Effectiveness of milking management practices for SCC and TBC levels in milk

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ABSTRACT - We aimed to evaluate the impact of milking management practices on milk quality though somatic cell count (SCC) and total bacterial count (TBC). By means of a survey that included farmers associated to two cooperatives, namely Cosulati in the state of Rio Grande do Sul and Castrolanda in the state of Paraná, the hypothesis that milking handling practices impact SCC and TBC levels negatively, regardless of the technological development level of the farm, was analyzed. For such, the quantile regression method was used to estimate models for the 10th, 50th, and 90th quantiles. The results confirmed that SCC and TBC levels decrease as the number of practices increases. However, only three practices impact SCC and TBC levels significantly, and their impact is not related to the farm technological level, according to tests performed by quantile regression. Finally, the study showed that some milk quality and management practices affect somatic cell count and total bacterial count more than others, which consists of using the correct active principle during dry cattle, post-dip, and the water temperature control periods.

Keywords: dairy, farm, production system

Introduction

Milk is a nutrient-rich food containing proteins, fats, vitamins, and mineral salts in its composition that are essential for human nutrition (Guerreiro et al., 2005). One of the major aspects that ensures consumer health refers to milk quality (Ruegg, 2003; Baggio and Montanini, 2017). Among the phases in the milk production chain, activities developed internally on the farms are very susceptible and capable of affecting the final product quality and, consequently, the technical and economic performances of the subsequent phases. Generally, the biggest technological and managerial limitations within the milk chain are found in the dairy farms themselves (Gottschall et al., 2002).

Quality milk results from complete milking, with appropriate hygiene conditions, and healthy animals (Brasil, 2011). Somatic cell count (SCC) and total bacterial count (TBC) are parameters used to indicate and monitor milk quality (Langoni, 2013). These indicators demonstrate and summarize information subjacent to the practices at the farm level (Gonzalez et al., 2004; Silva et al., 2010; Langoni et al., 2011) and are relatively cheap and of easy execution. That is why regulatory agencies are always attentive to them and have determined that until the mid-2019s, SCC and TBC levels must be kept under the limit of 500 thousand cells and 300 thousand colony forming units (cfu) per milliliter of milk, respectively (Brasil, 2018).

Even though a Normative Proceeding has been in force since 2011, there are farms in Brazil that still show low productive efficiency and SCC and TBC levels above the top limit established by legislation.
This is suggestive of flaws in the production process at a management level and leads to significant losses, low animal yield, and decrease in the final product lifespan (Lagger et al., 2000; Fagundes et al., 2006; Simões et al., 2015). In search of competitiveness, the milk processing industry has encouraged quality milk delivery through a premium price (Gonzalez et al., 2004). Therefore, there has been a potential increased efficiency at a farm level, inasmuch as under better sanitation and management conditions, there is better exploitation of the animal productive potential (Oliveira et al., 2001; Reis et al., 2001).

As to milking, many practices have been adopted by best milk production systems around the world, being consistently associated with the reduction of both SCC and TBC levels (Gonzalez, 2002; Zanella et al., 2011; Picoli et al., 2014). Studies have also shown that the production systems adopted on farms affect SCC and TBC levels (Lopes Junior et al., 2012; Cervo et al., 2017). In addition, other studies have evidenced that these counts are associated to the dairy farmer and farm characteristics. In their study, Lopes Junior et al. (2012) identified the technological level and continuity of activity as a function of the age profile of the groups directly affecting SCC and TBC levels. For Cervo et al. (2017), most production differences are based on process management and the use of genetic, nutritional, and herd health technologies.

In this study, the effects of the milking management practices on SCC and TBC milk levels will be tested in two producing regions in southern Brazil. The hypothesis is that milking management practices affect SCC and TBC levels above and beyond control variables, influencing their scores as they are practiced (for more or less). Studies aiming to identify efficiency levels generally use regression to the mean (Nascimento et al., 2012), which may not be enough because the effect of a specific variable on SCC and TBC levels may depend on these levels being low or high. Therefore, to avoid this limitation, quantile regression models were used to estimate different models for the quantiles of interest.

Material and Methods

In this study, a hierarchical approach, which controls the effects of variables such as the production system adopted, farm location, and other variables that might interfere with quality indicator levels, will be used. The present study consisted of a dairy farmer survey associated to two cooperatives located in southern Brazil. In all, 159 dairy farmers were interviewed, 106 of whom associated to the Cosulati cooperative in the state of Rio Grande do Sul and 53 associated to the Castrolanda cooperative in the state of Paraná. The choice of the number of farmers came from the total number of associates belonging to each of the cooperatives, considering 10% of the total number of associates in each. The sample was stratified in quartiles according to production in average liters per day from each cooperative. The interviewed farmers were selected within the cities with the biggest representativeness of producers for each stratum.

The data collection tool consisted of 22 closed questions, which were previously tested in a pilot study to identify eventual formulation errors. The tool was applied personally to farmers in January and February of 2017 by previously trained staff to standardize the questionnaire application.

For this study, we used parts of sections I and II of the tool, which referred to the farms socioeconomic data (section I) and practices and managements adopted in the milking process (section II). All the questions were answered in dichotomic scale (yes or no), for the items described (Table 1).

After collection, data were transferred to a statistical software (Stata/IC 12-0), and then analyzed through ordinary least squares (OLS) regression models (Hair et al., 2005). Quantile regression was used, since OLS regression disregards the possibility of SCC and TBC impact in a differentiated way on the farms. Therefore, quantile regression allowed the estimate of different models, one for each quantile, aiming to identify differences among coefficients (differences in the effect of variables on productive unit efficiency levels) (Nascimento et al., 2012).

Farm hygienic-sanitary quality indicators were obtained from supranational cooperatives. Milk reports and extracts from 12 months before the interview were performed, from which the annual SCC
(cells per milliliter) and TBC (cfu per milliliter) averages were obtained. Sixteen farmers (n = 16) were excluded from the sample, since they were no longer associated to the cooperatives at the moment the reports were written, which made it impossible to access the SCC and TBC indicators of these producers. Through influential observation diagnosis (outliers), a few other farmers were eliminated from the sampling (n = 12), which resulted in a total sample of 131 observations for SCC and 125 for TBC. As SCC and TBC indicators were asymmetric, they were brought back to normality through logarithmization (log10), as indicated by Hair et al. (2005).

The control variables used were: Production system, with 1 = Extensive system; 2 = Semi-confined system; and 3 = Confined system; Region where the sampling was performed, with 1 = Castro (Paraná) and 2 = Pelotas (Rio Grande do Sul); Farmers’ education, with 1 = Incomplete elementary school, 2 = Complete elementary school; and if the milk activity was the main source of income on the farm (more than 50% of total income), with 1 = Yes and 2 = No. We used dummies (dichotomous) variables to quantify all independent qualitative instead of factors. For example, the estimated coefficient of “System Type” informs the effect of the adoption of other types of systems in relation to the extensive system (the base system). The role of control variables is only for improving the quality of our counterfactual analysis with regressions.

Finally, the study central hypothesis was tested, first by means of hierarchical linear regression analysis to verify the existence of a functional relation between dependent variables (SCC and TBC). The sum of management practices (total of practices used in milking management, varying from 0 to 10) was regressed at the levels of SCC and TBC; then, with the set of individual practices used on each farm, inserted simultaneously into the regression model.

**Results**

By and large, it was possible to realize that the farmers associated to Castrolanda were more likely to adopt milking management practices than the ones associated to the Cosulati Cooperative in Rio Grande do Sul (Table 1).

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1. The extensive system is the one in which the cattle are most often out on the field.
2. The semi-confined system is the one in which the cattle remains out on the field for a few hours a day and receives supplementation on the trough at milking, remaining in the stables or installations the rest of the time.
3. Confined system is the one in which the cattle remains permanently inside stables or installations.
The dairy farmers who made up the sample (Table 2) were mostly middle-aged men with low education and a relatively extensive experience in the milking activity. Sampled farms mostly adopted the extensive production system, but there was a pronounced distinction when it came to animal and economic performance indexes between the two sampled locations, as there was a bigger distinction regarding farm characteristics rather than farmers. In the Pelotas area, farms were smaller with reference to the number of lactating animals, and the production (technical) performance and economic indexes were also inferior to the ones obtained by the Castro farmers.

Table 2 - Characterization of farmers and farms sampled in Pelotas (RS) and Castro (PR), 2017

| Control                     | Pelotas (RS) | Castro (PR) | Total |
|-----------------------------|--------------|-------------|-------|
| Production system (%)       |              |             |       |
| 1 – Extensive               | 86.7         | 84.9        | 86.1  |
| 2 – Semi-confined           | 13.7         | 0           | 8.9   |
| 3 – Confined                | 0            | 15.1        | 5.1   |
| Schooling (%)               |              |             |       |
| 1 – Incomplete elementary   | 72.1         | 29.4        | 58.1  |
| 2 – Complete elementary     | 27.9         | 70.6        | 41.9  |
| Milk as main source of income (%) |            |             |       |
| 1 – Yes                     | 73.3         | 86.0        | 77.4  |
| 2 – No                      | 26.7         | 14.0        | 22.6  |

| Farmer characterization      |              |             |       |
| Age (years)                 | 48.51        | 43.92       | 47.03 |
| Sex                         |              |             |       |
| Male (%)                    | 33.3         | 22.6        | 29.7  |
| Female (%)                  | 66.7         | 77.4        | 70.3  |
| Dairy activity experience (years) | 27.47      | 17.66       | 24.29 |

| Farm characterization        |              |             |       |
| Prevailing breed in dairy herd |            |             |       |
| Jersey (%)                  | 45.2         | 20.8        | 36.9  |
| Holstein (%)                | 41.3         | 52.8        | 45.2  |
| Mixed breed (%)             | 12.5         | 15.1        | 13.4  |
| Mixed (Jersey and Holstein) (%) | 0           | 9.4         | 3.2   |
| Other (%)                   | 1.0          | 1.9         | 1.3   |
| Number of lactating cows (heads) | 17.13    | 76.60       | 37.10 |
| Liters produced by cow per day (L/day) | 11.44    | 17.29       | 13.39 |
| Area (hectares)             | 50.37        | 67.78       | 56.06 |
| Liters produced by hectare (L/ha/year) | 3,422.93  | 15,964.09   | 7,521.35 |
| Daily production (L)        | 239.75       | 1,718.96    | 729.68 |
| Average price received (R$/L) | 0.83       | 1.33        | 1.00  |
| Average monthly income (R$) |              |             |       |
| Up to R$ 2,000.00 (%)       | 41.9         | 0           | 28.2  |
| From R$ 2,000.00 to R$ 10,000.00 (%) | 40.0     | 27.4        | 35.9  |
| Over R$ 10,000.00 (%)       | 18.1         | 72.5        | 36.0  |
| Milk as main source of income? | 73.3       | 86.0        | 77.4  |
| Quality                     |              |             |       |
| SCC (average, in number of cells/mL of milk) | 527.53   | 331.76      | 453.37 |
| TBC (average, in cfu/mL of milk) | 684.43  | 10.03       | 669.49 |

Number of dairy farmers: 105 (Pelotas) 53 (Castro) 158 (Total)

SCC - somatic cell count; TBC - total bacterial count.
In the present study, it can be suggested that milking practices are effective to improve milk quality (decrease of SCC and TBC levels). As an initial test for this hypothesis, the impact of the sum of the practiced managements and of this squared variable on SCC and TBC levels is shown. It was found that the number of practices affects both levels negatively, which implies in milk quality improvement, as suggested by the hypothesis (Table 3).

When the squared number of practices (second block of variables) is inserted, the coefficient is significant and positive for both SCC and TBC. It is understood, therefore, that when the number of practices increases, there is a growing improvement in milk quality (by lowering SCC and TBC levels). Thus, more practices imply milk quality improvement at increasing rates, showing a non-linear relation between management practices and milk quality.

The estimated coefficient results, through the quantile regression for the 10th, 50th, and 90th quantiles, predicted SCC levels from a set of control variables and individualized milking management practices (Table 4).

Through the Wald test applied to each of the explanatory variables, it was not possible to reject the null hypothesis statement of equality between quantile coefficients (Table 5). This implies that the general model (second column) estimated by OLS applies to the whole sample.

The Wald test was applied to check if there were differences in milking management practices between quantiles (10th, 50th, and 90th) to predict TBC levels. Again, the test response was negative, which shows that average regression was valid for the entire sample (Table 6).

In spite of this result, average regression allows the identification of some practices, which, for the evaluated farms, were more critical to SCC and TBC decrease than others. Therefore, to these farmers, the practices would be minimum managements adopted on the farms so that SCC and TBC indicators would have their scores reduced, in addition to the effects controlled by covariates (Region, Production system type, Education, and Milk as main source of income). As mentioned before, these practices act on the whole sample distribution, regardless of SCC and TBC levels each farm shows. Such result can be treated as a “Minimum data set” of managements that discriminate milk quality. Figure 1 sums up these managements, emphasizing that water temperature control, post-dip, and knowledge of the active principle to be used during the cattle dry period are the only managements that can individually predict milk quality beyond covariates.

| Table 3 - Estimated regression parameters by ordinary least squares for somatic cell count (SCC) and total bacterial count (TBC) |
|---------------------------------------------------------------|
| Variable                                      | Model       |
|                                              | SCC         | TBC         |
|                                              | (1)         | (2)         | (1) | (2) |
| Regions\(^1\)                                  | -0.144      | -0.197      | -0.571*** | -0.613*** |
| System type\(^2\)                              | -0.006      | -0.019      | 0.067     | 0.059     |
| Schooling\(^3\)                                | 0.047       | 0.033       | -0.051    | -0.065    |
| Milk as main source of income\(^4\)            | -0.127      | -0.105      | -0.067    | -0.046    |
| Number of practices\(^5\)                      | -0.375***   | -0.988***   | -0.301*** | -0.825*** |
| Number of practices squared\(^6\)              | 0.680***    | 0.577***    |           |           |
| N                                            | 129         | 129         | 123       | 123       |
| Adjusted R²                                   | 0.224       | 0.248       | 0.707     | 0.727     |

\(^1\) Castro region = 1 and Pelotas region = 2.
\(^2\) System type (extensive; semi-confined; confined); base system = extensive system.
\(^3\) Schooling (incomplete elementary school and complete elementary school).
\(^4\) Milk as main source of income (yes or no).
\(^5\) Number of management practices used by the producer.
\(^6\) Number of management practices squared.

\* Significant at 10%; ** significant at 5%; *** significant at 1%.
Table 4 - Estimated parameter regressions for somatic cell count

| Variable                                | General (OLS)¹ | 10th quantile | 50th quantile | 90th quantile |
|-----------------------------------------|----------------|---------------|---------------|---------------|
| Categorization education level          | −0.01          | 0.04          | −0.02         | −0.07         |
|                                         | (0.02)         | (0.12)        | (0.04)        | (0.11)        |
| System type                             | −0.00          | −0.01         | −0.00         | 0.03          |
|                                         | (0.03)         | (0.06)        | (0.05)        | (0.12)        |
| State                                   | −0.04          | −0.03         | −0.02         | 0.02          |
|                                         | (0.03)         | (0.11)        | (0.04)        | (0.07)        |
| Main source of income                   | −0.05          | −0.10         | −0.04         | −0.07         |
|                                         | (0.04)         | (0.13)        | (0.06)        | (0.15)        |
| Uses pre-dip                            | 0.02           | −0.09         | 0.04          | 0.05          |
|                                         | (0.06)         | (0.34)        | (0.09)        | (0.27)        |
| Uses post-dip                           | −0.03          | −0.03         | −0.01         | −0.01         |
|                                         | (0.06)         | (0.29)        | (0.08)        | (0.24)        |
| Controls water temperature hygiene      | −0.21***       | −0.26         | −0.24***      | −0.18         |
|                                         | (0.05)         | (0.15)        | (0.08)        | (0.22)        |
| Does individual milk control            | −0.03          | −0.05         | −0.04         | 0.00          |
|                                         | (0.05)         | (0.15)        | (0.08)        | (0.20)        |
| Does individual quality records          | 0.06           | 0.07          | 0.07          | −0.01         |
|                                         | (0.07)         | (0.25)        | (0.10)        | (0.25)        |
| Does mastitis return control             | 0.06           | 0.05          | 0.06          | 0.00          |
|                                         | (0.04)         | (0.10)        | (0.05)        | (0.17)        |
| Uses CMT biweekly                       | −0.00          | −0.06         | 0.01          | 0.01          |
|                                         | (0.04)         | (0.12)        | (0.06)        | (0.14)        |
| Knows dry active principle              | −0.06          | 0.02          | −0.08         | −0.06         |
|                                         | (0.04)         | (0.18)        | (0.06)        | (0.19)        |
| Does water quality test                  | −0.07          | −0.02         | −0.14**       | −0.10         |
|                                         | (0.04)         | (0.19)        | (0.07)        | (0.11)        |
| Does dark bottom mug test               | −0.01          | 0.04          | 0.01          | −0.07         |
|                                         | (0.04)         | (0.14)        | (0.06)        | (0.19)        |
| Constant                                | 3.00***        | 2.82***       | 3.00***       | 3.19***       |
|                                         | (0.10)         | (0.37)        | (0.15)        | (0.31)        |
| Observations                            | 129            | 129           | 129           | 129           |
| R²                                      | 0.35           |               |               |               |

¹ Coefficients obtained by Ordinary Least Squares (OLS) method; pattern error between parenthesis.
* Significant parameters at 10%; ** significant parameters at 5%; *** significant parameters at 1%.

Table 5 - Wald test for the three quantile regressions (10th, 50th, and 90th quantiles), somatic cell count (SCC), and total bacterial count (TBC)

| Variable                                | SCC          | TBC          |
|-----------------------------------------|--------------|--------------|
| Uses pre-dip                            | 0.89         | 1.36         |
| Uses post-dip                           | 0.81         | 0.16         |
| Controls water temperature hygiene      | 0.31         | 1.65         |
| Does individual milk control            | 0.16         | 0.14         |
| Does individual quality records          | 0.16         | 0.71         |
| Does mastitis return control             | 0.85         | 4.17         |
| Uses CMT biweekly                       | 0.34         | 1.97         |
| Knows dry active principle              | 0.52         | 0.71         |
| Does water quality test                  | 0.45         | 0.23         |
| Does dark bottom mug test               | 1.12         | 1.69         |

CMT - California Mastitis Test.
Table 6 - Parameter estimate of the regressions estimated for total bacterial count

| Variable                                      | General (OLS)¹ | 10th quantile | 50th quantile | 90th quantile |
|-----------------------------------------------|----------------|---------------|---------------|---------------|
| Categorization education level                | −0.09          | −0.09         | −0.02         | −0.11         |
|                                               | (0.06)         | (0.28)        | (0.13)        | (0.13)        |
| System type                                   | 0.11           | 0.10          | 0.07          | 0.16          |
|                                               | (0.08)         | (0.50)        | (0.16)        | (0.18)        |
| State                                         | −0.63***       | −0.31         | −0.62***      | −0.83***      |
|                                               | (0.07)         | (0.35)        | (0.12)        | (0.10)        |
| Main source of income                         | −0.15          | 0.10          | −0.21         | −0.14         |
|                                               | (0.10)         | (0.50)        | (0.19)        | (0.22)        |
| Uses pre-dip                                  | 0.04           | −0.13         | 0.07          | −0.23         |
|                                               | (0.16)         | (0.68)        | (0.28)        | (0.31)        |
| Uses post-dip                                 | −0.35**        | −0.16         | −0.36         | −0.18         |
|                                               | (0.14)         | (0.66)        | (0.26)        | (0.19)        |
| Controls water temperature hygiene            | −0.48***       | −0.94         | −0.39         | −0.22         |
|                                               | (0.14)         | (0.64)        | (0.26)        | (0.19)        |
| Does individual milk control                  | −0.08          | −0.02         | 0.03          | −0.27         |
|                                               | (0.13)         | (0.31)        | (0.26)        | (0.31)        |
| Does individual quality records               | 0.17           | −0.03         | −0.01         | 0.36          |
|                                               | (0.17)         | (0.78)        | (0.34)        | (0.35)        |
| Does mastitis return control                  | −0.06          | −0.07         | −0.01         | −0.15         |
|                                               | (0.09)         | (0.34)        | (0.18)        | (0.14)        |
| Uses CMT biweekly                             | 0.03           | 0.03          | −0.02         | 0.08          |
|                                               | (0.09)         | (0.36)        | (0.18)        | (0.15)        |
| Knows dry active principle                    | −0.29***       | −0.08         | −0.34*        | −0.16         |
|                                               | (0.10)         | (0.53)        | (0.20)        | (0.24)        |
| Does water quality test                       | −0.01          | −0.17         | 0.06          | 0.01          |
|                                               | (0.12)         | (0.46)        | (0.22)        | (0.19)        |
| Does dark bottom mug test                     | 0.05           | −0.17         | 0.02          | 0.19          |
|                                               | (0.10)         | (0.33)        | (0.20)        | (0.18)        |
| Constant                                      | 4.02***        | 2.98***       | 3.97***       | 4.61***       |
|                                               | (0.25)         | (1.08)        | (0.48)        | (0.44)        |
| Observations                                  | 123            | 123           | 123           | 123           |
| R²                                            | 0.78           |               |               |               |

CMT - California Mastitis Test.
¹ Coefficients obtained by Ordinary Least Squares (OLS) method.
Pattern error between parentheses.
* Significant parameters at 10%; ** significant parameters at 5%; *** significant parameters at 1%.

Figure 1 - Minimum data set of milking managements to access milk quality, somatic cell count (SCC), and total bacterial count (TBC).
Discussion

As the Wald test checked the hypothesis that estimated coefficients are the same for the three quantiles (Cameron and Trivedi, 2005), the independent variable impact was the same for all farms analyzed – both the ones that presented low SCC and TBC levels, as well as those that presented high levels.

The results obtained are in agreement with those found in literature (Fonseca and Santos, 2000; Guido et al., 2010), which refer to these managements as essential to milk microbial count. The post-dip practice prevents pathogen transmission among animals in the herd, reducing cross contamination, in addition to contributing to the udder protection when the sphincter is most susceptible to contamination by environmental microorganisms following milking (Santos and Fonseca, 2001).

The practice of controlling water temperature for milking equipment sanitation, for example, has been reported to reduce contamination and mesophyll microorganism counts, which originate in deficiencies in milking equipment washing and sanitation (Fonseca and Santos, 2000; Cavalcanti et al., 2010). Interestingly, this practice was the only one which could predict SCC. Water temperature control is a decisive factor for detergent and sanitizer effectiveness (Reis et al., 2017), i.e., water at an appropriate temperature for each step of the sanitation, and detergent use are essential so that the chemical product can perform its potential and the correct sanitation of the equipment.

Managing the animals’ dry period by knowing the specific antibiotic therapy for each case proved to be critical for milk quality. This is due to the real mastitis control possibility (Bradley et al., 2011). The dry period offers high risk for the occurrence of new infections. In every milk exploration, the dry-off protocol should be based on the knowledge of the health condition of the udder in each herd, specifically guiding towards adequate treatment and use of antibiotic therapy (Bexiga et al., 2005), as the main strains of microorganisms affecting the herd are known. The knowledge of strains and, consequently, of the most efficient and specific treatments is obtained by the isolation and identification of microorganisms that are present in the animals’ udder as well as antibiograms. In this study, the farmers’ knowledge of the active principle required in the dry period influenced TBC levels. The dry-off procedure, when performed with adequate antibiotics directed towards dry cattle treatment, enables an increase in the therapeutic success rate as compared with treatments performed during the lactation period, the reduction of incidence of new cases, fewer economic losses, and reduced animal culling (Owens and Nickerson, 2011).

Finally, it can be highlighted that, even if several milking managements did not show a significant impact over SCC and TBC levels, the more managements are performed, the lower the SCC and TBC levels will be. Such result is an indicator that milk quality is a result of multiple interactive processes. In this study, due to sampling, interactive models were not used. Yet, this is a possibility for future studies. It was also evident that, if there is a set of practices to prioritize, water temperature control during equipment washes, post-dip execution, and the correct choice of products to be used during the cattle dry period must be taken into account.

Conclusions

As the number of managements practiced by dairy farmers increases, SCC and TBC decrease, and this is not linear. It is important to highlight that the effect of the milking management beyond the effects of other variables that might affect the approached indicators were tested in this study, as is the case of the regions studied, the type of system exploited, the exclusive dedication to the activity, and farmers’ education. By understanding the effect of milking management practices at an individual level over SCC and TBC levels, a minimum data set of relevant managements to monitor milk quality on the farms was obtained. This minimum set of practices consists of using the correct active principle during the cattle dry period, post-dip, and water temperature control. Additionally, these management practices have shown to be equally relevant to explain efficiency levels (milk quality) of all farmers within the sample spectrum.
Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.O. Daneluz, M.D. Canever, H.G. Lima and R.F. Bermudes. Data curation: M.O. Daneluz, M.D. Canever and H.G. Lima. Formal analysis: M.O. Daneluz, M.D. Canever and H.G. Lima. Funding acquisition: M.O. Daneluz, M.D. Canever and H.G. Lima. Investigation: M.O. Daneluz, M.D. Canever and H.G. Lima. Methodology: M.O. Daneluz, M.D. Canever, H.G. Lima and F.G. Ribeiro. Project administration: M.O. Daneluz, M.D. Canever and H.G. Lima. Resources: M.O. Daneluz and M.D. Canever. Software: M.O. Daneluz and M.D. Canever. Supervision: M.O. Daneluz, M.D. Canever and H.G. Lima. Validation: M.O. Daneluz, M.D. Canever, H.G. Lima and F.G. Ribeiro. Visualization: M.O. Daneluz, M.D. Canever and H.G. Lima. Writing-original draft: M.O. Daneluz, M.D. Canever, H.G. Lima, R.F. Bermudes and F.G. Ribeiro. Writing-review & editing: M.O. Daneluz, M.D. Canever, H.G. Lima, R.F. Bermudes and F.G. Ribeiro.

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