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Quality of native goat's milk from a multivariate perspective

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Abstract

The aim of this study was to assess the relationship between physical components and the fatty acid (FA) profile of goat's milk, compare the physical and chemical variables of the milk using canonical correlation, and verify the contribution of the physical variables in predicting the FAs in the milk, contributing to the selection of the FA profile by the correlated response based on the physical profile of the milk. A total of 24 native goats, weighing 42 ± 4 kg and 30 ± 3 days into lactation, were used. The data were subjected to canonical analysis to identify the variables that best discriminate. The main objective of this procedure is to find the best set of variables to compose the discriminant function. Four components were needed to explain 70.68% of the total variation in the physical variables and FA profile in the milk of native goats. The percentage of variance accumulated in the first two factors is 41%. The most important variables in the first factor were the short-chain FAs, namely, caproic (6C), caprylic (8C), capric (10C), and lauric (C12), which are responsible for giving the milk its characteristic and even unpleasant odor, depending on their concentration. New studies with a larger sample of data should be carried out to better understand the relationships between physical and chemical variables in the milk of native goats as well as other breeds of goats for comparison. In addition, the weak simple correlations between the variables studied contributed to the results obtained.

Keywords: fatty acids; CLA; fat globule diameter; canonical discriminant; fat globules.

Practical Application: Studies with this approach can allow for a better understanding of the relationships between physical and chemical variables in milk and the possibility of obtaining gains through a correlated response, which can result in faster genetic gains.

1 INTRODUCTION

In addition to its economic and social importance, milk is rich in a large number of nutrients that are essential for growth and maintaining a healthy life, acting as a preventive measure against a number of diseases such as obesity, insomnia, arthritis, and osteoporosis (Rozenberg et al., 2016). Children, the elderly, and people allergic to the milk of other species are the biggest consumers of goat's milk, and this potential is due to the fact that goat's milk has peculiar physicochemical properties and comprises more than 100,000 different types of molecules with specific functions (Jaiswal & Worku, 2022).

Due to the difficulties of more specific laboratory studies and the high costs of defining the fatty acid (FA) profile of milk, the selection of these characteristics can be done indirectly based on the physical characteristics of milk, such as total fat, quantity and size of fat globules, protein content, and total casein in milk, thereby saving time and human and financial resources (Santos et al., 2021). Studies with this approach can provide a better understanding of the relationships between physical and chemical milk variables and the possibility of making gains through correlated responses, which can result in faster genetic gains. Growing interest in improving the content of conjugated linoleic acid (CLA) in food products has arisen recently due to the discovery of its potential as an anticancer, antiatherogenic, antidiabetic, and antiobesity agent (Persson et al., 2022).

There is a strong relationship between the physical and chemical composition of milk, and these relationships are known in cattle (Communod et al., 2013), but in goats, the studies are preliminary and do not use the multivariate approach, exploring the bivariate approach through the study of simple correlations between pairs of characteristics, allowing conclusions that are not always the most appropriate. There are few studies relating the physical characteristics of milk, production, and milk constituents, especially when the multivariate approach is used. However, there is a need to advance studies into the characteristics related to milk and its constituents, which could make a major contribution to the sustainable use and valorization of milk-derived products.

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Canonical discriminant analysis is a multivariate technique for reducing data dimensionality, similar to the principal components technique and canonical correlation analysis. Ho ever, this technique is a specialty of discriminant analysis and is used to represent several populations in a small subspace (Guedes et al., 2018). Some studies have already been carried out using principal component analysis in cow's milk quality (Akbar et al., 2021; Conte et al., 2022; Correddu et al., 2020; Muniz et al., 2022). The aim of this research was to evaluate the relationship between physical components and the FA profile of goat's milk, compare the physical and chemical variables of the milk using canonical correlation, and verify the contribution of the physical variables in predicting the FAs in the milk, contributing to the selection and profile of FAs through the correlated response based on the physical profile of the milk.

2 MATERIAL AND METHODS

2.1 Animal

This study was approved by the Animal Ethics Committee of UFPB (protocol no. 6167/18). Twenty-four multiparous native goats, weighing 42 ± 4 kg and 30 ± 3 days into their lactation, were used. They were kept in a confinement system for 60 days, housed in a covered shed and in individual wooden stalls equipped with a feeding and drinking trough.

2.2 Diet

The diets were adjusted to comply with the NRC (2007) for lactating goats producing 2.0 kg of milk per day and 4% fat, with a 55:45 volume:concentration ratio. The experimental diet was offered ad libitum at 7.30 a.m. and 4.30 p.m. as a complete mixture of tifton hay, ground maize grain, soya meal, vitamin/ mineral supplements, and urea.

2.3 Milk production and physicochemical analysis

Milking was carried out manually throughout the experiment, twice a day at 6 a.m. and 3 p.m., including 15 days of adaptation and data collection, and the milk was weighed (kg day-1). Before milking, the goats' udders were washed with chlorinated water, dried with paper towels, and then tested for mastitis (black-bottomed cup test). After each milking, the goats' teats were dipped in a 2% iodine solution.

The morning production samples were stored in a refrigerated environment (4°C) to be mixed with the afternoon milk, forming a sample made up of goats per day. From the whole milk obtained from an animal (kg day⁻¹), an aliquot of 200 mL was taken (with the participation of samples proportional to the morning and afternoon milking) to analyze the physicochemical characteristics.

After being placed in identified plastic bottles, the samples were slowly pasteurized at 65°C for 30 min (Brasil, 2001) and frozen at -4°C (in a freezer) for later analysis. Fat (%), protein (%), and lactose (%) were analyzed using the Master Complete® Milk Analyser (AKSO®, São Leopoldo, Rio Grande do Sul, Brazil) under specific technical conditions.

2.4 Analysis of fatty acid profile

The samples of 100 mL of feite were centrifuged in the cold $(15,000 \times g$ for 5 min) to separate the fat from the other nutrients. At the end of the centrifugation, 2.0 g aliquots were withdrawn for extraction (Folch et al., 1957). The transesterification into fatty acid methyl esters (FAMEs) was performed according to the procedure described by Hartman and Lago (1973). FAMEs were analyzed using gas chromatography-flame ionization detector (GC-FID) (TraceTM 1310, Thermo Fisher Scientific, USA) equipped with a SPTM-2380 capillary column (60 $m \times 0.25$ mm ID and 0.25 µm film thickness). The different peaks were identified in comparison with standard FAMEs (Supelco® 37 Component FAME Mix, USA), and quantification was based on their respective peak areas and was normalized.

2.5 Morphometric analysis of milk fat globules

Diameter (lm) and the number of fat globules per milliliter of milk were determined directly in each sample of fresh milk, as described by Martini et al. (2013). In brief, each sample of fresh milk was diluted 1:100 in distilled water and stained by adding a 0.1% solution of Acridine Orange (Sigma-Aldrich, Milan, Italy) in a 0.1 M phosphate buffer (pH 6.8); the ratio of milk and staining solution was 10% (v/v). The analysis was performed by a fluorescence microscope Leica Ortomat Microsystem (Leika SPA, Milan, Italy) equipped with a camera (TiEsseLab, Milan, Italy) and an Image software TS View 2.0 (C & A Scientific, Manassas, VA). The globules were grouped into three size categories: small globules with a diameter of 5 lm.

2.6 Statistical analysis

Pearson correlations were estimated between all the variables using the correlation procedure in SAS (Statistical Analysis System, version 9.1.3). The number of components was chosen based on their eigenvalues, using the criterion suggested by Kaiser (1960) as cited by Mardia et al. (1979), which consisted of including only those components whose eigenvalues were greater than 1. The analyses were carried out using the Statistica software (version 8.0). The data were subjected to canonical analysis to identify the variables that best discriminate. The main objective of this procedure is to find the best set of variables to compose the discriminant function. Statistical analyses were performed using the Statistica 8.0 software.

3 RESULTS

Four components were needed to explain 70.68% of the total variation in the physical variables and FA profile in the milk of native goats (Table 1). The percentage of variance accumulated in the first two factors is 41%, which may reflect the weak structure of the correction matrix between the variables studied.

The most important variables in the first factor were the short-chain FAs, namely, caproic (6C), caprylic (8C), capric (10C), and lauric (C12) (Table 2), which are responsible for giving milk its characteristic and even unpleasant odor, depending on their concentration.

The second factor is represented by the physical characteristics of the milk: diameter and size of small and medium fat globules. The communality, which measures the contribution of the factors to explaining the total variance of each variable, was all greater than 0.7, with the exception of the number of fat globules, milk production in lactation, and total fat.

Table 1. Factors, eigenvalues variation, and cumulative variation of physical variables and the fatty acid profile of native goat's milk.

Factors	Eigenvalue	Variation %	Cumulative variation %
	4.506	23.04	23.04
	3.987	18.21	41.25
	3.07	15.67	56.92
	2.98	13.76	70.68

The bivariate correlations between the milk's physical variables and FAs are shown in Table 3. Most of the correlations between the variables were low, and some were close to zero. Medium correlations were observed between milk casein and some FAs, with the highest value obtained between casein and C18 $1C9 (0.52) (p < 0.05)$. A medium and significant correlation was also observed between fat globule size and some FAs, with a higher correlation between small and medium globules with n3 and large globules with CLA and C6 (Table 3).

The general structure of the simple correlation matrix between the set of variables (Table 3) is weak, making it difficult to interpret the data together. The study of canonical correlations can contribute to a better interpretation and explanation of the relationships between variables, as they make it possible to build groups of variables and assess the relationships between

NFG: number of fat globules; SFG: small fat globules; MFG: medium fat globules; LFG: large fat globules.

Table 3. Simple correlations between physical variables and fatty acids in the milk of native goats.

Variables	n ₃	n6	$n6$ $n3$	CLA	C6	C8	C10	C12	C14	C16	C18	C ₁₈ 1C ₉
Diameter	0.27	-0.08	-0.27	-0.27	0.26	0.19	0.13	0.00	-0.12	0.02	-0.11	0.09
NFG	-0.17	-0.09	0.00	-0.05	-0.14	-0.19	-0.19	-0.24	-0.06	0.24	-0.05	0.19
SFG	$-0.37*$	-0.03	0.24	0.15	-0.14	-0.05	-0.04	0.01	0.02	-0.08	0.14	-0.07
MFG	$0.38*$	0.10	-0.20	-0.07	0.07	-0.03	-0.02	-0.02	0.03	0.11	-0.12	0.04
LFG	0.10	-0.21	-0.24	$-0.35*$	$0.31*$	0.30	0.22	0.04	-0.20	-0.05	-0.11	0.14
Milk	0.03	0.03	0.05	0.09	0.03	-0.02	0.05	0.22	0.18	-0.08	-0.11	0.00
Fat	-0.03	0.18	0.20	0.12	-0.15	-0.11	-0.14	-0.22	-0.27	0.10	0.16	-0.07
Protein	0.14	-0.10	-0.11	-0.29	0.09	0.14	0.09	0.09	-0.24	-0.07	-0.03	0.15
Casein	0.07	$-0.42*$	$-0.37*$	-0.01	$-0.35*$	$-0.34*$	$-0.39*$	$-0.39*$	-0.26	-0.01	0.09	$0.52*$
SS	-0.05	0.01	0.06	-0.11	0.06	0.15	0.11	-0.04	-0.18	0.15	0.00	-0.14

**p* < 0.05; NFG: number of fat globules; SFG: small fat globules; MFG: medium fat globules; LFG: large fat globules; SS: soluble solids.

and within groups (canonical variables). Based on the groups formed, the canonical pairs that best explain the relationships and predictive power of the variables studied are defined.

This study found a high canonical correlation between the first two canonical pairs (V1W1 and V2W2). The corrected canonical correlation between the first canonical pair was high (0.79) (*p* < 0.01) and a canonical correlation of 0.77 (*p* < 0.05), respectively, between the second canonical pair (Table 4) and the likelihood ratio test indicated the first two variables as significant. Hence, it is possible to reduce the sample space by around 90%, since the original 22 variables were reduced to 2 (significant canonical pairs). In addition to the overall relationships observed between the canonical pairs, it is possible to check the variables with the greatest contribution to the definition of each canonical variable, their correlation with a given canonical pair, and the contribution of each individual variable to the variation in each opposite canonical variable and vice versa.

Table 4 therefore shows the canonical coefficients (canonical weights) for the two groups of canonical variables (Vi and Wi). The magnitude of the canonical coefficients shown in Table 4 determines the relative contributions of each variable to the canonical correlation between the pairs of canonical variables. The sign (positive or negative) indicates how these variables affect the opposite canonical variable.

Thus, it was observed that diameter, globule size (small and medium globules), and casein were the variables with the greatest contribution (canonical weight) to the formation of the V1 and V2 variables (Table 5). However, the negative relationship between V1 and casein indicates that as the amount of casein increases, the amount of FAs decreases. However, an increase in the diameter and quantity of small globules results in an increase in the proportion of FAs present in the milk. The FAs C18_1C9, c18, c16, c14, c12, and c6 contributed most to W1 and W2. In general, most FAs are reduced as the amount of C8 increases.

Table 6 shows a negative correlation between FAs (W1) and casein, medium globules, number of fat globules, diameter, and a small but positive correlation with milk-soluble solids and small globules. The correlations for the other variables are almost zero, especially for the first canonical variable (W1). The second canonical FA variable (W2) showed a negative correlation with soluble solids and large fat globules and a positive correlation with milk production during lactation.

The canonical redundancy analysis indicated that none of the first canonical pairs are good predictors of the group of opposing variables. In general, the physical variables cannot be used to predict FAs since the first two pairs of canonical variables from the physical data explain less than 15% of the variation observed in FAs. Even so, the multiple quadratic correlation indicates that the physical variables have good predictive power for the content of C18_1C9 (0.4335), poor predictive power for C10 (0.23), C8 (0.22), n6_n3 (0.18), C6 (0.15), and omega6 and C12 (0.11), and no predictive power for the other FAs studied.

4 DISCUSSION

Quantifying the direct environmental effects on milk production is difficult, as milk production is also strongly affected by other factors such as nutrition, lactation period, and age, which may or may not be directly linked to environmental factors (Fuquay, 1981). Around 65% of the FAs in milk triacylglycerols are saturated, ranging from 65.9 to 71.9% (Silva & Gioielli, 2009).

In goat's milk, the smaller-diameter fat globules favor the formation of fine, soft clots that allow the clots formed to disintegrate

V: predictor variable (number of fat globules, diameter, soluble solids, small, medium and large fat globules, milk, fat, protein, and casein); W: criterion variables (omega3, omega6, n6_n3, CLA, C6, C8, C10, C12, C14, C16, C18 C18_1C9).

\mathbf{X}_{\cdot}	V1	V ₂		W1	W ₂
Number of fat globules	-0.557	-0.193	n ₃	-0.180	0.279
Diameter	1.226	0.843	n6	-0.058	-0.097
Soluble solids	0.448	-0.614	$n6_n3$	0.181	0.426
Small fat globules	1.707	4.565	CLA	0.024	0.488
Medium fat globules	0.290	3.383	c6	-1.116	0.995
Large fat globules	0.000	0.000	c8	1.632	-1.763
Mik	0.010	0.440	c10	-0.931	0.746
Fat	0.125	-0.036	c12	-0.702	1.269
Protein	-0.569	0.373	c14	-0.243	1.140
Casein	-0.722	-0.041	c16	-1.268	0.179
			c18	-1.664	1.885
			c18 1C9	-1.706	1.110

Table 5. Standardized canonical coefficients (canonical weights) of the variables in each set Xi and Yi for each canonical variable.

more quickly, which facilitates the digestive process. Small globules predominate, with 65% having a diameter of less than 3 μm. For this reason, goat's milk has twice as many fat globules as cow's milk, but with greater digestive power, which differentiates it from cow's milk and is of great importance from a nutritional point of view as it reduces the time it takes to pass through the stomach and intestines due to easier digestion (Prosser, 2021).

 Studies carried out on bovine milk have reported that the amount of single FAs tends to change based on the dimensions of the secreted milk fat globules (MFGs) (Martini et al., 2006). However, these relationships have been scarcely investigated in other species such as sheep (Martini et al., 2008, 2010) and goats (Argov-Argaman et al., 2016). Modifying the MFG size could have a significant impact on the nutritional value, technological, and organoleptic characteristics of milk (Martini et al., 2008) and could provide products with specific dietary features.

The size of the fat globules in milk affects the technological and sensory properties and nutritional quality of dairy products, as it interferes with the ripeness, softness, and structure of the cheese, as well as the stability of dairy products, and is of great importance to industries and health (Costa et al., 2021; Martini et al., 2016). Freire et al. (2022) assessed the fat globule size of goat's milk and observed a higher percentage of small fat globules (58%), followed by medium fat globules (38%).

The third factor comprised myristic (14C), palmitic (16C), and stearic (18C) FAs; the latter two together with C10 being the most important from a quantitative point of view in goat's milk since goat's milk fat has a higher proportion of short-chain FAs (6–16 carbons). Hence, the FA profile should be analyzed with caution, as its composition generally varies according to the animal's nutrition, breed, stage of lactation, and other factors (Prosser, 2021).

Despite the great nutritional importance of CLAs, it made up the fourth factor, along with linoleic acid (n6) and the ratio of (n6-n3). CLA is a healthy component of milk fat, predominantly found in ruminant milk. It is recognized as beneficial to human health, acting to prevent various chronic diseases such as cardiovascular disease, hypertension, and obesity (Adak & Gabbar, 2011). The CLA content in milk fat can vary from 0.3 to 1.0% and is influenced by the seasons,

feed, food processing, and the stage of lactation of the animals (Prandini et al., 2007).

Therefore, in situations similar to this research and where it is not possible to collect all the variables used in this study, priority should be given to those highlighted in the first two components. This is because the first component retains the largest portion of the total variation in the data, the second retains the second largest portion, and so on for the other components (Cruz & Regazzi, 2003).

5 CONCLUSION

The physical variables studied were not good predictors of milk FAs. Few studies have been carried out with this approach, so new studies with a larger sample of data should be conducted to better understand the relationships between physical and chemical variables in the milk of native goats, as well as other breeds of goats, for comparison. In addition, the weak simple correlations between the variables studied contributed to the results obtained.

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