

REGULAR ARTICLE

Influence of good practices training and milking systems on standard plate count of dairy properties in a municipality in the interior of São Paulo state

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Statements and Declarations

Data availability

All data will be shared upon request.

Institutional Review Board Statement

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

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Author contribution

FMR: Conceptualization, Experimental data collection, Data storage, Literature review, Manuscript writing, Funding acquisition; INFS: Experimental data collection, Data storage, Literature review, Manuscript writing; SVBF: Literature review, Manuscript writing, Manuscript revision; CPC: Data storage, Data analysis, Manuscript revision; PABML: Conceptualization, Experimental data collection, Data storage, Manuscript writing, Manuscript revision, Supervision, Funding acquisition.

Abstract

Milk is a product of great economic, social, and nutritional importance for the Brazilian population. However, the lack of proper hygiene and sanitization during the milking process, whether manual or mechanical, the equipment used in the process, and the storage of milk, coupled with a lack of access to knowledge on good sanitary practices, represents a significant risk to the quality and quantity of milk produced and marketed. Thus, this study aimed to verify the influence of good practices training in milking and the type of milking (manual or mechanical) on the standard plate count (SPC) concentration in milk from small-scale dairy farms in Bastos-SP, Brazil. Three sample collections were carried out: one before and two after a good sanitary practice training in the milking process to assess its impact on the microbiological quality of production from 14 dairy farms. An exploratory and statistical analysis of the milk results was conducted. Values exceeding the national legislation's accepted limits for SPC were found, and no significant difference was observed between the pre- and post-training collections or between the types of milking. The study demonstrated that the intervention methodology could influence producer behavior, considering that the single intervention used was group training, which did not effectively result in the implementation and maintenance of good sanitary practices in the milking process.

Keywords

Family farming; Food microbiology; Good manufacturing practices; Milk quality; Rural extension.



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Introduction

The dairy supply chain stands out as a fundamental ally for nutrition and societal development by providing nutritionally rich food to consumers. Responsible for the production and availability of milk and dairy products for populations worldwide, this sector plays an essential role in food security, with its products classified as a priority in meeting recommended dietary levels (FAO; IFAD; UNICEF; WFP; WHO, 2022).

Milk, an animal-based food, is traditionally included in the basic diet of Brazilians and serves as an important source of calcium and proteins. According to the Brazilian Society of Food and Nutrition (SBAN), milk and its derivatives are rich in proteins, vitamins, and minerals, with an emphasis on calcium, which has a higher absorption rate in dairy products compared to plant-based foods, reinforcing its importance in the diet. Additionally, there is a proven relationship between the consumption of milk and dairy products and the bone and muscle health of individuals (Amancio, 2015). In this context,

food safety, which includes the quality and nutritional composition of the food, becomes essential for preserving the health of Brazilian consumers.

To ensure this quality, Brazil has strict legislation defining the physicochemical and organoleptic characteristics of milk and its derivatives. Normative Instruction (IN) 76/2018, currently in effect and established by the Ministry of Agriculture and Livestock (MAPA), sets qualitative and quantitative parameters for the refrigeration of milk post-harvest and during transportation. These parameters include sensory characteristics, such as a uniform white color and characteristic odor, as well as physicochemical criteria and a standard plate count (SPC) limit of 300,000 CFU/mL (5.477 log₁₀, as considered for normalized data) for raw milk refrigerated in individual tanks (MAPA, 2018).

Exceeding the SPC concentration can compromise public health and reduce milk quality, hindering processing and putting the production sector at risk (Zareie, 2016). The main sources of microbial contamination include factors inherent to

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the interior and exterior of lactating cows' udders, procedures carried out during the milking process, as well as the equipment used for milk collection and storage. Additionally, factors such as feed, soil, feces, and surrounding air, along with the stages of milking, transport, processing, and distribution of milk, are also critical to contamination by microorganisms (Ahmed, 2022).

Good Agricultural Practices (GAP) are essential to prevent contamination and ensure the quality and safety of milk, providing a product suitable for consumption (Beloti et al., 2020). Clean milk, in addition to being safe and nutritious, has high commercial value, resulting in high-quality products, good yields for coproduct production, and a positive financial impact on those involved in the production chain (Ahmad et al., 2020). GAPs are directly related to proper sanitation throughout the entire process and ensuring an environment conducive to milking, promoting animal welfare and health and, consequently, the quality of the product generated.

In this sense, the concept of sustainable development emerges as a response to the scarcity of natural resources and concerns for future generations, aiming to reconcile economic growth with the conscious use of resources (Maia, 2002), tying this article to the Sustainable Development Goals (SDGs) (ONU, 2015) number 12 (Responsible Consumption and Production) and 2 (Zero Hunger and Sustainable Agriculture), which are directly related to food security and the promotion of sustainable sanitary practices, contributing to the production of nutritious and sustainable food.

Thus, the objective of this study was to evaluate the influence of good practices training and the type of milking on the concentration of standard plate count (SPC) in the milk produced on small-scale farms in the municipality of Bastos, Brazil.

Materials and methods

Location and profile of the rural properties

The study was conducted in the municipality of Bastos, in the state of São Paulo, in partnership with the Bastos Dairy Producers Association (APRULB), which has 64 associated producers classified as small or medium-scale production properties. These properties either use a mechanical milking system or a manual milking system. Based on producer registration data, 14 dairy producers who met the criteria established by the research group for participation were randomly selected. The criteria were: being registered with the association, being a small-scale production property, expressing interest in participating in the research, having the property located in the municipality of Bastos or in proximity, performing manual or mechanical milking, having a storage tank on the property or delivering the milk in a drum on the same day of milking to the association's plant.

Milk collection

After selecting the producers, milk samples were collected from the individual tanks on the properties or from individual drums used by the producers who delivered their milk to APRULB before it was mixed with the milk from the community tank.

In total, milk samples were collected from seven properties using manual milking and seven properties using mechanical milking, resulting in 14 milk samples collected in duplicate for laboratory analysis. There were three collections over three

non-consecutive days: one collection before the good hygiene practices training with no prior guidance, one collection 20 days after the good hygiene practices training with the possibility of occasional guidance, and one final collection 50 days after the training with the possibility of occasional guidance, as outlined in the schedule shown in Figure 1.

The milk samples were collected before being mixed with the community tank at APRULB, placed in sterile glass vials with a capacity of 50 mL, and stored in a cooler to maintain refrigeration from the moment of collection until the laboratory analysis.

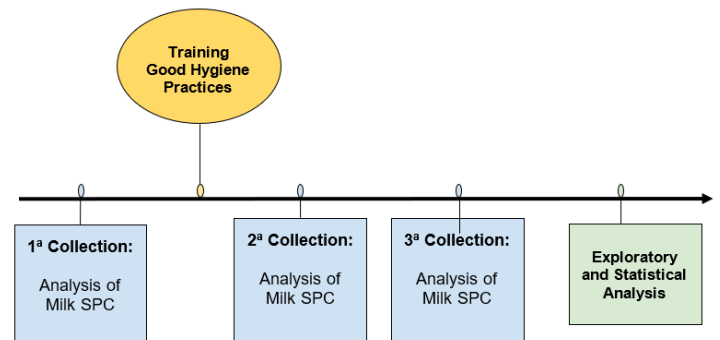


Figure 1. Schedule for data collection and milk sample collection from small-scale dairy producers in the municipality of Bastos, SP.

Training on good hygiene practices

The good practices training was conducted by the team from the Kamby Extension Program (FCE/Unesp), which aims to improve milk production in Tupã and the surrounding region, both in quantitative and qualitative aspects, through the democratization of knowledge and the exchange of experiences among participants. The training was pre-scheduled with APRULB and announced in advance to ensure maximum participation and was open to all dairy producers in the municipality of Bastos, both members and non-members, as well as other interested parties.

The training covered the partial results from the first sample collection as an initial awareness tool, providing scientific background on the microbiological quality of milk in alignment with Brazilian food quality regulations (MAPA, 2018), and, finally, guidance on sanitation and hygiene during the milking process, based on the Milking Management Good Practices Manual, developed by Rosa et al., (2014), which provides instructions on the steps and actions the producer should follow during the milking process to ensure the production of a high-quality product free from contamination.

The manual finally provides a summarized step-by-step guide with 26 procedures for carrying out milking. The information was presented in a contextualized and easily understandable manner to ensure the comprehension of participating producers, using models, teaching materials, items, and products commonly used during milking for simulations, as well as a dedicated time for addressing questions during the training. Additionally, specific guidance was provided to interested producers on the collection days and contact methods via social media applications were made available to reduce the distance between the responsible team and the producers.

Analysis of the microbiological quality of milk

The milk samples were sent to the School of Sciences and Engineering (FCE) at Unesp, Tupã Campus, and were analyzed at the Chemistry Laboratory and the Biology Laboratory within six hours of collection.

Following the methodology outlined in IN 62, published on August 26, 2003, by MAPA, which addresses quality standards and hygiene, storage, and transportation regulations to ensure the safety of raw refrigerated, pasteurized, and type A milk in Brazil, and preceding IN 76/20, the sample was diluted in duplicate in sterilized peptone water. Subsequently, a dose was selected to be homogenized in a Plate Count Agar (PCA) culture medium within sterile Petri dishes, which were then incubated at 36°C for 48 hours (BRASIL, 2003).

After the incubation period, the colony-forming units (CFU) were counted following the methodology of Franco and Landgraf (2008), which determines the selection of the dilution that resulted in the lowest count, within the range of 25 to 250, and with the least handling during the analysis. The data from the readings were tabulated for subsequent statistical analysis.

According to IN 76/2018 (MAPA, 2018), which addresses the microbiological composition of raw milk in individual tanks, the permissible limit for SPC is 300,000 CFU/mL of milk. Therefore, this was used as the maximum threshold to determine whether the sample was contaminated or not. For better data comprehension, a logarithmic transformation was applied to the scale to simplify it, meaning the results were converted to a \log_{10} scale, making the maximum contamination limit for the samples $5.477 \log_{10}$ CFU/mL.

Data Analysis

For the analysis of experimental data from the Standard Plate Count (SPC), which quantified the Colony-Forming Units (CFU) per milliliter of each sample, quantitative and statistical methods were applied. Initially, the results from each collection were \log -transformed (\log_{10}) to reduce the numerical scale and facilitate data interpretation. The samples were classified as contaminated or not contaminated based on CFU values, and the number of contaminated samples before and after the hygiene practices training was compared, as well as between the milking methods (manual and mechanical).

An exploratory analysis was conducted to understand the behavior of the data resulting from the milk sample analyses. For the statistical analysis, the Tukey test was used with a 95% confidence level to check for significant differences between the collections made before and after the training, as well as to compare the milking methods. Graphs and tables were created to present the results clearly and accessibly.

Results and discussion

Exploratory data analysis

After conducting the three milk collections from the volunteer producers in the project, the results were tabulated following logarithmic transformation for better visualization of the acquired data. A maximum limit of $5.477 \log_{10}$ CFU/mL was considered.

In general, by considering data concentration based on the types of collections and differentiating them by milking method, it is possible to observe the data concentration from Figure 2. The graph illustrates the distribution of SPC data

across different collection stages and milking types. The wider areas in the "violin" plots indicate where the values are most concentrated, making it easier to compare variations between classifications and combinations. This visualization reveals whether a particular collection method or milking type has a higher or lower concentration of high or low values.

In the pre-training stage, it is observed that manual milking has a narrow range of data concentrations within the limit and a wider range of data concentrations above the limit. In contrast, mechanical milking concentrates most of the data below the limit, with only one outlier above the limit. These initial results suggest that mechanical milking yielded better outcomes than manual milking in this phase.

As the collections progress, a gradual increase in data above the limit is noted for mechanical milking, indicating a more heterogeneous distribution. Manual milking, although showing an increase in values above the limit during the POS20 stage, shows a significant reduction of these values in POS50. If we disregard the last collection, the PRÉ and POS20 stages indicate superior performance of mechanical milking compared to manual milking, partially aligning with the results of Kusumah et al. (2023). This analysis helps to understand the origin of the previously presented averages and assess the impact of the maximum and minimum values on the percentage and exploratory analyses.

Contrary to the initial expectations of the project, this exploratory analysis shows an increase in SPC values throughout the collections, despite the Good Practices Training being conducted. According to Nyokabi et al. (2021), The lack of adherence to Good Hygiene Practices may negatively impact the quality of the milk produced, preventing the product from reaching its maximum potential, both nutritionally and commercially. Therefore, the data initially suggest that the implementation of the guidance provided was not effective.

Despite these initial observations and the apparent differences in the graphs and exploratory analyses, it was crucial to conduct a statistical analysis to determine whether these variations were statistically significant. Only with this validation was it possible to interpret the impacts of the milking methods with greater confidence.

After the initial exploratory data analysis, a statistical cluster analysis was conducted using Tukey's method with a 95% confidence interval. In other words, the goal was to determine whether there were significant differences between the collections or milking methods in terms of improvement, deterioration, or stability throughout the project. Tables 1 and 2 present the results obtained from the analysis of the pre- and post-training collections and the milking methods, where groupings sharing the same letter are not significantly different from each other.

Statistical analysis using Tukey's method revealed that, in both cases, for both the collections and the milking methods, there was no statistically significant difference. This suggests that the previously observed percentage variations likely represent the natural variability of the data, rather than an effect caused by the type of guidance or milking method, with approximately 95% certainty.

Considering these results, the good hygiene practices training did not have an impact on the overall group of selected

volunteers. In other words, although some volunteers showed improvements throughout the project, when considering the entire group of selected producers, there were no overall improvements. This result can be explained from the perspective presented by the Moya et al. (2019), in which this behavior was associated with psychosocial factors such as trust in the trainer and cultural factors such as previous learning, resulting in lower adherence to the applied guidance. The authors further complement that, in addition, the lack of resources, continued education, and the individual perspective of the producer on the matter also contribute to these impacts.

According to the dissertation of the work conducted by Santos (2023), by following five producers over five collection cycles, it was possible to observe a statistically significant decrease in SPC throughout the project due to the guidance methodologies employed. The author used tools such as targeted guidance and a validated checklist, along with laboratory results, to engage with the producers and ensure adherence to good hygiene practices. This approach demonstrates the effectiveness of follow-up that builds a connection with the producer, shows the feasibility of new practices, and applies a targeted methodology.

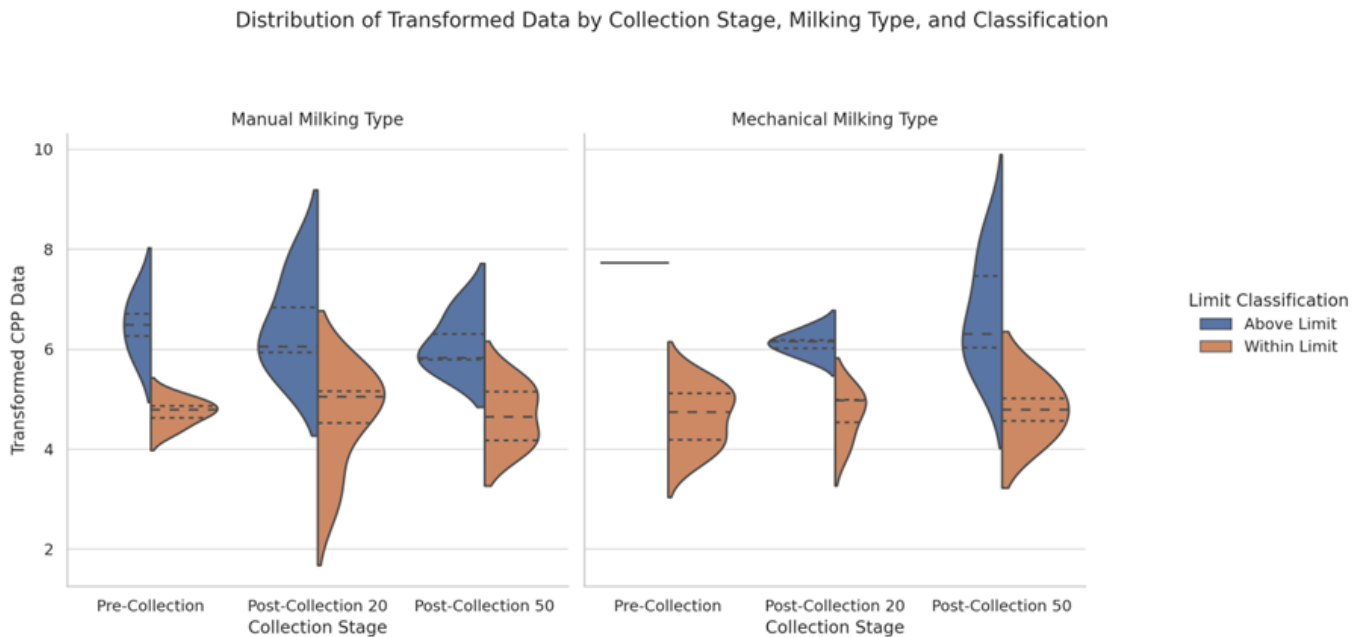


Figure 2. Distribution of experimental data classified according to the established maximum limit.

Table 1. Average SPC values (log10 CFU/mL) of milk collected from 14 dairy properties before and after a training on good hygiene practices.

Collection	AVERAGE (log10 UFC/mL)
Pre-training	5,117 ± 1,054a
Post-training (20 days)	5,501 ± 1,050a
Post-training (50 days)	5,739 ± 1,201a

*Average with the same letter do not differ from each other according to Tukey's Test (p>0.05)

Table 2. Average SPC values (log10 CFU/mL) of milk collected from 14 dairy farms monitored before and after guidance on good hygiene practices with different milking systems (mechanical and manual).

Milking Method	AVERAGE (log10)
Mechanical	5,584 ± 1,031a
Manual	5,321 ± 1,180a

*Average with the same letter do not differ from each other according to Tukey's Test (p>0.05)

Conclusions

The current work demonstrates that the intervention methodology can influence the producer's behavior, given that the only intervention used was collective training. Based on the results presented and discussed, it was possible to verify that neither the good hygiene practices training applied to the producers, nor the type of milking method used impacted the SPC results throughout the study.

Although, during the exploratory analysis, the data showed percentage variations indicating an increase in SPC over the collections and a higher average SPC concentration with mechanical milking, the Tukey statistical test demonstrated that these differences were not statistically significant. In other words, the variations observed in the exploratory analysis were, in fact, due to the natural variation of the data and methods, rather than being influenced by the type of milking or the good hygiene practices training.

Therefore, for future work, it is possible to consider more targeted and assertive interventions for each producer, more individualized follow-up, and a longer period of data collection and monitoring.

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