquartile range, arithmetic and geometric means, and standard deviations were also calculated for the above-mentioned variables.

Subsequently, correlation analysis between milk composition and quality (fat, protein, SCC, and TBC) and PBMQ (in milk-equivalents), aiming to understand their relationships, was performed. Accordingly, the Spearman correlation coefficient (nonparametric method) was used, considering that SCC and TBC were variables that did not present normal distributions, which is an assumption for Pearson correlation analysis.

Next, a multiple regression analysis to verify which variables were significant with respect to the PBMQ paid to the farmer was performed. In addition, this analysis also enables the acquisition of subsequent estimates of PBMQ based on the milk quality and composition. For this, PBMQ in milk-equivalents was used as a response variable, whereas fat, protein, SCC, and TBC were used as predictor variables in a generalized linear mixed model. In such a model, a heterogeneous first-order autoregressive covariance structure was used to model the unequally spaced repeated measurements of the month within the year (we excluded one month, for reasons described in detail in the following section), which presented lesser values for Bayesian information criterion and Akaike information criterion. The final model was as follows (1):

$$y_{ij} = \beta_0 + TBC\beta_1 + SCC\beta_2 + Prot\beta_3 + Fat\beta_4 + \delta_{ij} + \varepsilon_{ij} \quad (1)$$

where, y_{ij} is the value of the PBMQ by litre of milk in milk-equivalents for the month i at the year j, $i = 12$, and $j = 4$; β_0 is the intercept, an average value common to all as observations; $TBC\beta_1$ is the fixed effect of the bulk tank *TBC*; $SCC\beta_2$ is the fixed effect of the bulk tank *SCC*; $Prot\beta_3$ is the fixed effect of the bulk tank protein content in milk; $Fat\beta_4$ is the fixed effect of the bulk tank fat content in milk; δ_{ij} is the random effect of the month within the year; and ε_{ij} is the random error.

For the multiple regression analysis, the assumptions of homogeneity of variances, residual normality, linearity, and multicollinearity were tested. Plots of the standardized residuals versus adjusted predicted values were used to test homogeneity of variances, whereas normal probability plots of standardized residuals and Shapiro-Wilk's test (p value = 0.66) were used to test normality, and plots of standardized residuals against predictor variables (fat, protein, SCC and TBC) were used to test linearity (Koop *et al*., 2009). A variance inflation factor (VIF) was used to test multicollinearity, where VIF = 1 indicates that predictor variables are not correlated, VIF between 1 to 5 indicates moderate correlation, and VIF between 5 to 10 indicates high correlation (Cohen *et al*., 1983). The VIF verifies the degree to which one predictor variable can predict the other predictor variables present in the model. Nevertheless, an outlier for the month of October 2014 (value of 0.114 milk-equivalents) was excluded to meet all the assumptions.

Lastly, a PCA was performed aiming to characterize the observations (12 months of the year) using the arithmetic means for the months of the year in the studied period considering the variables of milk composition and quality, and PBMQ. For this analysis, we used all the data; i.e. we used the data for October 2014 in the average calculation. The PCA enables reduction of dimensions using a linear combination between the original variables to create new variables that are termed principal components, which maintain the information of all the original variables (Manly, 2004). A correlation matrix between original variables was used to perform PCA. The two first principal components were used to construct the *biplot* graph, which presents the original variables and observations together (Gabriel, 1971).

All the analyses were performed using the SAS software (SAS, 9.1 SAS/2012, 2012). Descriptive and exploratory analyses were performed using the SAS PROC MEANS and SAS PROC SGPLOT, respectively. The correlation analysis was performed using SAS PROC CORR, whereas the multiple regression analysis was conducted using SAS PROC GLIMMIX and the assumptions verification was performed using SAS PROC UNIVARIATE and SAS PROC REG (multicollinearity: VIF). Finally, the PCA was performed using the SAS PROC PRINCOMP. Statistical significance was considered at $P < 0.05$.

RESULTS

From the descriptive statistical analysis for protein, fat, SCC, TBC, and PBMQ (Table 2) it can be observed that TBC and SCC presented values considered distant between arithmetic means (TBC = 146 229 cfu.mL^{-1} and SCC = 304 312 cells.mL^{-1}), geometric means (TBC = 51 647 cfu.mL^{-1} and SCC = 285 000 cells.mL^{-1}), and medians (TBC = 35 500 cfu.mL^{-1} and SCC = 285 500 cells.mL^{-1}), which is an indication that such variables not presented normality. In this case, the use of geometric mean or median is more adequate than the arithmetic mean to describe the values of SCC and TBC because they are not affected by outliers, which are common in these

Table 2. Descriptive statistics for variables of milk composition and quality and payment based on milk quality related to the studied period[1]

Variable	N	Arithmetic Mean	Standard Deviation	Minimum	Median	IR	Maximum	Geometric Mean
TBC (cfu.mL^{-1})	48	146 229	211 667	7000	35 500	212 500	811 000	51 647
SCC (cells.mL^{-1})	48	304 312	114 760	139 000	285 500	154 000	696 000	285 000
Fat (g.kg^{-1})	48	33.38	2.29	25.30	33.55	2.30	37.60	33.30
Protein (g.kg^{-1})	48	31.41	1.38	28.30	31.55	2.15	34.70	31.38
PBMQ (R$)	48	0.078	0.043	0.000	0.076	0.080	0.145	0.077
PBMQ (milk-equivalent)	48	0.061	0.034	0.000	0.060	0.063	0.114	0.061

[1]Study period – January 2013 to December 2016, N – Number of observations, IR – Interquartile range, TBC – Total bacterial count, SCC – Somatic cell count, PBMQ – Payment based on milk quality.

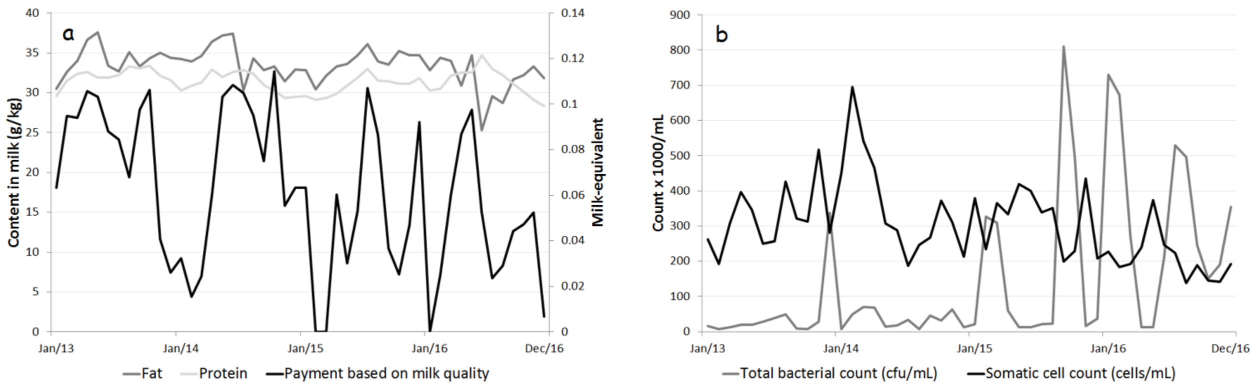

Figure 1. Descriptive behaviour for: (a) the variables of milk composition (fat and protein content) and payment based on milk quality (in milk-equivalents), and for (b) the variables of milk quality (somatic cell count and total bacterial count) over the studied years (January 2013 to December 2016)

variables. Considering the other variables, the average of PBMQ during the studied period was R$ 0.078 and 0.061 milk-equivalents, whereas the mean of protein and fat content in milk was 31.41 and 33.38 g.kg^{-1}, respectively. Interquartile range is the range of the middle 50 % of the data and is a dispersion measure often used with the median and it is indicating that the dispersion seems to be higher for TBC than for the other variables. Moreover, fat and protein content presented a more stable behaviour over the years studied than PBMQ, which exhibited greater variation over the months (Figure 1a). In comparison, SCC had a more stable behaviour than TBC, which presented notable peaks of increase in months of the year 2016, possibly reflecting a high rate of latent mastitis (Figure 1b).

From the multiple regression analysis, considering the PBMQ paid to the farmer and the milk quality and composition, we found that all the variables (fat, protein, SCC, and TBC) significantly affected the PBMQ received (p-value <0.0001 for all) (Table 3). Moreover, the model presented a root mean squared error value of 0.013 milk-equivalents and a determination coefficient (R^2) of 0.85, which is a good measure indicating that most of the variation in the PBMQ (85 %) is due to the milk composition and quality. The multiple regression analysis also provides a model that enables an estimation of the possible PBMQ paid considering the milk composition and quality. Such a model is below (2):

$$PBMQ = -0.368 - (1.110^{-7} \times TBC) - (1.640^{-7} \times SCC) + (0.005 \times Fat) + (0.010 \times Prot) \qquad (2)$$

where *PBMQ* is the payment based on milk quality (milk-equivalents); *TBC* is the bulk tank total bacterial count (cfu.mL^{-1}); *SCC* is the bulk tank somatic cell count (cells.mL^{-1}); *Fat* is the % of fat in the bulk tank milk; and *Prot* is the % of protein in the bulk tank milk.

Table 3. Multiple regression analysis estimates (± standard error) related to payment based on milk quality (in milk-equivalent) and milk composition and quality

Variable	Estimated parameter	Standard error	P-value	R^2
Intercept	-0.368	0.052	< 0.0001	0.85
TBC (cfu.mL^{-1})	-1.110^{-7}	1.045^{-8}	< 0.0001	
SCC (cells.mL^{-1})	-1.640^{-7}	2.010^{-8}	< 0.0001	RMSE[3]
Fat (g.kg^{-1})	0.005	0.001	< 0.0001	0.013
Protein (g.kg^{-1})	0.010	0.001	< 0.0001	

TBC – Total bacterial count, SCC – Somatic cell count, R^2 – Determination coefficient, RMSE – Root mean squared error.

Table 4. Spearman's correlation coefficients for variables of milk composition and quality and payment based on milk quality

	PBMQ	TBC	SCC	Fat	Protein
PBMQ	1				
TBC	-0.66*	1			
SCC	0.10	-0.41*	1		
Fat[1]	0.32*	-0.29*	0.51*	1	
Protein[1]	0.51*	-0.28	0.13	0.31*	1

PBMQ – Payment based on milk quality (milk-equivalent), TBC – Total bacterial count (cfu.mL^{-1}), SCC – Somatic cell count (cells.mL^{-1}), [1]Values in g.kg^{-1}, *Significant correlation at the level of 5 % of probability.

In regards to the correlation coefficients (Table 4), TBC, fat and protein showed significant correlation with PBMQ. Protein and fat presented positive correlation (r = 0.51 and r = 0.32, respectively), whereas TBC presented negative correlation (r = -0.66). In addition, the variables also showed significant inter-correlations, where SCC presented positive correlation with fat (r = 0.51) and negative correlation with TBC (r = -0.41), whereas fat presented positive correlation with protein (r = 0.31) and negative correlation with TBC (r = -0.29).

In sequence, PCA was then performed, from which the two first principal components were selected. The first principal component represented 50.2 % of the total data variance and showed an eigenvalue of 2.51, whereas the second principal component represented 33.9 % of total data variance and presented an eigenvalue of 1.69 (Table 5). Together, these two principal components explained 84.0 % of the total data variance. Moreover, the first principal component represents an inverse relationship between PBMQ (0.51) and fat (0.43) versus TBC (-0.57); which were the variables with higher loadings into this principal component. The second principal component represents an inverse relationship between PBMQ (-0.42) and protein (-0.54) with SCC (0.59) and fat (0.40), which were the variables with higher loadings into this component.

The *biplot* graph presents a relationship between the variables (fat, protein, SCC, TBC, and PBMQ) considering the averages for the months of the year in the four studied years (Figure 2). The months of January, February, and March (summer months) presented equal characteristics, which were high TBC and SCC with low-protein content and PBMQ. Conversely, the months of June, July, and August (winter months) presented an inverse pattern compared to the summer months, which was high-protein content and PBMQ with low SCC and TBC. May and April presented high-fat content and SCC with low-protein content and TBC, whereas September presented high-protein content and TBC with low-fat content and SCC (inverse behaviour). October and November were the months that were plotted over the axis of the first principal component, remaining near the general average of the observations for this principal component but presenting an inverse relationship whereby October showed high-protein content and PBMQ with low-fat content and SCC, whereas November showed low-protein content and PBMQ with high-fat content and SCC. December was a month that was plotted over the axis of the second principal component axis, remaining near the general average of the observations for this principal component but showing high TBC with low-fat content and PBMQ. In general, a contrast was observed between summer months and winter months, demonstrating a seasonal effect related to the milk composition and quality and the PBMQ.

Table 5. Eigenvalues and eigenvectors related to principal component analysis of variables using the correlation matrix*

Component	Eigenvectors				
	TBC	SCC	Fat[1]	Protein[1]	PBMQ
Component 1	-0.569	0.277	0.431	0.393	0.509
Component 2	-0.161	0.587	0.400	-0.542	-0.419

	Component 1	Component 2
Eigenvalues	2.51	1.69
Proportion	50.19 %	33.85 %
Cumulative	50.19 %	84.04 %

*Variables include milk composition and quality and payment based on milk quality, TBC – Total bacterial count (cfu.mL^{-1}), SCC – Somatic cell count (cells.mL^{-1}), [1]Values in g.kg^{-1}; PBMQ – Payment based on milk quality (milk-equivalent).

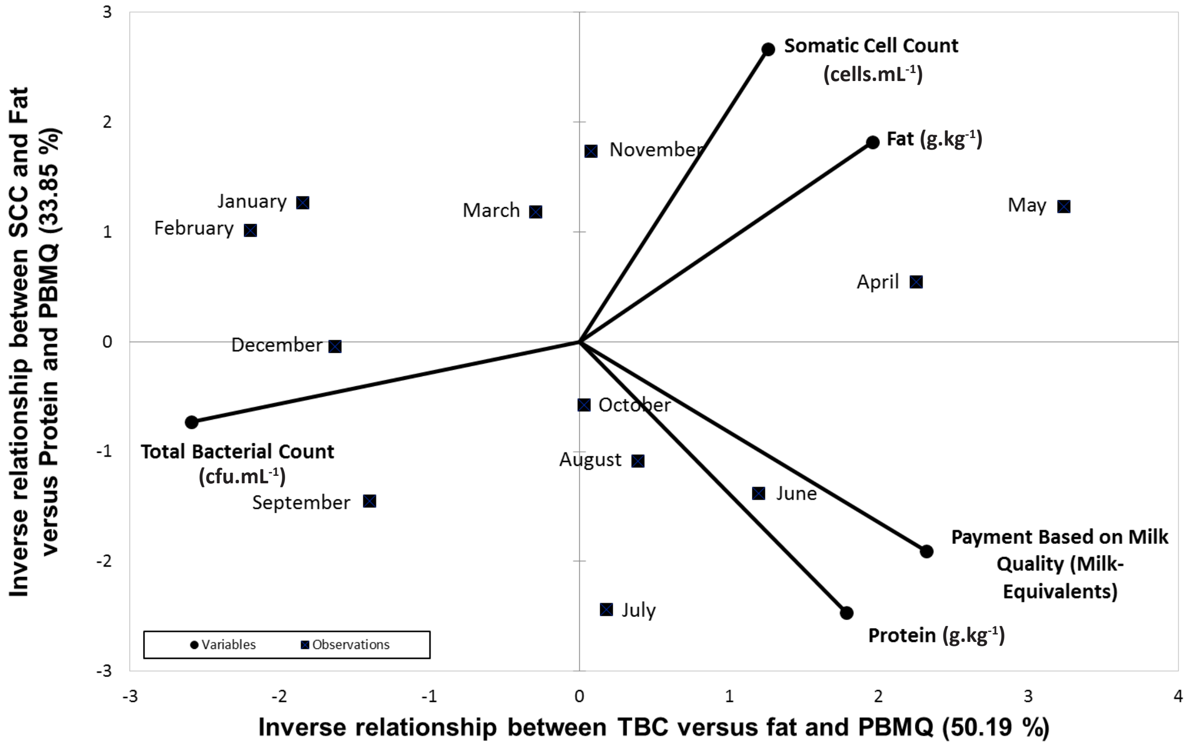

Figure 2. ***Biplot*** **graph obtained from principal component analysis performed using the correlation matrix of the variables of milk composition and quality and the payment based on milk quality. SCC: Somatic cell count (cells.mL^{-1}); PBMQ: Payment by milk quality (milk-equivalents); TBC: Total bacterial count (cfu.mL^{-1})**

DISCUSSION

In this study, we aimed to clarify the relationship between variables of milk composition and quality with the PBMQ, taking into consideration the variation caused by seasonality. The data comprised of milk composition, quality and PBMQ from a dairy farm for a four-year period that was submitted to a PPBMQ applied by the dairy company that purchased its milk. The payment table (Table 1) was based in a mixed payment system, where penalties and bonuses were applied together for all the variables included in the program (fat, protein, SCC and TBC). Busanello *et al*. (2017a) analysed information about PPBMQs used by the main dairy companies in Brazil and found that there are no any payment systems in this country based only on penalties. However, some studies have shown that although mixed payment systems (bonuses and penalties together) were effective in motivating and sensitising the farmers to improve milk composition and quality, payment systems based only on penalties were most effective because such systems induced loss aversion (Valeeva *et al*., 2007; Nightingale *et al*., 2008; Huijps *et al*., 2010; Saenger *et al*., 2013).

In Brazil, since 29th December 2011 and after in 26th November 2018, the Normative Instructions 62, 76 and 77 (IN-Brazil) (Brasil, 2011; Brasil, 2018a,b, respectively) established threshold standard values for milk composition and quality. In the Southern Region, maximum geometric mean values for SCC of 500 000 cells.mL^{-1} and TBC of 300 000 cfu.mL^{-1} were recommended as well as minimum arithmetic mean values for a protein content of 29.00 g.kg^{-1} and fat content of 30.00 g.kg^{-1}, considering such measures in a three-month period with one analysis within each month.

In the present study, geometric means for SCC and TBC remained within the thresholds required by IN-Brazil and, for both, geometric means and medians remained close in comparison with the arithmetic mean, it demonstrated more distant values showing the same behaviour as reported by Busanello *et al.* (2017a) for SCC. In addition, the values of the protein content and fat also remained within the required threshold (31.41 g.kg^{-1} and 33.38 g.kg^{-1}, respectively).

Results obtained from multiple regression analysis showed that all the studied variables strongly affected the PBMQ value paid to the farmer. Therefore, our findings demonstrate the possibility to simulate the PBMQ value (milk-equivalents) using equation (2) considering the threshold values recommended by IN-Brazil, it can also demonstrated for some countries such as the United Kingdom (UK) and Northern Ireland (e.g. SCC: 500 000, 180 000, and 195 000 cells.mL^{-1}; TBC: 300 000, 30 000, and 17 000 cfu.mL^{-1}; protein: 29.00, 32.90, and 32.30 g.kg^{-1}; and fat: 30.00, 40.90, and 40.00 g.kg^{-1}, respectively for IN-Brazil, UK, and Northern Ireland). Such data were obtained from the National Mastitis Council (NMC, 2013), Cassoli and Machado (2016), Cassoli *et al*. (2016), and the Agriculture and Horticulture Development Board − Dairy (2017) for UK, IN-Brazil (Brasil, 2011; Brasil, 2018a,b) for Brazil, and Department of Agriculture, Environment and Rural Affairs (2016) for Northern Ireland, respectively. From the equation (2) with these values, we obtained PBMQ values of -0.032, 0.144, and 0.133 milk-equivalents for IN-Brazil, UK, and Northern Ireland, respectively.

Such simulation shows us that even the farmers reaching the recommended threshold imposed by the Brazilian government might have a reduction in their milk price because of the PPBMQs. When the quality and composition of Brazilian milk are compared with those of other countries, Brazil still has a considerable limitation to improve milk composition and quality. If Brazilian milk quality were nearer to that of the UK and Northern Ireland, for example, the farmers could attain an increase in their milk price, as was the case in the simulation. However, although milk composition and quality standards exist in Brazil, the farmers that produce and the dairy companies that purchase the milk outside the threshold are not punished in any way by the government. It may be one of the reasons why SCC and TBC improvements have stagnated in the country in recent years (Cassoli and Machado, 2016; Cassoli *et al*., 2016; Busanello *et al*., 2017a,b).

In our study, important correlations between PBMQ and protein, fat, or TBC were found. Increase in the protein and fat content results in an increase in PBMQ, whereas the increase in TBC results in a decrease in PBMQ. Correlations between the other variables are widely found in the literature. For example, the positive relationship between SCC and fat content is a result of the reduction in milk production due to mastitis, concentrating that component in milk (Çinar *et al*., 2015; Stürmer *et al*., 2018). A weak relationship between fat and protein content also was evidenced by Nistor *et al*. (2014).

Multiple linear regression further showed that increase in the SCC results in a decrease in PBMQ as well. Kvapilík *et al*. (2017) also found that SCC, TBC, protein, and fat content affected the milk price paid to the farmers. In contrast, Roma Júnior *et al*. (2009) found a higher PBMQ in autumn, whereas we found higher PBMQ in winter (due to high protein content and low SCC and TBC) and lower PBMQ in summer (due to low protein content and high SCC and TBC). The impact of seasonality on TBC and SCC is heavily discussed in the literature although this primarily involves questions related to hygiene and health of the cows, as well as the influence of the seasons of the year due to the climatic variation (Nightingale *et al*., 2008; Heck *et al*., 2009; More, 2009; Fagan *et al*., 2010; Tančin, 2013; Simioni *et al*., 2014; Hill and Wall, 2015; Tančin *et al*., 2018). Also, there is a relationship between the management of the herds and welfare of the cows, which affect the hygiene of the herds and the milk quality (Sant'Anna and Costa, 2011). Moreover, the practice of withdrawal the first three milk jets can reduce the TBC and SCC in milk (Tančin *et al*., 2006) as it was done in our study.

Fat and protein content are variables that influence positively on PBMQ unlike of SCC and TBC. Thus, fat and protein content favour the bonuses, while SCC and TBC favour the penalties (Roma Júnior *et al*., 2009; Simioni *et al*., 2014). Roma Júnior *et al*. (2009) found that SCC is the main variable causing penalties, while Simioni *et al*. (2014) found that TBC was main variable resulting in penalties. Nevertheless, though PPBMQs induce to an improvement of SCC and TBC, for fat and protein content other factors are also important (Botaro *et al*., 2013).

The PCA shows an inverse relationship between the summer (January, February and March) and winter months (June, July and August) for protein content, TBC, and PBMQ variables. In the other months, a varied behaviour was observed. The greater advantage of PCA compared with univariate approaches is that PCA considers the multiple relationships between the variables and within the observations (in this case, months), which enables the derivation of more general conclusions.

To our knowledge, no other studies have utilised any multivariate approaches involving a relationship between PBMQ and milk composition and quality. With respect to protein content, Heck *et al*. (2009) also found lower values in the summer (33.90 $g.kg^{-1}$) compared to winter (35.60 $g.kg^{-1}$). In our study, the intake of pastures is favoured in winter because they are based on tempered species, whereas in summer the pastures are based on tropical species. Moreover, the seasons of the year affect the milk production and composition (Fagan *et al*., 2010), with heat stress also having an impact because of the concomitant decrease of nutrient intake (Polsky and von Keyserlingk, 2017). Also, Stürmer *et al*. (2018) found that the climatic variables are responsible for 10.2 % of the variation in composition, quality, pricing, and production of milk.

In general, our results indicate that PBMQ is directly affected by the change in milk composition and quality along the seasons. Consistent with this, Roma Júnior *et al*. (2009) mentioned that seasonality should be considered into the formulation of PPBMQs. Nevertheless, although various countries apply PPBMQs even for other mammalian species as sheep and goats (e.g. France, Italy, Portugal, Greece, and Spain [Pirisi *et al*., 2000; Pirisi *et al*., 2007]), Brazil has only taken small steps to create effective PPBMQs that induce the improvement of bovine milk composition and quality. In addition, few countries include lactose in theirs PPBMQs, such as Ireland and the United States (Sneddon *et al*., 2013), whereas in Brazil lactose has already been identified as showing considerable variation in bulk tank milk samples from dairy farms (variation due to SCC, parity and seasons), which suggests that lactose should also be used in PPBMQs (Alessio *et al*., 2016).

The PPBMQs, together with the production conditions, are determination factors toward improving the profitability and sustainability of the dairy farmers (Michaličková *et al*., 2014; Michaličková *et al*., 2017). Good animal practices that enable the improvement of milk composition and quality lead to improvement of the economic results, mainly due to an increase of the bonuses (Banga *et al*., 2009; Paixão *et al*., 2014; Teixeira Júnior *et al*., 2015). However, effort is required from all the stakeholders within the dairy sector to reach this result, with the lack of reliance between dairy farmers, government, technicians, and dairy companies appearing to constitute one of the most important issues (Devitt *et al*., 2013).

Finally, our study revealed important findings suggesting that PBMQ is influenced by seasonal variation in the milk composition and quality. Nevertheless, our study contains some limitations. The data used represent only the exclusive reality of a single dairy farm for a four-year period. It is possible that the results are not generalisable to those from individual farms and PPBMQs applied by other dairy companies. However, the use of more data from additional farms and dairy companies with different PPBMQs will likely lead to more complex data manipulation and statistical analysis. It is also possible that the effect of different PPBMQs and their results on PBMQ might lead to a different statistical approach. Nevertheless, we consider that these found results are of considerable importance to understand the effects of seasonal variation of milk composition and quality on the PBMQ.

CONCLUSION

Milk compounds (fat, protein, SCC, and TBC) significantly affect the final value of the PBMQ paid to the farmer. Moreover, there is a seasonal effect on PBMQ, wherein winter months (June, July, and August) the PBMQ is higher and in summer months (January, February and March) it is lower. In addition, there is a general negative relationship between SCC and TBC with PBMQ and a positive relationship between protein and fat content with PBMQ. Finally, dairy farmers can increase their PBMQ received in the summer by improving the nutritional management of the herds that enables an increase in milk protein, as well as improving hygiene and mastitis management to reduce SCC and TBC. Moreover, dairy companies probably should consider a separated formulation of PPBMQs according to the seasons of the year (summer and winter months).

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