Contents lists available at ScienceDirect



Animal The international journal of animal biosciences



The effects of implementing management practices on somatic cell count levels in bovine milk



R.K.R. Vieira^a, M. Rodrigues^a, P.K.S. Santos^a, N.B.C. Medeiros^a, E.P. Cândido^b, M.D. Nunes-Rodrigues^{a,*}

^a Postgraduate Program in Animal Production in the Amazon - Campus Parauapebas, Federal Rural University of the Amazon-UFRA, Cep 68515000, PA 275, Km 13, Parauapebas, Pará, Brazil ^b Capanema Campus, Federal Rural University of the Amazon-UFRA, Capanema, Cep 5514-6434, Av. Barão de Capanema, Pará, Brazil

ARTICLE INFO

Article history: Received 6 July 2020 Received in revised form 19 December 2020 Accepted 4 January 2021 Available online 18 February 2021

Keywords: Amazon Management Milk quality Principal component analysis Somatic cell count

ABSTRACT

Somatic cell count (SCC) can be used as a proxy for the prevalence of mastitis in a herd, reflecting the hygiene conditions and management practices on dairy farms, and thus an indicator of milk quality. In this study, we investigated how the adoption of management practices in milking systems can contribute to the reduction of SCC levels and improve milk quality. We collected data regarding management practices from 91 dairy farms in three municipalities of southeastern Pará: Parauapebas, Curionópolis, and Eldorado dos Carajás. Fifty milliliters of milk from each farm were collected in bottles containing bronopol, to preserve SCC. An exploratory factorial analysis (EFA) was performed to reduce the number of variables (management techniques) on dairy farms to some latent factors. We then used the selected factors to estimate the bovine mastitis management index to classify farms according to their use of technology and management techniques. Our results showed that most of the farmers (65.9%) used management techniques inefficiently in their systems, resulting in a significant loss of product quality, while only 3.3% had adopted the full set of techniques. The EFA results demonstrated that simple management practices including regular cleaning of the milking lines, a strip cup test, the California mastitis test, and washing teats with water before milking could be adopted to improve milk quality. However, in scenarios where the regulations become more rigorous, most farmers are unable to meet the maximum allowable SCC requirements, necessitating management innovations to reduce SCC. Therefore, the dissemination of knowledge, technical assistance, and access to new technologies is essential for improving management practices, and thus milk quality.

© 2021 The Authors. Published by Elsevier Inc. on behalf of The Animal Consortium. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Implications

Somatic cell count can be used as a proxy for the prevalence of mastitis in a herd, and thus an indicator of milk quality. In this sense, somatic cell count reflects the hygiene conditions and management practices used on dairy farms and can be used to identify producers who are likely to comply with the regulatory practices designed to increase milk quality. Therefore, by monitoring somatic cell count antigen levels in milk, it is possible to target farms in need of more knowledge, technical assistance, and financing to improve their production methods.

Introduction

The high prevalence of mastitis in dairy herds worldwide results in significant economic losses for the entire production chain via reduced milk quality and production, disposal of low-quality milk, culling of chronically ill animals, and the costs of medication and veterinary services (Aiemsaard et al., 2011; Jagielski et al., 2014). The somatic cell

Corresponding author.
E-mail address: nunes.mdnunes@gmail.com (M.D. Nunes-Rodrigues).

count (**SCC**) is used as an indicator of subclinical mastitis (Kehrli and Shuster, 1994), and for monitoring the milk quality and health of the mammary glands, and thus, possible economic losses (Mendes et al., 2010).

Somatic cells in milk are, in part, leukocytes, which are composed of scaling cells from the secretory epithelium as well as defense cells that migrate from the blood to the alveoli during infection (Machado et al., 2000). When the mammary is infected with pathogens, the immuno-logical reaction increases leukocyte counts in milk (Sá et al., 2018). Most leukocytes are natural defense cells called neutrophils (Almeida et al., 2011). These cells migrate to the mammary gland and are normally found in breast tissues and secretions during the onset of inflammation and may even remain in chronic processes (Brito et al., 1997; Carneiro et al., 2009).

Implementing the required management practices on farms reduces the prevalence of mastitis, leading to a decrease in bacterial load within the environment (Hohmann et al., 2020), reducing possible transmission of bacteria, and protecting the teat ends (Lopes et al., 2017). Changes in the cow's environment throughout the lactation phase and exposure of healthy quarters to microorganisms during the milking process cause contamination between animals (Radotits et al., 2007;

https://doi.org/10.1016/j.animal.2021.100177

1751-7311/© 2021 The Authors. Published by Elsevier Inc. on behalf of The Animal Consortium. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keefe, 2012). The absence of preventive management techniques increases the risk of mastitis. Costa et al. (2019) identified that farms with high SCC do not use techniques such as pre- and post-dipping, California mastitis tests (**CMT**) or SCC tests, and hygienic procedures such as the use of paper towels to dry mammary glands or cleaning milking lines (**MLs**).

Animal production and land use are correlated with low productivity in the Brazilian Amazon (Bowman et al., 2012; Souza and Gomes, 2015; Sparovek et al., 2018). These inefficient systems led to our hypothesis that most farmers do not use adequate management practices in dairy production, consequently increasing the cases of mastitis in the herd. In this study, we investigated how the adoption of management practices in milking systems can contribute to reducing SCC levels and improving milk quality in dairy farms in the Southern Amazon.

Material and methods

Study area and data source

This study is analytical with a quantitative and qualitative approach. We performed an investigation in the southeast of Pará in three municipalities: Parauapebas, Curionópolis, and Eldorado dos Carajás. The climate of the region is characterized by a tropical wet–dry (Aw) climate according to the Köppen and Geiger (1928) classification, and is hot and humid, with a well-defined dry-hot period with annual temperatures above 28°C and an average annual rainfall of 1800 mm.

We selected dairy farms at random and collected the data using questionnaires to understand the production techniques in place. The questionnaire had 30 questions (multiple choice) subdivided into three blocks: characteristics of production, herd characteristics, and milking management. Ninety-one farmers answered the questionnaires between August 2018 and March 2019.

After receiving the forms, we collected a composite sample in expansion tanks and/or milk drums from each of the 91 farms. Fifty milliliters of milk from each sample was transferred to appropriate bottles containing a tablet each of 8 mg of bronopol (2-bromo-2-nitro-1,3-propanediol) at a concentration of 0.02% to preserve the samples (International Dairy Federation [IDF], 2006) until their arrival at the Milk Quality Laboratory of the Food Research Center of the School of Veterinary and Zootechnics of the Federal University of Goiás in Goiânia, GO, Brazil, belonging to the Brazilian Network of Milk Quality Control Laboratories. Bronopol has bactericidal properties and is the main preservative used in the physicochemical analysis of milk (Monardes et al., 1996; Gonzalo et al., 2004; Sánchez et al., 2005; National Center for Biotechnology Information [NCBI], 2020). Before collecting the samples, the milk was homogenized. In expansion tanks, homogenization was performed using a mechanical stirrer, and in drums using a homogenizer, immersing it in the milk for 10 s, and collecting it with stainless steel shells. The collected samples were kept at a temperature between 1 and 7°C for 72 h until they arrived at lab. All procedures described in this methodology are based on the recommendations of the IDF to guide the performance SCC analyses, described in ISO 13366-2/IDF 148-2 of 2006 (IDF, 2006; Brazil, 2018).

Statistical analysis

We performed an exploratory factorial analysis (EFA) to reduce the initial number of variables to a smaller group of factors. We applied the VARIMAX factor rotation method to ensure orthogonal rotation to maximize the factor loads for the variables of the model. The EFA included the following variables: i) production system (PS); ii) type of milking (TM); iii) milking place (MP); iv) milking line (ML); v) examination of first jets with a strip cup test (SCT), vi) California mastitis test (CMT), vii) the presence of calves during milking (PC), viii) teat washing with water before milking (TW); ix) pre- and post-dipping (PPD); x) immersion of teat liners in solution between milking animals (ITS);

and xi) treatment of cows with no milk (**TD**). All selected variables were dichotomous, representing the presence or absence of practice or productive condition (Table 1). It was necessary to introduce a polychoric correlation matrix into the EFA since selected variables were dichotomous. Algebraic expressions for EFA is presented in detail in Jolliffe (2002). Bartlett's test of sphericity was applied to check if the correlation matrix was significantly different from an identity matrix (P < 0.05). In addition, the Kaiser–Meyer–Olkin (**KMO**) index was calculated to test the general consistency of the data in EFA (values above 0.5 is expected to validate the analysis).

To compare the rural producers interviewed in this research with the factors obtained, we estimated a factor score for each observation and each factor using the Thompson regression method (Hair et al., 2014). Rural producers were divided into two groups to assess the effects of management practices on compliance with the legislation (Normative Instruction of the Ministry of Agriculture, Livestock and Supply no. 76, of November 26, 2018): i) SCC within the legal limit—less than 500000 SC/ml—and ii) SCC above the legal limit (above 500 000 SC/ml). We also performed simulations with a progressive reduction of SCC to analyze scenarios in the event of an increase in the rigor of legislation. Simulated scenarios were chosen based on the historical of Brazilian Normative Instructions, which aim to reduce the SCC threshold for milk.

After obtaining the factorial scores for each milk producer, we could then classify them using the bovine mastitis management index (BMMI), which assesses the use of management technologies to control bovine mastitis. To determine the number of factors, we selected only the factors based on the Kaiser criterion (eigenvalues > 1).

To calculate the index of each productive unit, we multiplied each factor score by the variance of the associated factor. Factor scores were estimated using linear regression (Thompson method). Then, the scores for each observation and factor were normalized to a scale of 0 to 1 (Eq. (1)).

$$BMMI_i = \sum_{j=1}^p \frac{\lambda_j}{\sum_{j=1}^p \lambda_j} e_{ij} \tag{1}$$

where:

 λ : eigenvalue factor. j = 1, 2, ..., p.

e: Normalized factor score for each observation of each factor. i = 1, 2, ..., n; and j = 1, 2, ..., p.

We established three categories for the BMMI according to the results of the producer: i) score \leq 0.33: minimal or absent management, for producers who used little or no recommended milking and hygiene

Table 1

Description and statistics for variables used in exploratory factorial analysis (EFA). Target animal: bovine milk production unit.

Variable	Expected	Value 1 (count)	Value 1 (% of total)
PS	0 = Pasture without irrigation; $1 =$ Irrigated pasture.	30	33.0
TM	0 = Manual; $1 =$ Mechanized.	19	20.9
MP	0 = stockyard; $1 = $ Milking parlor.	16	17.6
ML	0 = No; 1 = Yes.	9	9.9
SCT	0 = No; 1 = Yes.	37	40.7
CMT	0 = No; 1 = Yes.	14	15.4
PC	0 = No; 1 = Yes.	86	94.5
TW	0 = No; 1 = Yes.	33	36.3
PPD	0 = No; 1 = Yes.	15	16.5
ITS	0 = No; 1 = Yes.	7	7.7
TD	0 = No; 1 = Yes.	12	13.2

Abbreviations: PS = production system; TM = type of milking; MP = milking place; ML = milking line; SCT = strip cup test; CMT = California Mastitis Test; PC = presence of calf during milking; TW = teat washing with water before milking; PPD = pre- and post-dipping; ITS = immersion of teat liners in solution between milking animals; TD = treatment of cows with no milk.

practices; ii) score between 0.33 and 0.67; moderate management, for producers who used between five and six appropriate practices, and iii) score \geq 0.67: specialized management, for producers who used all the recommended practices. The closer the score was to 1, the greater the use of management techniques capable of reducing the occurrence of bovine mastitis in productive units. The EFA, Thompson method, and correlations between variables and BMMI score were performed using software R, version 3.5.3 (R Core Team, 2019).

Results

The current legal maximum allowable SCC for the northern region of Brazil (SCC < 500000 SC/ml) was achieved in 86.8% of the interviewed producers. We used principal component analysis of the variables listed in Table 1 to evaluate the influence of management techniques and their relation to SCC for each producer. We found that if the legal requirements became more stringent, the number of producers capable of meeting SCC thresholds decreased to 65.9, 36.3, and 12.1% of farmers as SCC requirements were lowered to less than 400 000, 200 000, and 100 000 SC/ml, respectively. For each variable, we could observe a connection between dairy farms and factor score loads (Fig. 1), in which primarily the first and second factors were correlated with lower SCC farms.

The majority of sampled farms produced 50 l of milk or less per day (48.35% of farms); 32.97% of farms produced between 50 and 100 l of milk per day; and 18.68% of farms collected more than 100 l of milk per day. The herds were composed of 80.22% crossbred animals and undefined breeds and 19.78% Girolando cattle.

To explore the influence of management practices on the control of bovine mastitis, we calculated the BMMI and classified rural producers according to their use of management techniques. We calculated the polychoric correlation matrix (Supplementary Table S1) to obtain the principal components of the variables in Table 1. Only factors with eigenvalues above 1 were considered, resulting in four factors (Table 2). The accumulated variance of the four factors was 92%, indicating a

Table 2

Factor loads, factor variance and cumulative variance for variables included exploratory factorial analysis (EFA)—after VARIMAX rotation. Target animal: bovine milk production unit.

Variable	Factor loadings			
	Factor 1	Factor 2	Factor 3	Factor 4
PS		0.95		
TM		0.69		
MP		0.86		
ML	0.76			
SCT	0.83			
CMT	0.90			
PC			-0.97	
TW	0.95			
PPD	0.87			
ITS		0.63		
TD				0.89
Factor variance (%)	40	26	14	13
Cumulative variance (%)	40	66	80	92

Abbreviations: PS = production system; TM = type of milking; MP = milking place; ML = milking line; SCT = strip cup test; CMT = California Mastitis Test; PC = presence of calf during milking; TW = teat washing with water before milking; PPD = pre- and post-dipping; ITS = immersion of teat liners in solution between milking animals; TD = treatment of cows with no milk.

good fit. Bartlett's test of sphericity ($\chi^2 = 746.57$, P < 0.05) and KMO index (0.58) showed that the EFA was adequate for the selected data.

The first factor included the variables ML, SCT, CMT, TW, and PPD, which are the techniques most correlated to the reduction of SCC in dairy farms with 40% of explained variance. The second factor contains four variables (PS, TM, MP, and ITS) and explains 26% of the variance. Factors three and four (TD) contained only one variable each, while it is notable that the three factors showed a negative correlation, indicating that SCC levels tended to increase in systems with the presence of a milking calf.

After estimating the factor, the BMMI was calculated for each producer, which was classified into three groups. The higher the BMMI,

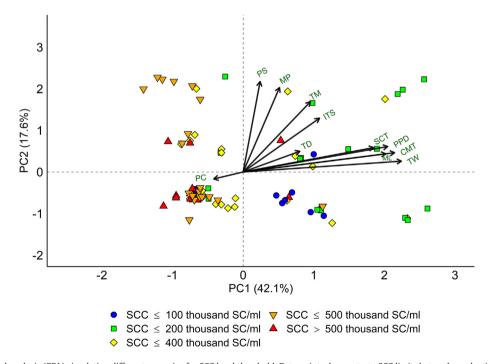


Fig. 1. Exploratory factorial analysis (EFA) simulating different scenarios for SCC legal threshold. Data points demonstrate SCC limit that each production unit can meet in case of institutional change. Vertical and horizontal axes unit represent the factor scores for each farm. Arrows represent the direction and magnitude of variables according to each principal component. Target animal: bovine milk production unit. Abbreviations: PS = production system; TM = type of milking; MP = milking place; ML = milking line; SCT = strip cup test; CMT = California Mastitis Test; PC = presence of calf during milking; TW = teat washing with water before milking; PPD = pre- and post-dipping; ITS = immersion of teat liners in solution between milking animals; TD = treatment of cows with no milk; PC = principal component; SCC = somatic cell count.

Table 3

BMMI index of the use of management techniques for mastitis control. Target animal: bovine milk production unit.

-	BMM Min	l Max	Classification	% of respondents	Average SCC (thousand SC/ml)
	0.00 0.33	0.33 0.67	Minimal or absent management Moderate management	65.9 30.8	385 281
	0.67	1.00	Specialized management	3.3	179

Abbreviations: BMMI = bovine mastitis management Index; SCC= somatic cell count.

the greater the number of management techniques favorable to the reduction of SCC (Table 3). Our results indicated that most producers do not carry out appropriate management practices in this sector (65.9% classified as minimal or absent), whereas only 3.3% used a good number of appropriate techniques. The average SCC was 40.4% lower in farms that used a method that included immersing teat liners in solution; 21.7% lower in farms where the ML is mechanized; and 48.5% lower in properties that perform pre- and post-dipping. However, farms where the calf is present during milking showed an SCC 3.4% higher than that in farms where the calf was not present.

Regarding milking mechanization, we observed that only 20.9% of dairy farms use mechanized milking, in which milk removal is performed using an apparatus that has a vacuum pump to suck the milk and take it to the storage unit. Non-mechanized farms used hand milking. In addition, in 82.4% of the properties, the milking procedure is carried out in the stockyard, which is not a suitable place for milking, while all farms that utilized mechanical milking procedures performed them in a milking parlor.

The results of BMMI can be compared with the number of favorable practices developed for each farm. Farms in the minimal or absent management group adopted three or fewer practices, while specialized management farms used nine or more practices. Farmers whose scores put them in the BMMI index category of minimal or absent management were found to have a higher average SCC, while farmers whose scores placed them in the specialized management category presented a lower average SCC.

Discussion

For the variable PS, farms that used irrigated pasture showed small differences in mean SCC as compared to farms that did not use irrigation. This difference may be due to the fact that the microorganisms that cause mastitis thrive in the increased humidity and higher temperature (Pinho Manzi et al., 2012) during the season when irrigation systems are in operation (Ribeiro et al., 2008). However, there is no research proving that the irrigation of pastures influences SCC levels; consequently, the mean difference in SCC between the systems was small. Studies have shown that some pathogens originate in the environment, and have been isolated in the pasture, soil, and water, such as Prototheca zopfii (Pore et al., 1983; Anderson and Walker, 1988; Costa et al., 1997) and P. wickerhamii (Pore et al., 1983). Nevertheless, irrigation is not necessary in the region as it is possible to maintain forage throughout the year with proper management, even during low rainfall seasons, while irrigated systems require constant SCC monitoring to maintain compliance with maximum legal levels and to prevent the advancement of subclinical to clinical mastitis.

In the properties analyzed, the animals go to the pasture soon after being milked, and since these systems have high humidity, dirt, and organic matter, they favor the penetration of microorganisms, increasing the chances of udder infection (Oliveira et al., 2012). An alternative to avoid infection could be to provide food after milking, to stimulate the animals to remain upright until the closure of the teats sphincter (Costa et al., 1998). Its closure occurs in less than 2 h (Prestes et al., 2002) after milking, in addition to the application of post-dipping.

It has been shown that mechanical milking with a closed circuit and bucket foot occurring in a milking parlor, where both work simultaneously with the vacuum pump to suction the milk and transport it from the milking unit to a storage unit, results in reduced SCC levels (Netto et al., 2009), probably because producers who perform mechanized milking have better knowledge of management practices and more qualified labor available than traditional producers. Our results showed that the groups with moderate and specialized management have, on average, lower reported values of SCC, a result also confirmed by Vallin et al. (2009). However, when people working in dairies do not have adequate knowledge about the procedures for using, operating, and maintaining milking equipment, there is an increase in SCC, as stated by Bozo et al. (2013). In addition to these factors, failures in mechanical milking equipment, such as changes in vacuum, pulsation, over-milking, and deficiency in liner disinfection, cause SCC levels to fluctuate, affecting the integrity of the teat canal (Reis et al., 2018).

The screen cup test allows the first jets of milk to be examined, checking the formation of lumps in the milk that can lead to the diagnosis of clinical mastitis, while CMT allows the identification of animals with subclinical mastitis. This procedure gives the farmer decision-making power to address the treatment of cows with chronic mastitis, to select them for disposal, and to implement a correct sequence of animals to be milked (ML); that is, animals with high levels of SCC or cases of infections will be milked last (Hovinen and Pyörälä, 2011) thus reducing the risk of contamination through milking, equipment, and animals. This study demonstrated that dairy farms that adopt this practice significantly reduced their SCC antigen levels, which is an important and simple practice to spread by knowledge diffusion in the regional milk supply chain.

The immersion of teat liners in antiseptic solution is a preventive measure that should be used after milking between animals to reduce SCC levels (Junqueira et al., 2020). The procedure aims to avoid contamination of animals via utensils and equipment at the time of milking, as liners can function as an element for transferring microorganisms from an infected cow to a healthy cow. Keeping control of the entry of animals through a ML also makes it possible to control the entry of pathogens, especially contagious ones, as animals can be a source of infection for other animals on the property, transmitting microorganisms at the time of milking (Langoni et al., 2011). This measure, if used well, reduces the incidence of diseases in dairy farming systems.

Washing with water and disinfecting the teats before (pre-dipping) and after (post-dipping) milking are essential for the control and prevention of diseases in dairy herds, as they are methods that reduce contamination in teats (Oliver et al., 1993; Ribeiro et al., 2006; Hohmann et al., 2020). These procedures reduce the number of microorganisms that could eventually penetrate the teat canal during milking through the teat sphincter and trigger an inflammatory process (Oliveira et al., 2012). Thus, pre-dipping is a useful tool for reducing teat skin contamination (Miguel et al., 2012).

The presence of a milking calf was the only variable that demonstrated a negative correlation, suggesting that producers who keep the calf at the time of milking may increase SCC in milk. However, this variable is still controversial in the literature, as studies have pointed to the suction by the calf as a factor that decreases SCC due to a reduction in residual milk in the teat canal and microorganisms that cause mastitis (Rasmussen and Larsen, 1998). However, Brito et al. (2000) reported the occurrence of higher levels of infection of the mammary gland when there is management of the use of calf suckling to stimulate the letdown of milk. Our data demonstrated that the calf presence increased the levels of SCC in milk, since this practice favors the contamination of the teats; however, the difference was not large when compared with farms that did not have a calf presence.

The requirement for compliance with Brazilian legislation regarding bovine mastitis is the maximum allowable SCC. However, if the legislation becomes more stringent, reducing the maximum allowable SCC would reduce the number of producers able to meet the requirements. A hypothetical scenario in which SCC limit changes to 100 SC/ml would require a large increase in the level of technology adopted in farms and specialized labor combined with good management practices, since most of the visited farms either do not use adequate management practices in the milking system or have an SCC above 100 SC/ml.

This research demonstrates that when producers use more management practices, SCC levels are reduced, as evidenced by the specialized management groups, which displayed lower SCC averages. These practices are complimentary and should be used to reduce SCC antigen levels. The correct use of procedures in milking significantly reduces the occurrence of bovine mastitis, thus enabling milk production with better quality and within the limits provided by the legislation.

The variables of factor one (ML, SCT, CMT, teat washing with water before milking, and pre- and post-dipping) are practices that favor the greatest reduction in CCS and, therefore, can be part of priority projects and knowledge dissemination policies. Practices such as pasture irrigation and the calf presence, although they did not lead to significant changes in CCS levels, should be adopted with care to avoid becoming sources of increased CCS.

To continuously improve milk quality and increase efficiency on dairy farms, the dissemination of appropriate management techniques through the supply chain is necessary. Policies for technical assistance are important; however, the private sector can also contribute by enforcing the adoption of standards by farmers. Finally, record-keeping of SCC at the farm level is necessary to track the improvement of milk quality, as well as requiring the development of strategies to increase SCC analysis, which is not adequately enforced in this region.

Supplementary materials

Supplementary data to this article can be found online at https://doi. org/10.1016/j.animal.2021.100177.

Ethics approval

Not applicable.

Data and model availability statement

Data are publicly and available in the Mendeley Data Repository: (doi:10.17632/xbk6x9rrnb.1).

Author ORCIDs

R. K. R. Vieira: 0001-5306-9624. M. Rodrigues: 0003-3879-6115. P. K. S. Santos: 0002-4363-6821. N. B. C. Medeiros: 0001-5233-6639. E. P. Cândido: 0002-5235-6768. M. D. Nunes-Rodrigues: 0003-0008-0912.

Author contributions

RKR Vieira: Conceptualization, Methodology, Data curation, Writing —original draft preparation, Visualization, Investigation, Validation, Review & editing. M Rodrigues: Methodology, Data curation, Software, Writing—original draft preparation, Review & editing. PKS Santos: Writing—original draft preparation, Visualization, Investigation, Validation, Review & editing. NBC Medeiros: Conceptualization, Methodology, Data curation. EP Cândido: Conceptualization, Methodology, Visualization, Investigation, Validation. MD Nunes-Rodrigues: Conceptualization, Methodology, Data curation, Writing—original draft preparation, Investigation, Validation, Review & editing, Supervision.

Declaration of interest

None.

Acknowledgements

The authors acknowledge the municipal government of Parauapebas, the Rural Production Secretariat, for supporting the field survey of this study and to Editage (www.editage.com) for English language editing.

Financial support statement

This work was supported by the FAPESPA—Fundação Amazônia de Amparo a Estudos e Pesquisas, with one grant of Postgraduate Program in Animal Production in the Amazon—Campus Parauapebas, Federal Rural University of the Amazon-UFRA.

References

- Aiemsaard, J., Aiumlamai, S., Aromdee, C., Taweechaisupapong, S., Khunkitti, W., 2011. The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745. Research in Veterinary Science 91, e31–e37. https://doi.org/10.1016/j.rvsc.2011.01.012.
- Almeida, L.M., Almeida, M.Z.P.R.B., Mendonça, C.L., Mamizuka, E.M., 2011. Novel sequence types (STs) of *Staphylococcus aureus* isolates causing clinical and subclinical mastitis in flocks of sheep in the northeast of Brazil. The Journal of Dairy Research 78, 373–378. https://doi.org/10.1017/S0022029911000379.
- Anderson, K.L., Walker, R.L., 1988. Sources of Prototheca spp. in a dairy herd environment. Journal of the American Veterinary Medicine Association 193, 553–556.
- Bowman, M.S., Soares-Filho, B.S., Merry, F.D., Nepstad, D.C., Rodrigues, H., Almeida, O.T., 2012. Persistence of cattle ranching in the Brazilian Amazon: a spatial analysis of the rationale for beef production. Land Use Policy 29, 558–568. https://doi.org/ 10.1016/j.landusepol.2011.09.009.
- Bozo, G.A., Alegro, L.C.A., Silva, L.C., Santana, E.H.W., Okano, W., Silva, L.C.C., 2013. Suitability of somatic cell count and total bacterial count in raw refrigerated milk to legislation standards. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65, 589–594.
- Brazil, 2018. Livestock and supply 2018 normative instruction no. 77. Retrieved on 3 December 2020, from. https://www.in.gov.br/materia/-/asset_publisher/ Kujrw0TZC2Mb/content/id/52750141/do1-2018-11-30-instrucao-normativa-n-77de-26-de-novembro-de-2018-52749887.
- Brito, J.R.F., Caldeira, G.A.V., Verneque, R.D.S., Paiva Brito, M.A.V., 1997. Sensitivity and specificity of the California mastitis test as a diagnostic tool for subclinical mastitis in quarter somatic cell count estimation. Pesquisa Veterinaria Brasileira 17, 49–53. https://doi.org/10.1590/S0100-736X1997000200002.
- Brito, J.R.F., Brito, M.A.V.P., Verneque, R.S., 2000. Bacterial counts on the surface of the teats of cows milked under different methods of udder preparation, including cows milked by hand and stimulated by suckling a calf. Ciência Rural 30, 847–850.
- Carneiro, D.M.V.F., Domingues, P.F., Vaz, A.K., 2009. Innate immunity of the bovine mammary gland: response to infection. Ciência Rural 39, 1934–1943. https://doi.org/ 10.1590/S0103-84782009005000106.
- Costa, E.O., Melville, P.A., Ribeiro, A.R., Watanabe, E.T., Parolari, M.C.F.F., 1997. Epidemiologic study of environmental sources in a Prototheca zopfii outbreak of bovine mastitis. Mycopathologia 137, 33–36. https://doi.org/10.1023/A:1006871213521.
- Costa, E.O., Ribeiro, A.R., Watanabe, E.T., Melville, P.A., 1998. Infectious bovine mastitis caused by environmental organisms. Journal of Veterinary Medicine 45, 65–71.
- Costa, G.M., Mesquita, A.A., Rocha, C.M.B.M., Bruhn, F.R.P., Andrade, R.S., Custódio, D.A.C., Braz, M.S., Pinto, S.M., 2019. Risk factors for high bulk milk somatic cell counts in dairy herds from Campos das Vertentes region, Minas Gerais state, Brazil: a case-control study. Pesquisa Veterinaria Brasileira 39, 606–613.
- Gonzalo, C., Boixo, J.C., Carriedo, J.A., San Primitivo, F., 2004. Evaluation of rapid somatic cell counters under different analytical conditions in ovine milk. Journal of Dairy Science 87, 3623–3628. https://doi.org/10.3168/jds.S0022-0302(04)73500-6.
- Hair, J.F., Black, W.C., Babin, B.J., Anderson, R.E., 2014. Multivariate data analysis. 17th edition. Pearson Education Limited, Harlow, UK.
- Hohmann, M.-F., Wente, N., Zhang, Y., Krömker, V., 2020. Bacterial load of the teat apex skin and associated factors at herd level. Animals 10, 1647. https://doi.org/10.3390/ ani10091647.
- Hovinen, M., Pyörälä, S., 2011. Invited review: udder health of dairy cows in automatic milking. Journal of Dairy Science 94, 547–562. https://doi.org/10.3168/jds.2010-3556.
- International Dairy Federation (IDF), 2006. Milk Enumeration 394 of somatic cells (International Standard.ISO 13366-2/IDF148-2). Retrieved on 3 December 2020, from. https://www.pau.edu/msrlibrary/iso/pdf/iso_13366-2_2006_ed2_en_40260_3_cpdf. pdf.
- Jagielski, T., Puacz, E., Lisowski, A., Siedlecki, P., Dudziak, W., Międzobrodzki, J., Krukowski, H., 2014. Short communication: antimicrobial susceptibility profiling and genotyping of *Staphylococcus aureus* isolates from bovine mastitis in Poland. Journal of Dairy Science 97, 6122–6128. https://doi.org/10.3168/jds.2014-8321.

Jolliffe, I.T., 2002. Principal component analysis. 2nd edition. Springer-Verlag, New-York, NY, USA.

- Junqueira, N.B., Salina, A., Oliveira, G.C., Mettifogo, E., Joaquim, S.F., Guimarães, F.F., Dalanezi, F.M., Langoni, H., 2020. Detection of clinical bovine mastitis caused by mycoplasma bovis in Brazil. The Journal of Dairy Research 87, 306–308. https://doi.org/ 10.1017/S0022029920000205.
- Keefe, G., 2012. Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. Veterinary Clinics of North America: Food Animal 28, 203–216.
- Kehrli, M.E., Shuster, D.E., 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. Journal of Dairy Science 77, 619–627. https:// doi.org/10.3168/jds.s0022-0302(94)76992-7.

Köppen, W., Geiger, R., 1928. Klimate der Erde. Verlag Justus Perthes, Gotha, Germany.

- Langoni, H., Sakiyama, D.T.P., Guimarães, F.F., Camossi, L.G., Silva, A.V., 2011. Somatic cell and aerobic mesophylic microorganisms counts in organic raw milk produced in Botucatu-SP, Brazil. Veterinaria e Zootecnia 18, 653–660.
- Lopes, M.A., Demeu, F.A., Costa, G.M., Rocha, C.M.B.M., Bruhn, F.R.P., 2017. Representatividade de diferentes fatores no impacto econômico da mastite em rebanhos leiteiros. Boletim de Indústria Animal 74, 135–147. https://doi.org/ 10.17523/bia.v74n2p135.
- Mendes, C.G., Sakamoto, S.M., da Silva, J.B.A., Jácome, C.G.M., Leite, A.Í., 2010. Physicalchemical analysis and fraud research in informal milk sold in the city of Mossoró-RN. Ciência Animal Brasileira 11, 349–356. https://www.revistas.ufg.br/vet/article/ view/1146.
- Miguel, P.R.R., Pozza, M.S.S., Caron, L.F., Zambom, M.A., Pozza, P.C., 2012. Incidence of contamination in the process of getting milk and susceptibility to antimicrobial. Semina: Ciências Agrárias 33, 403–416. https://doi.org/10.5433/1679-0359.2012v33n1p403.
- Monardes, H.G., Moore, R.K., Corrigan, B., Rioux, Y., 1996. Preservation and storage mechanisms for raw milk samples for use in milk-recording schemes. Journal of Food Protection 59, 151–154. https://doi.org/10.4315/0362-028X-59.2.151.
- National Center for Biotechnology Information (NCBI), 2020. PubChem compound summary for CID 2450, Bronopol. Retrieved on 3 December 2020, from. https:// pubchem.ncbi.nlm.nih.gov/compound/Bronopol.
- Netto, A.S., Fernandes, R.H.R., Azzi, R., Lima, Y.V.R., 2009. Comparative study of milk quality from manual and mechanical milking. Revista Do Instituto de Ciências Da Saúde 27, 345–349.
- Oliveira, J.M.B., Vanderlei, D.R., Moraes, W.S., Brandespim, D.F., Mota, R.A., Oliveira, A.A.D. F., Medeiros, E.S., Pinheiro Júnior, J.W., 2012. Fatores de risco associados à mastite bovina na microrregião Garanhuns, Pernambuco. Pesquisa Veterinaria Brasileira 32, 391–395. https://doi.org/10.1590/S0100-736X2012000500005.
- Oliver, S.P., Lewis, M.J., Ingle, T.L., Gillespie, B.E., Matthews, K.R., 1993. Prevention of bovine mastitis by a premilking teat disinfectant containing chlorous acid and chlorine dioxide. Journal of Dairy Science 76, 287–292.
- Pinho Manzi, M., Nóbrega, D.B., Faccioli, P.Y., Troncarelli, M.Z., Menozzi, B.D., Langoni, H., 2012. Relationship between teat-end condition, udder cleanliness and bovine

subclinical mastitis. Research in Veterinary Science 93, 430-434. https://doi.org/ 10.1016/j.rvsc.2011.05.010.

- Pore, R.S., Barnett, E.A., Barnes, W.C., Walker, J.D., 1983. Prototheca ecology. Mycopathologia 81, 49–62. https://doi.org/10.1007/BF00443909.
- Prestes, D.S., Filappi, A., Cecim, M., 2002. Factors affecting mastitis susceptibility: a revision. Revista Da FZVA 9, 118–132.
- R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Radotits, O.M., Blood, D.C., Gay, C., 2007. Clínica Veterinária. Um tratado de doenças dos bovinos, ovinos, suínos, caprinos e eqüinos. 9th edition. Guanabara Koogan, Andares Rio De Janeiro, Brazil.
- Rasmussen, M.D., Larsen, H.D., 1998. The effect of post milking teat dip and suckling on teat skin condition, bacterial colonisation, and udder health. Acta Veterinaria Scandinavica 39, 443–452.
- Reis, E.M.B., Lopes, M.A., Demeu, F.A., Bruhn, F.R.P., Lima, A.L.R., De Benedicto, G.C., Pelegrini, D.F., 2018. Characterization of family-owned dairy farms in the western amazon. Semina: Ciencias Agrarias 39, 2233–2246. https://doi.org/10.5433/1679-0359.2018v39n5p2233.
- Ribeiro, M.G., Costa, E.O., Leite, D.S., Langoni, H., Garino Júnior, F., Victória, C., Listoni, F.J.P., 2006. Virulence factors in *Escherichia coli* strains isolated from bovine mastitis. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 58, 724–731.
- Ribeiro, M.G., Motta, R.G., Paes, A.C., Allendorf, S.D., Salerno, T., Siqueira, A.K., Fernandes, M.C., Lara, G.H.B., 2008. Peracute bovine mastitis caused by *Klebsiella pneumoniae*. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 60, 485–488. https://doi.org/ 10.1590/S0102-09352008000200031.
- Sá, J.P.N., Figueiredo, C.H.A., Sousa Neto, O.L., Roberto, S.B.A., Gadelha, H.S., Alencar, M.C.B., 2018. The main microorganisms that cause bovine mastitis and its consequences in the milk production chain. Revista Brasileira de Gestão Ambiental 12, 1–13.
- Sánchez, A., Sierra, D., Luengo, C., Corrales, J.C., Morales, C.T., Contreras, A., Gonzalo, C., 2005. Influence of storage and preservation on fossomatic cell count and composition of goat milk. Journal of Dairy Science 88, 3095–3100. https://doi.org/10.3168/jds. S0022-0302(05)72991-X.
- Souza, G.S., Gomes, E.G., 2015. Improving agricultural economic efficiency in Brazil. International Transactions in Operational Research 22, 329–337. https://doi.org/10.1111/ itor.12055.
- Sparovek, G., Guidotti, V., Pinto, L.F.G., Berndes, G., Barretto, A., Cerignoni, F., 2018. Asymmetries of cattle and crop productivity and efficiency during Brazil's agricultural expansion from 1975 to 2006. Elementa: Science of the Anthropocene 6, 25. https:// doi.org/10.1525/elementa.187.
- Vallin, V., Beloti, V., Battaglini, A., Tamanini, R., Fagnani, R., Angela, H., Silva, L., 2009. Milk quality improvement after implantation of good manufacturing practices in milking in 19 cities of the central region of Paraná. Semina: Ciências Agrárias 30, 181–188. https://doi.org/10.5433/1679-0359.2009v30n1p181.