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Relationship between milk production system and milk traits and somatic cell counts in Brazilian Murrah buffaloes: a multivariate analysis

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ABSTRACT. The purpose of this study was to evaluate the monthly milk production and quality of buffaloes from two milk production systems in the Brazilian northeast using the multivariate analysis: principal component analysis (PCA). A total of 2,506 individual milk recordings were performed in two production systems, containing information on milk production (kg day^{-1}), fat, protein, lactose and total solids counts and somatic cell count (SCC). There were positive correlations between the fat content and the contents of total solids (TS) and protein, and of TS and protein. From the PCA, two main components (PC1 and PC2) were identified, explaining 67.71% of the total variation. The fat, protein, lactose and ST level, represented by PC1, explain 46.18% of the total variance, and were an indicator of milk nutritional quality. The CP2, composed of milk production, SCC and production systems, explains 21.53% of the total variance, and was indicative of herd health. PCA results may be useful in dairy buffalo breeding programs, and a reduced number of variables are necessary to assess the nutritional quality of milk and herd health.

Keywords: *Bubalus bubalis*; mastitis; milk recording; score of somatic cells.

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Introduction

In the last few decades, buffalo breeding has increased and acquired more importance in the scientific surveys carried out in Brazil and other countries. This is mainly due to characteristics of the buffalo in terms of the production of animal protein (milk and meat). Moreover, many dairy products are produced with their milk, such as butter, yogurt, and different types of cheese (Marques et al., 2011; Michelizzi et al., 2010; Rangel et al., 2014).

Several studies of dairy buffalo have focused on evaluation the chemical milk composition of the milk, microbiological quality of the milk, and characterization of pathogens related to mastitis, which cause great economic losses in the dairy industry worldwide (Andrea et al., 2015; Botton et al., 2019; Coimbra-e-Souza, Brito, Chamon, Laport, & Giambiagi-de-Marval, 2017; Fabres-Klein, Aguilar, Silva, Silva, & Ribon, 2014; Lee et al., 2014). All this information has contributed to understanding the process of feeding of the dairy herd and to identifying failures that might compromise the qualitative value of the produced milk. This is also of great important to evaluating the hygienic management during milking and the health of the mammary gland of lactating cows (Busanello et al., 2017; Dias et al., 2017; Paixão et al., 2017) in the milk production system, thus enabling the development of adequate management practices to maintain milk quality and ensuring the supply of safe products for human consumption.

Multivariate statistical analysis has become a valuable tool for evaluating the relationship between milk production, milk composition and quality as well as dairy performance in animal production systems (Botton et al., 2019; Shah et al., 2018; Todde, Murgia, Caria, & Pazzona, 2016). This type of analysis consists of the multiple analyses of variables in the context of a single relationship or a set of relationships (Hair, Black, Babin, Anderson, & Tatham, 2009); this is unlike univariate analysis, which contemplates variables in an isolated way.

Principal Component Analysis (PCA) is a multivariate analysis technique that transforms several related variables into a set of uncorrelated variables (components). PCA is an analytical data technique that obtains linear transformations of a group of variables correlated in such a way that certain ideal conditions are reached, and one of these conditions is that the transformed variables are uncorrelated. It is expected that the various linear combinations constructed by PCA analysis capture the maximum extent of the variance, with the first and the second generated components being the best summary of the linear combinations in the data, providing structural simplification of the data. Thus, PCA can use the main components in place of the original variables without losing information as demonstrated in the studies by Hair et al. (2009), Moura et al. (2010) and Shah et al. (2018).

A study carried out in milk production systems that applied the PCA technique demonstrated that the first principal component was an indicator of milk nutritional quality, and the second component was related to the hygienic and sanitary quality of milk (Bodenmüller Filho et al., 2010). In milk production systems, PCA has been used to identify variables, thus avoiding efforts unnecessary by others (Moura et al., 2010). Therefore, the purpose of this study was to use the multivariate technique of principal component analysis to evaluate milk records from two Brazilian buffalo milk production systems to identify components that could explain the correlations between the set of variables.

Material and methods

In this study milk production records from buffaloes of the Murrah breed reared in two production systems in the state of Pernambuco, Brazil were used. In the first system, the management system was semi-extensive (SP1), in which the animals grazed on pickets of *Brachiaria humidicola* and received supplementation in a trough consisted of sugarcane tip, brewery residue (yeast), mineral salt and commercial concentrate. In the second system, the buffaloes were extensively raised (SP2), on *Brachiaria humidicola* pastures. In both production systems, milk recordings were performed monthly, totaling 2,506 records, from 362 buffaloes. Milk production (MY) were measured on each farm and milk samples for the determination of the constituents (fat, protein [PRO], lactose [LAC] and total solids contents [TS]) and somatic cell count (SCC; cells mL^{-1}).

The collected milk aliquots were sent to the laboratory in sterile flasks containing the bronopol for maintenance of milk properties in isothermal boxes containing recyclable ice for keep the temperature at 4 to 7°C. The SCC was assessed with an electronic meter by laser flow cytometry (Somacount 3000, Bentley Instruments®) whereas milk composition analysis was performed using automated Bentley 2000 equipment (Bentley Instruments®), by infrared absorption. These analyses were carried out at Laboratory of the Northeastern Dairy Management Program (PROGENE) located in Recife, PE, Brazil.

SCC data were transformed to somatic cell score (SCS), to achieve the normality and homogeneity of variances (Ali & Shook, 1980), using the formula proposed by Dabdoub and Shook (1984), in which:

$$SCS=[\log2(CCS/100{,}000)]+3$$

Linear score classes (LSC) were also created, ranging from 0 to 9 scores.

The following data was used for the statistical analyses: buffalo number, milk production at the monthly control, date of each control, fat, PRO, LAC and TS, SP1 and SP2, somatic SCS and LSC. Initially, the data were analyzed using the descriptive statistical analyses of the mean, standard error of the mean and coefficient of variation. A Pearson correlation matrix was constructed with the original variables, since a principal components analysis (PCA) is effective if there is a correlation between the variables, as it is indicative of redundant information; therefore, a few factors would be able to explain much of the total variability (Moura et al., 2010).

Finally, the PCA method was used to determine the minimum number of factors that would account for the maximum variance in the data. The technique used to establish the number of factors to be extracted was based on the criterion of Hair et al. (2009), in which each variable contributes with a value 1 for the total eigenvalue; thus only components that have eigenvalues greater than 1 are considered significant, and all components with eigenvalues smaller than 1 are considered insignificant and discarded. The statistical software Statgraphics Plus 5.1 was used for all statistic procedures.

Results and discussion

The average milk yield of the herds was 4.81 ± 0.04 kg per day (Table 1). This value is slightly higher than the averages reported by Jorge et al. (2005), in Brazil, Mendoza and Benítez (2009), in Cuba, and Misra, Rao, and Ravishankar (2010), in India. For the milk components (fat, PRO, LAC and TS), the mean values found were close to the values reported by Jorge et al. (2005) in Brazil.

The mean SCC value was 269.593 (cells mL^{-1} x 1,000) (Table 1). According to Moroni et al. (2006), values above 200,000 cells/mL are indicative of intramammary infection; below this value, the animals would be considered healthy. In this sense, the average observed in this study indicates a warning signal to producers. By relating the SCC values and linear score (LSC), 44 samples, or 17.62% of the total, had values above LSC 5 (> 200,000 cells mL^{-1} of milk), such that in the studied herds studied there, there was a high prevalence of healthy buffaloes (Table 2).

The two presented production systems (SP1 and SP2) had 5.61 and 23.86% of the samples above LSC 5 (data not shown), indicating that the first herd can serve as a basis for the diffusion of techniques (e.g., milking technique, drying buffaloes and cleaning equipment) used for the applied sanitary management of milking buffaloes.

Another important factor observed in this study was the negative correlation of the SCC with the lactose content (r = -0.36) as shown in the correlation matrix (Table 3). Previous studies have demonstrated the existence of a negative correlation between SCC and lactose content in cows' milk. The lactose content of buffalo milk decreased proportionately with milk production with high somatic cell score (Alessio et al., 2016). A high negative correlation between SCC and lactose contents corroborated with other authors that suggest the lactose level information can be used as an indicative change in milk components due to mastitis (Silanikove, Shapiro, & Shinder, 2009). Lactose follows this effect because it is synthesized in the mammary gland from glucose and galactose. Therefore, milk from animals with mastitis or high SCC have decreased lactose levels because there is a reduction in the synthesis activity in the mammary gland tissue (Silanikove et al., 2009).

Table 1. Number of observation (N), Mean (± SD), and coefficient variation (CV) for milk production, components and somatic cell count (SCC).

Variables	N	Mean (± SD)	CV(%)
Milk yield (kg day^{-1})	2506	4.81 ± 0.04	40.14
Fat (%)	2506	6.99 ± 0.03	24.17
Protein (%)	2506	4.01 ± 0.01	12.95
Lactose (%)	2506	4.72 ± 0.01	6.98
Total solids (%)	2506	16.85± 0.04	11.225
SCC (cell mL^{-1} x 1,000)	2506	269.59 ± 11.03	204.82

Table 2. Classes of Linear Score (LSC), and means, variation of SCC.

Class of Linear Score	N	Mean of SCC (x1000 mL^{-})	Variation (x 1000 cell mL^{-1})
1 (0 – 0.9)	142	17.7	12.5 – 24.5
2 (1 – 1.9)	149	36.1	25 – 49.5
3 (2 – 2.9)	210	73.3	50 – 99
4 (3 – 3.9)	278	144.7	100 – 199.5
5 (4 – 4.9)	291	287.5	101 – 399
6 (5 – 5.9)	204	558	400 – 791
7 (6 – 6.9)	106	1,110	805 – 1,589.5
8 (7 – 7.9)	37	2,035	1,601 – 2,844
9 (8 – 8.9)	12	4,212	3,349 – 5,367
10 (>9)	3	8,789	8,157 – 9,654.5

The correlation matrix (Table 3) shows a strong correlation between SCC and LSC; therefore, for future discussions, the variable LSC will be used to describe the influence of somatic cell levels on milk production and constituents. The lactose concentration ranged according to the increase in LSC (Figure 1). Other milk components also changed, however, only the lactose content showed a significant difference between the classes of score.

Table 3. Matrix of correlation of the variables analysed for caracterization of milk production of Murrah buffaloes.

Variables	MY	FAT	PRO	LAC	TS	SCC	LSC
FAT	- 0.39**						
PRO	- 0.19**	- 0.47**					
LAC	0.46**	- 0.65**	0.58**				
TS	- 0.32**	0.95**	0.62**	- 0.59**			
SCC	- 0.14*	0.05*	- 0.04	- 0.23*	- 0.02		
LSC	- 0.19*	0.08**	- 0.04*	- 0.29**	- 0.01	0.76**	
PS	- 0.11*	0.12**	- 0.35*	0.13*	-0.22**	0.21**	0.29**

MY (Milk production, kg day^{-1}); FAT, PRO, LAC, ST (content of fat, protein, lactose and total solids); SCC (somatic cells count); LSC (class of linar score); SP (production system). * $p < 0.05$; **$p < 0.01$.

Information obtained through the correlations between variables of productive interest can help in the definition of technical assistance actions, since the industry analyzes the quality of the raw material - milk - using several parameters, among them the volume of production, distribution, and the nutritional and hygienic-sanitary quality (Bodenmüller Filho et al., 2010). On the other hand, several characteristics come together to compose such evaluation parameters, and these can become redundant if they highly correlate with each other, increasing the number of variables to be studied, with a greater expense in terms of time and work for the researcher. In this sense, new research using multivariate statistical analysis techniques in the field of animal production is being explored, in order to increase the possibility of data exploration.

The correlation between milk yield and LSC was significant and negative (r = -0.19), similarly affecting fat and total solids contents, which was also verified by Barreto et al. (2010). Milk production is lower due to the involvement of the secretory tissue of the mammary gland; there is a decrease in the lactose content and an increase in fat content to total solids, as explained by the dilution factor. The fat and total solids contents presented a significant and positive correlation (r = 0.95). However, the correlation between fat and lactose was high and negative (r = -0.59). Lactose is the main osmotic component because it is the main component responsible for the extraction of water into milk. Thus, as the fat concentration increases with the decrease in milk production, a decrease in lactose concentration is also expected.

Analyzing the data through PCA, we identified a matrix which five components would explain 95% of the total variance of the data. When only considering components with an eigenvalue above 1 (Hair et al., 2009), in order to establish practical significance for the analysis, two components were retained, which, together, were able to explain 67,71 % of the total variance (Table 4). This demonstrates that the main components analysis was effective for this type of data and could thus summarize the number of characteristics responsible for the characterization of buffalo milk production systems into two components. Additionally, it implies that the two components may be used in place of the variables without losing relevant information, and the identification of these suggests two important dimensions to evaluate the inter-correlations in this production system.

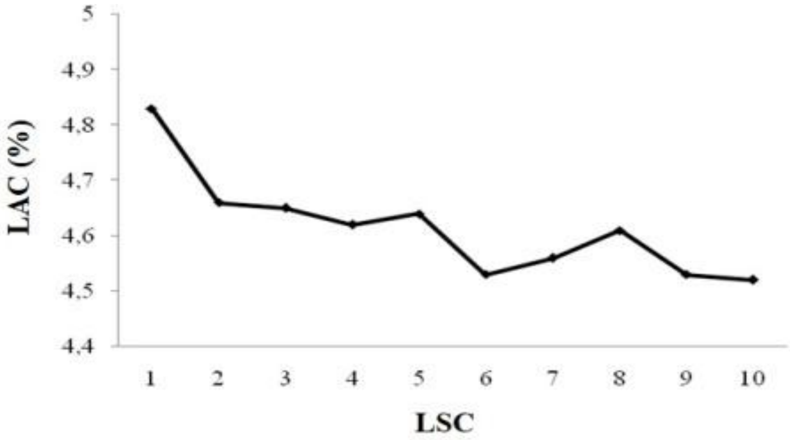

Figure 1. Percentagen of lactose (LAC) according to linear score class (LSC).

Table 4. Autovalues, individual proportion and cummulate variation of the data by principal component analysis.

Principal component	Autovalues	Total Variance (%)	Acummulate Variance (%)
CP1	3.23	46.18	46.18
CP2	1.51	21.53	67.71
CP3	0.78	11.09	78.80
CP4	0.68	9.72	88.51
CP5	0.49	6.99	95.51
CP6	0.30	4.28	99.79
CP7	0.01	0.21	100.00

The selection of the factors (principal components) is carried out in such a way that the first uses the largest possible proportion of the original variability, whereas the second factor has to resort to the maximum variability not possible in the first, and so on, such that the total factors will consist of those that acquire the percentage of variability (Moura et al., 2010). According to the results, the principal component 1 accounted for 46.18% of the total variance, and was identified as the component that is associated with nutritional quality, since the variables corresponding to fat, protein, lactose and total solids showed higher loads (auto vectors) for this component (Table 4). The second principal component was identified as being responsible for hygienic-sanitary and handling quality, since it involved milk production, the linear score class and the production system, accounting for 21.53% of the total variance. Some authors have used principal component analysis to evaluate groups of milk producers. For Bodenmüller Filho et al. (2010), 70.52% of the variance in milk production systems was explained by the first three factors.

The analysis of principal components, through a graph of projection of the variables (Figure 2), allowed for the analysis of the correlations inherent to the main components, projecting them in a factorial plane. Correlations can be interpreted as a function of the angle between the vectors, such that that, if close to zero, the correlation is very high and positive; if it is close to 180°, the correlation is also high but negative, and if the angle is about 90°, the variables are poorly related (Smith, Moreira, & Latrille, 2002). There was a strong correlation between fat, protein and total solids contents, characterizing the nutritional quality. The antagonism of these with the lactose content is remarkable. Presenting at an angle close to 180 ° and in opposite quadrants, milk production and LSC are represented in an antagonistic way, so that the lower the somatic cell load in milk, the better the buffalo's health, resulting in higher milk production. Similarly, the higher somatic cell count, the lower the lactose content, indicating a decrease in lactose levels as the health of the mammary gland is affected.

The analysis of these parameters can help in the discussion of the direction that should be taken for measures, such as for the nutritional adjustment of the buffaloes for better production, or to identify the group of animals that present with a high somatic cell count, since this variable (LSC) contributed the most in the negative variation of the production and of the constituents. Another important factor observed was the small angle formed between the LSC and the production system. Thus, it can be inferred that the management (nutritional, sanitary and milking) directly influenced the milk production results, according to previous explanations of the correlation matrix table.

Milk production is linked to many factors that are responsible for conferring good or bad quality on the product *in natura* as well as its derivatives, factors that range from the genetic formation of the herd to the transportation and marketing of the products. Practically, companies adopt two parameters that are usually used to evaluate the characteristics of milk: nutritional quality and hygienic quality.

The nutritional quality or nutritional value is represented by the physical-chemical evaluation; that is, it is represented by the quantification of the milk components that are of greater interest as the contents of fat, protein, lactose and total solids. The hygienic or sanitary evaluation is related to the somatic cell levels and the bacterial load present in the milk, with the first measure used as an estimate of the health of the mammary gland, both for the herds and individual animals, and the second to the hygiene of milking and processing.

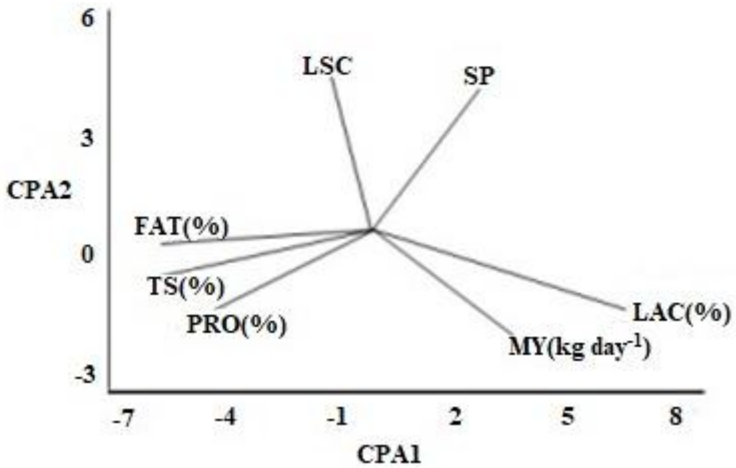

Figure 2. Variables projection: milk production (MY), content of fat (FAT), protein (PRO), lactose (LAC), total solids (TS) and linear score class (LSC).

Conclusion

In this study, we explained the relationship between milk production, chemical components and somatic cell counts in the milk of buffaloes reared in different production systems. The first principal component, represented by the fat, protein, lactose and total solids contents, was a good indicator of the nutritional quality of the milk, while the second principal component, composed of milk production and somatic cell counts, was more related to hygienic-sanitary quality. The somatic cell score and the production system explained variation in milk production and milk components, respectively.

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