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A principal component analysis required in technical assistance guidance for chilled raw milk producers

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ABSTRACT. The purpose of the present study was to evaluate the principal component analysis (PCA) to guide technical assistance regarding several dairy farms' issues, which includes improving microbiological quality and physical-chemical composition of raw refrigerated milk. Data of monthly analysis of fat, protein, lactose, dry defatted stratum, somatic cell count, total bacterial count, milk temperature of 8,101 samples of milk from expansion tanks and production of 78 farms located in the northern region of Minas Gerais, Brazil were processed. Descriptive statistical measures and Pearson correlation coefficient were estimated involving all evaluated traits during the dry and rainy seasons. In addition, multivariate analyses were performed using PCA. The results showed that two farm sites were negatively related to milk quality in both seasons. One farm stood out positively, being able to be used as a herd management model to drive technical assistance actions. Thus, PCA is efficient in simplifying large amounts of data, allowing simpler and faster technical herd management interpretation.

Keywords: dairy cattle; milk quality; multivariate analyses; PCA.

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Introduction

Milk quality regulations and payment schemes regarding criteria of quality and hygiene have led to improvement in microbiological quality and physical-chemical composition of milk produced. Furthermore, payment schemes based on quality benefit the dairy industry as well as the producer with bonus payments. Consumers also benefit by receiving dairy products with improved quality (Meneghini et al., 2016).

Routinely, dairy industries rely on a monthly detailed dataset of milk quality. In general, chemical composition, physical properties, and hygiene quality standards, such as milk yield, fat content, protein content, lactose content, dry defatted stratum, and somatic and microbial cell counts, determine the overall quality of raw milk. All of this collected information defines the prices paid to producers, and guides actions for technical assistance (Busanello et al., 2017). However, there is a demand for an efficient tool to convert the large amount of information collected into herd management actions. Thus, principal component analysis (PCA) might be an alternative to handle dairy production system data, and may also be used as a decision support tool by extended agencies.

PCA is a multivariate technique first described by Pearson (1901) and later applied by Hotelling (1933) in several areas of science. This technique consists of modelling covariance structure by linear transformation of the original variables into a significantly smaller set of uncorrelated components that explain most of the variability in the data. This may facilitate the technician's interpretation of the large amount of information because it allows an easy and rapid means of inferring insights concerning the peculiarity of each herd management system involving several traits simultaneously (Hongyu, Sandanielo, & Oliveira Junior, 2016).

PCA studies were also performed by Resende, Freitas, Pereira, Silva and Pereira (2016). Those authors evaluated the main elements of profitability for dairy farms through the Triângulo Mineiro and Alto Paranaíba regions. Cândido, Pimenta Filho, Gonzaga Neto, Santos and Moura (2015) also used this methodology to describe the Cariri da Paraiba's dairy production system. Milanês, Soares, Ribeiro and Carvalho (2020) applied the technique to identify the relationship and select the variables that best explain the total variation of the data of performance, as well as carcass data of Santa Inês sheep.

Thus, in order to facilitate the interpretation of herd management actions that influence the microbiological quality and physical-chemical composition of milk produced in the North of Minas Gerais, we evaluated the use of PCA as a tool to handle dairy farm datasets in order to better guide herd management, and to improve raw milk quality.

Material and methods

The work was conducted in accordance with ethical standards and approved by the Ethics Committee in Animal Experimentation, at Federal University of Minas Gerais (CEUA-UFMG) - Protocol nº 6/2015.

Milk microbiological quality and physical-chemical composition data from 78 farms located in the North of the Minas Gerais region were collected by the local dairy industry from January 2014 to July 2017. The farms were selected in the laboratory database evaluated because they represented the ones that showed the whole amount of data in the entire months of the analyzed periods. Samples were collected twice a month and microbiological and chemical milk composition analyses were carried out by a laboratory accredited by the Ministério de Agricultura, Pecuária e Abastecimento.

The predominant climate in the northern region of Minas Gerais, according to the classification of Köppen (1948), is Aw semi-arid with summer rains (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). According to Reboita, Rodrigues, Silva and Alves (2015) the region has two well-defined seasons, including dry and rainy, with an average annual temperature of 26°C and an average annual precipitation of 850 mm. Thus, we took into account the microbiological and chemical milk composition data that were collected over two seasons: 1) the rainy season from October to March and 2) the dry season from April to September. The dataset included 78 observations, including eight milk microbiological quality and physical-chemical composition traits. The evaluated traits were: milk yield (MY), defatted dry extract content (DDE), lactose content (LC), fat content (FC), protein content (PROT), temperature (TEMP), somatic cell counts (SCC) and total bacterial counts (TBC).

Descriptive analyses were performed, including mean, minimum and maximum values, standard errors, confidence intervals, and coefficients of variation for each evaluated trait. Pearson correlation coefficient estimates were also obtained to quantify any linear associations among the evaluated traits. The t-test was used to establish if the Pearson correlation coefficients were significantly different from zero, and, hence that there was evidence of an association between the two traits.

Subsequently, multivariate analyses were performed using PCA. After obtaining the principal components, the degree of influence of each trait on the principal components was verified by the correlation between each trait and the principal components being interpreted. From the principal components scores a two-dimensional dispersion plot was generated to visualize the dispersion of each herd (Hongyu et al., 2016). The statistical analyses were performed using the R program, through the functions ‘cor.test’ and ‘princomp’ (R Core Team, 2018).

Results and discussion

Among the chemical components, FC presented the highest coefficient of variation (Table 1) for both rainy and dry seasons, followed by PROT, LC, and DDE. The variables SCC, TBC, and MY showed high CV, indicating substantial diversity among the production systems evaluated. Ribeiro Neto et al. (2012) also found high CV values for SCC (115.68%) and TBC (116.29%) when evaluating environmental impacts on the chemical composition of refrigerated raw milk in the Northeast region.

Table 2 shows high positive correlations among DDE, LC, and PROT that may be partially explained by the fact that DDE is composed of carbohydrates, proteins, and minerals (Tronco, 2013). It was also observed that there was a significant correlation between FC and SCC in the rainy season, whereas LC and PROT levels decreased with increasing SCC across both seasons.

Milk storage temperature did not present a significant correlation with any other trait over the dry period. However, there was a small positive correlation with PROT in the rainy season. However, this estimate was of low magnitude and not of any practical significance (Table 2).

In order to determine the number of principal components, we observed Principal components (PCs) that obtained an eigenvalue > 1, according to the methodology proposed by Kaiser (1958) and Fraga et al. (2016). The first three PCs had eigenvalues > 1 and together they were responsible for 67.81% and 69.71% of the total variance for dry and rainy seasons, respectively (Table 3).

Table 1. Descriptive analyses for milk composition[1], somatic cells count (CCS), total bacteria count (TBC) e milk yield (MY) and raw milk storage temperature (TEMP) in rainy and dry season in the North of Minas Gerais from 2014 to 2017.

Traits[1]	Parameters[2]					
	Maximum	Minimum	Mean	SE	CV (%)	CI 95%
			Dry season			
MY (L prod^{-1}coleta^{-1})	2993.36	49.75	711.83	89.67	111.25	533.27 - 890.38
DDE (g 100^{-1} g)	9.26	8.37	8.77	0.02	2.23	8.73 - 8.82
LC (g 100^{-1} g)	4.69	4.19	4.47	0.01	2.63	4.45 - 4.50
FC (g 100^{-1} g)	4.35	2.71	3.88	0.03	6.81	3.82 - 3.94
PROT (g 100^{-1} g)	3.67	3.05	3.31	0.01	3.77	3.28 - 3.34
TBC (1000 UFC mL^{-1})	1194.22	8.87	266.50	34.02	112.73	198.76 - 334.24
CCS (1000 CS mL^{-1})	1819.50	173.64	491.70	33.79	60.68	424.43 - 558.98
Temp (°C)	4.75	2.91	3.71	0.04	9.61	3.62 - 3.79
			Rainy season			
VOL (L prod^{-1}coleta^{-1})	3010.84	36.91	718.42	85.91	104.93	547.32 - 889.52
DDE (g 100^{-1} g)	9.15	8.29	8.77	0.02	2.05	8.73 - 8.81
LACT (g 100^{-1} g)	4.69	4.25	4.52	0.01	2.41	4.45 - 4.54
FC (g 100^{-1} g)	4.47	2.71	3.66	0.03	8.35	3.59 - 3.73
PROT (g 100^{-1} g)	3.62	2.98	3.25	0.01	3.48	3.23 - 3.28
TBC (1000 UFC mL^{-1})	2479.48	6.25	426.95	61.76	126.94	303.94 - 549.96
CCS (1000 CS mL^{-1})	1870.17	219.29	521.04	36.66	61.75	448.02 - 594.06
Temp. (°C)	5.00	2.99	3.78	0.05	10.91	3.69 - 3.88

[1]Milk yield: MY; Defatted dry extract content: DDE. Lactose content: LC. Fat content: FC. Protein content: PROT; Total bacteria count: TBC; Somatic cells count:CCS. [2]Standard error: SE. Coefficient of variation: CV. Confidence interval: CI.

Table 2. Pearson correlation between quality and production traits of raw milk collected in dry (above diagonal) and rainy season (bellow diagonal) in the North of Minas Gerais.

Traits[1]	MY	CCS	DDE	LC	FC	PROT	TEMP	TBC
MY	-	0.0^{ns}	-0.26*	0.07^{ns}	-0.17^{ns}	-0.39**	-0.16^{ns}	-0.33**
CCS	0.09^{ns}	-	-0.43**	-0.43**	0.15^{ns}	-0.27*	0.09^{ns}	0.08^{ns}
DDE	-0.26*	-0.47**	-	0.75**	0.15^{ns}	0.86**	0.06^{ns}	0.19^{ns}
LC	-0.07^{ns}	-0.59**	0.79**	-	-0.02^{ns}	0.32**	-0.09^{ns}	0.02^{ns}
FC	0.13^{ns}	0.32**	0.01^{ns}	-0.19^{ns}	-	0.17^{ns}	0.10^{ns}	0.14^{ns}
PROT	-0.36**	-0.23*	0.85**	0.35**	0.15^{ns}	-	0.14^{ns}	0.24*
TEMP	-0.03^{ns}	0.03^{ns}	0.2^{ns}	0.08^{ns}	0.2^{ns}	0.24*	-	-0.07^{ns}
TBC	-0.26*	-0.08^{ns}	0.19^{ns}	0.17^{ns}	-0.12^{ns}	0.15^{ns}	-0.04^{ns}	-

[1]MY = Milk yield; CCS = Somatic cells count; DDE = Defatted dry extract content; LC = Lactose content; FC = Fat content; PROT = Protein content; TEMP = Milk storage temperature; TBC = Total bacteria count. nsNot significant at level of 5% by t test. *Significant at level of 5%. **Significant at level of 1% by t test.

Table 3. Eigenvalue (EGV), individual (PEI%) and cumulative explained variance (PEA%, in percentage) for every principal component obtained from milk microbiological and chemical composition trait that determine the milk quality for dry and rainy season in the North of Minas Gerais.

PC	Dry season			Rainy season		
	ATV	PEI%	PEA%	ATV	PEI%	PEA%
1	2.72	34.02	34.02	2.89	36.10	36.10
2	1.64	20.51	54.53	1.49	18.69	54.78
3	1.06	13.28	67.81	1.19	14.93	69.71
4	0.90	11.31	79.11	0.79	9.89	79.60
5	0.65	8.16	87.28	0.78	9.80	89.40
6	0.56	6.98	94.26	0.47	5.90	95.30
7	0.46	5.71	99.96	0.37	4.68	99.99
8	0.00	0.37	100.00	0.00	0.01	100.00

Table 4 presents correlations between the selected principal components and the original variables for dry and rainy seasons. In the dry season, the DDE, LC, PROT, and SCC variables presented higher correlations with PC1; FC showed the highest correlation with PC2, and MY and TBC were correlated with PC3. In the rainy season, PC1 was correlated with DDE, PROT, and LC; PC2 with MY; and PC3 with TEMP.

Farm number 18 presented the highest score for principal component 1 among the evaluated farms (Figure 1). Thus, milk samples from farm 18 presented satisfactory values of PROT, LC, DDE, and SCC. Cows raised on farms 53, 15, 07, and 33 presented satisfactory performance of LC, PROT, and SCC, but low values of FC. The herds on farms 50 and 70 presented acceptable values for FC, but unsatisfactory values of PROT, LC, and SCC.

Table 4. Correlation between principal components and milk quality traits collected in rainy and dry seasons in the North of Minas Gerais.

Traits1	Rainy Season			Dry Season		
	CP1	CP2	CP3	CP1	CP2	CP3
MY	-0.40	0.02	0.72	-0.40	0.63	-0.06
CCS	-0.64	-0.45	-0.33	-0.52	-0.53	0.03
DDE	0.95	-0.18	0.10	0.97	0.09	-0.05
LC	0.81	0.21	0.33	0.70	0.49	0.03
FC	-0.14	-0.79	0.09	0.18	-0.52	-0.06
PROT	0.77	-0.43	-0.14	0.86	-0.21	-0.09
TEMP	0.21	-0.60	0.14	0.08	-0.37	-0.83
TBC	0.32	0.19	-0.63	0.30	-0.51	0.60

[1] MY = Milk yield; CCS = Somatic cells count; DDE = Defatted dry extract content; LC = Lactose content; FC = Fat content; PROT = Protein content; TEMP = Milk storage temperature; TBC = Total bacteria count.

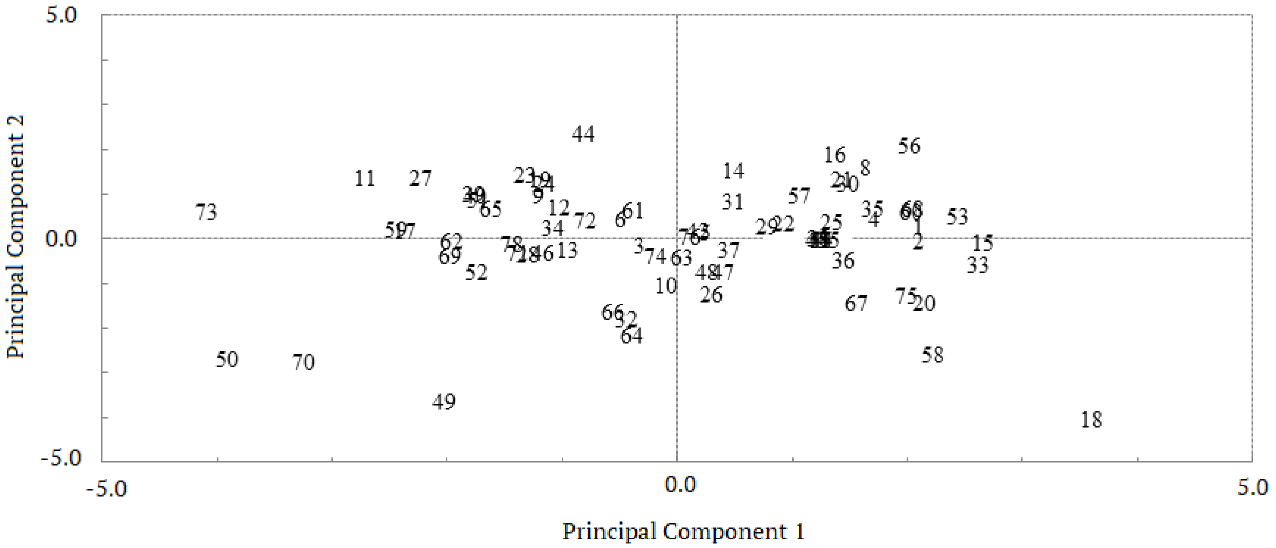

Figure 1. Scatter plot of 78 farms in relation to the top 1 and 2 principal components in rainy season.

By dispersing the principal components 1 and 3 represented in Figure 2, it is possible to classify the producers according to milk production levels, and nutritional and hygienic qualities (Table 4). The producers positioned at the positive end of both axes are those that produced the highest volume of milk with low TBC and nutritional values. At the negative ends in both axes were those producers who generate small amounts of milk with inferior nutritional and hygienic qualities.

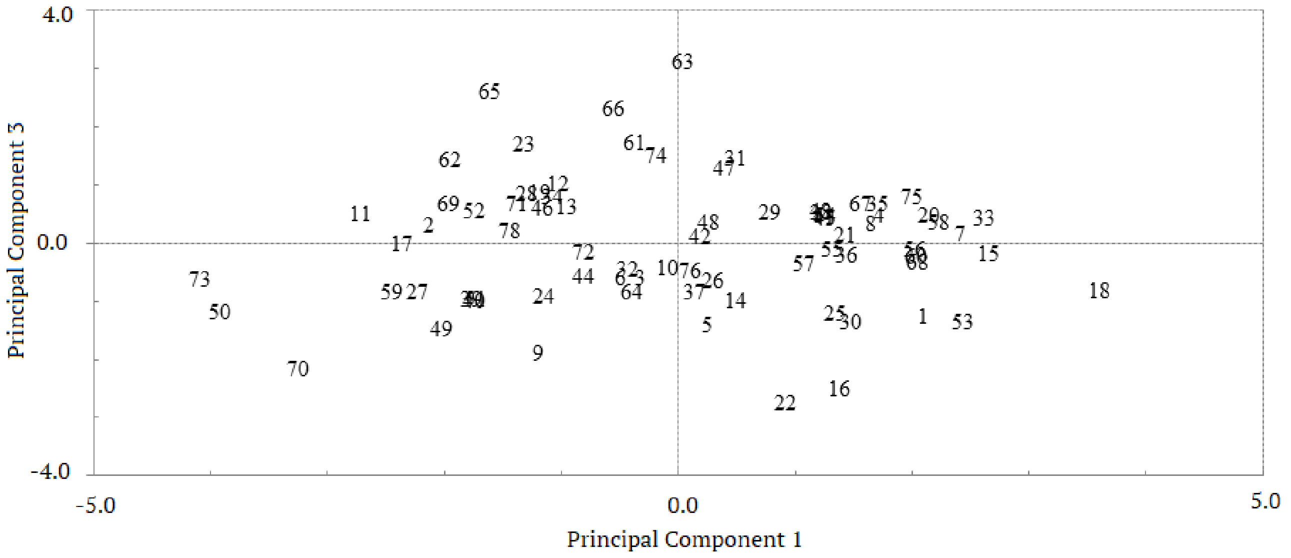

Figure 2. Scatter plot of 78 farms in relation to the top 1 and 3 principal components in rainy season.

Figure 3 illustrates the distribution of fat content and milk yield for each evaluated farmer. The farmers located at the PC2-negative and PC3-positive ends presented the best milk yield, low TBC, and satisfactory levels of fat content. It was noted that there were no producers at the ends of these axes at the same time. This indicates considerable improvements in milk production, nutritional composition, and hygienic-sanitary qualities.

The distribution of the producers is based on the characteristics of the nutritional quality and production during the dry period of the year (Figure 4). It was verified that producers located at the end of the positive quadrant of PC1 and PC2 produced the highest volume of milk, which was also of better nutritional quality in the cases studied over the dry season. It can be said that these producers can serve as positive references for other production systems because they presented milk of a relatively higher nutritional standard and have different production patterns, could serve as a basis for technical assistance actions of the dairy industry.

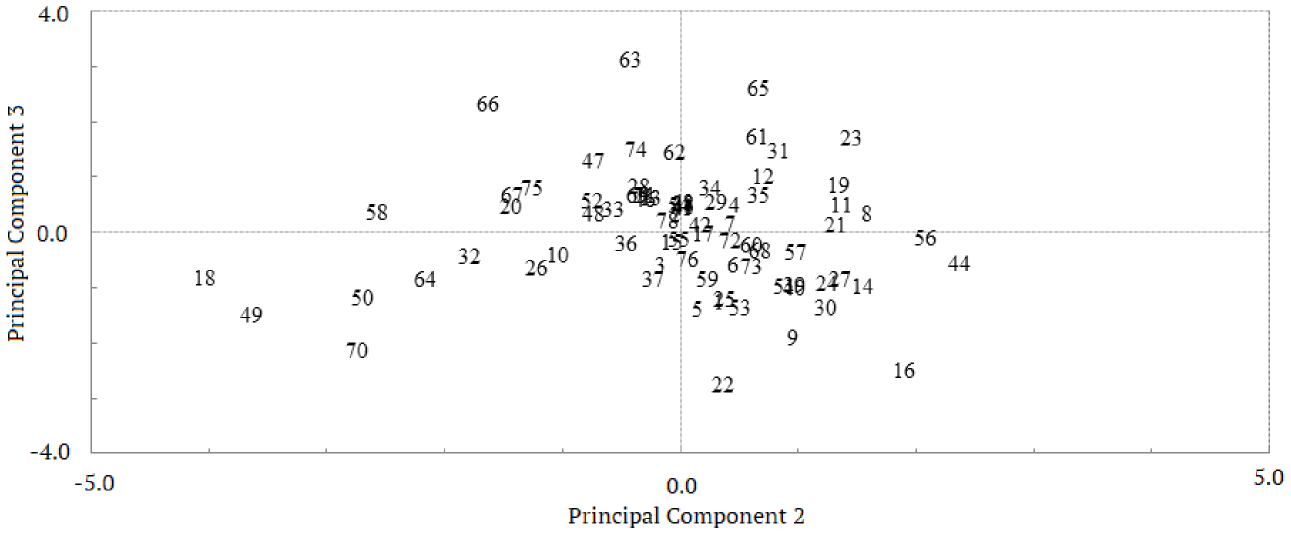

Figure 3. Scatter plot of 78 farms in relation to the top 2 and 3 principal components in rainy season.

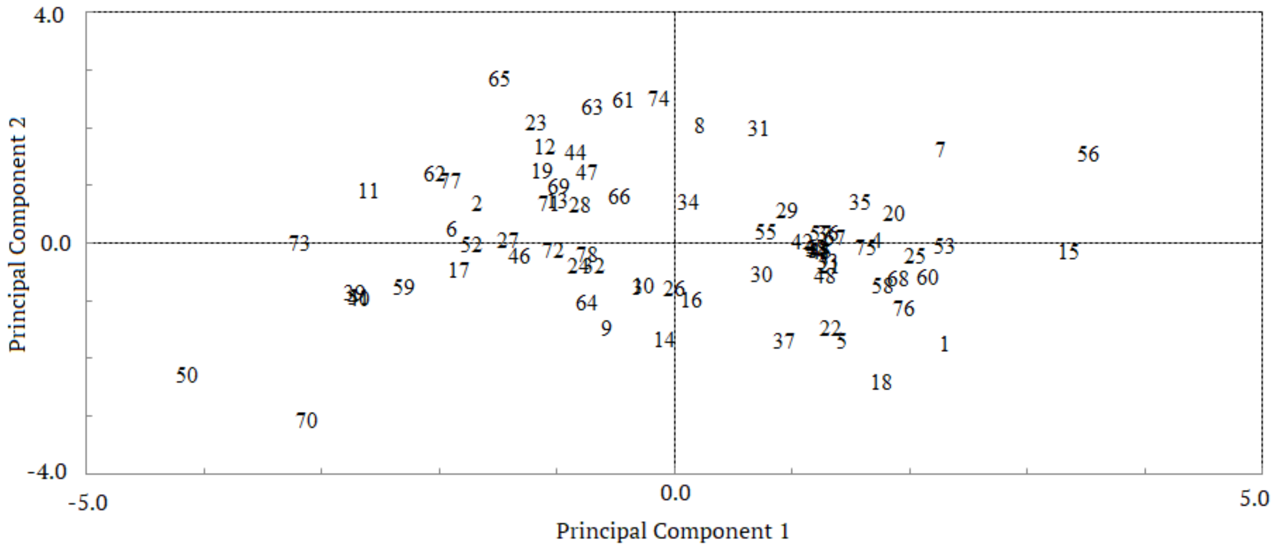

Figure 4. Scatter plot of 78 farms in relation to the top 1 and 2 principal components in dry season.

The dispersion of the first and third major components, as seen in Figure 5, illustrates the classification of the producers according to nutritional characteristics of the milk versus storage temperature of the product.

It was possible to indicate the distribution of milk producers according to the volume produced over the dry period and the parameters that determine the hygienic and sanitary qualities of milk (Figure 6). Producers with higher production volume and better milk quality are found at the ends of the PC2-positive and PC3-negative quadrants.

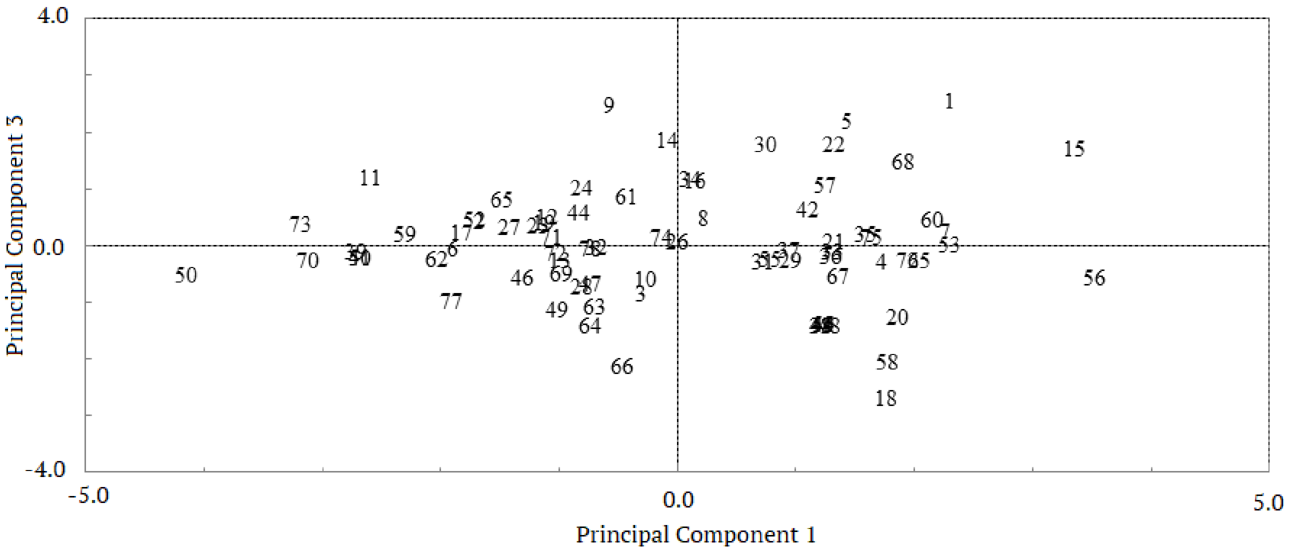

Figure 5. Scatter plot of 78 farms in relation to the top 1 and 3 principal components in dry season.

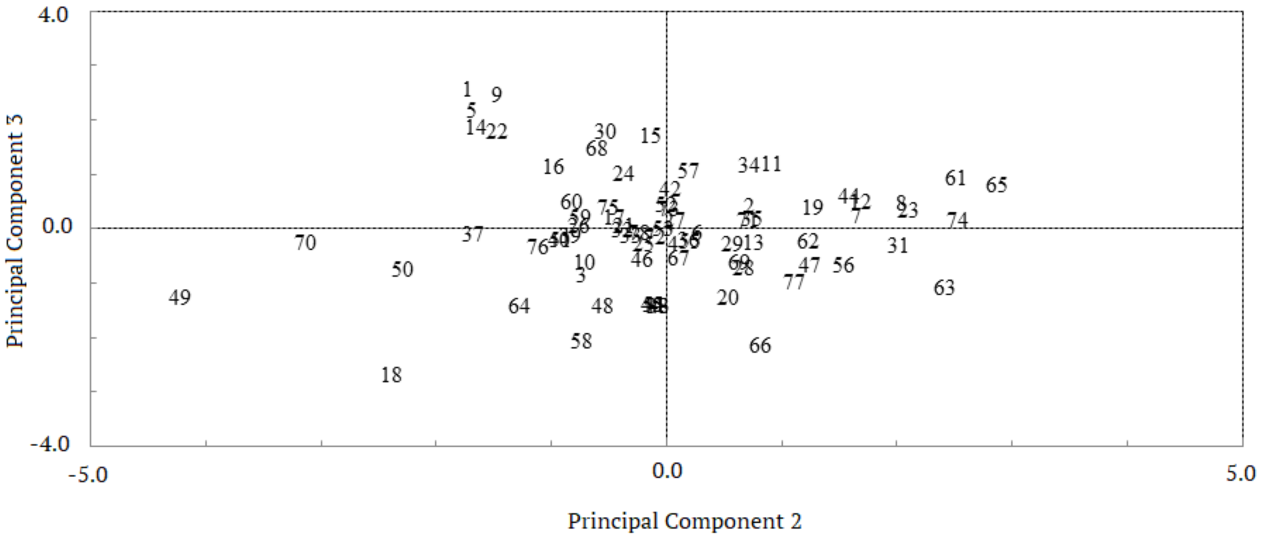

Figure 6. Scatter plot of 78 farms in relation to the top 2 and 3 principal components in dry season.

The FC estimates of 3.88 and 3.66% were obtained for the dry and rainy periods, respectively, and are higher than the minimum limit of 3% established by Normative Instruction 62 for milk (Brasil, 2011). Souza et al. (2015), observed an average of 3.52% in milk for fat content, during the study of F1 H / Z cows.

To increase or maintain the fat content, it is necessary to provide a higher roughage-concentrate ratio, supply fibers, adjust the particle size of forages and silage, supply oils and fats and include buffers in the diet (Sutton &Morant, 1989). Grant, Colenbrander and Mertens (1990) evaluated different dimensions of feed particles and observed that 2.6 to 3.1 mm provide the best values of fat in milk. Macleod, Grieve and McMillan (1983) states that the higher the roughage-concentrate ratio, the better the percentage of fat in milk.

The average protein contents of 3.31 and 3.25% were obtained for the rainy and dry periods, respectively (Table 1). The PROT estimate is in accordance with the values of 3.29% found by Cabral et al. (2016) when working with crossbred cows with production of 15 to 25 liters / milk / day in southwest Goiás, and similar to the average of 3.13% obtained by Henrichs, Macedo and Karam (2014) when they evaluated 6,692 raw milk harvested in refrigeration tanks in the Metropolitan Region of Curitiba (PR). Protein content is the main chemical parameter of milk in terms of dairy production, because the greater the amount of this constituent in milk, the greater the industrial yield (Paula Silva, Lucci, Dias & Santos, 2019).

The average lactose levels in the present study of 4.47 and 4.52% for dry and rainy seasons, respectively, were close to the values of 4.42 and 4.40% cited by Henrichs et al. (2014) and Ribas et al. (2014), respectively.

According to Lin et al. (2016) LC is the main carbohydrate in milk and plays a primary role in milk production because it represents the main osmotic constituent in milk and draws water into the mammary epithelial cells.

The SCC estimates of 491,000 cells mL^{-1} and 521,000 cells mL^{-1} were obtained for the dry and rainy seasons (Table 1). These values are lower than the estimate of 564,95 cells mL^{-1} obtained by Ribeiro Neto et al. (2012). Those authors analyzed samples from 48 Jersey cows at different stages of location in the metropolitan region of Curitiba (PR). However, the SCC values obtained here were similar to the limit of 500,000 cells mL^{-1} required by Normative Instruction Nº 07 (Brasil, 2016).

Hot and humid climates associated with poor hygiene conditions may facilitate proliferation of microorganisms, leading to high exposure to cows to agents causing mastitis. Consequently, with higher somatic cell counts, increased risks of clinical mastitis exist. Moreover, reductions in milk yield due to infection of the mammary gland may be more pronounced than the reduction in fat synthesis, resulting in lower fat concentration (Henrichs et al., 2014).

The TBC estimates of 266,000 CFU mL^{-1} and 426,000 CFU mL^{-1} were obtained for the dry and rainy seasons, respectively, and were lower than the value of 987,000 CFU mL^{-1}cited by Henrichs et al. (2014). The TBC values obtained for the rainy season were higher than the maximum of 300,000 CFU mL^{-1} established by Normative Instruction No. 07 (Brasil, 2016). In contrast, for the duration of the dry season, TBC values were lower than the limit required by the current Normative Instruction, indicating some concern in, and demanding action from, the dairy industry and also technical assistance.

According to studies by Vargas et al. (2014) the reduction in lactose content as the values of SCC increased may be due to disorders of the mammary gland, resulting in less biosynthesis of, or alterations to cell membrane permeability causing higher losses of lactose in the bloodstream. Still in agreement with the referred authors, infections of the mammary gland might contribute to increased bacterial milk contamination. These microorganisms may use lactose as their main substrate for proliferation, resulting in lower LC content.

According to Fraga et al. (2016), if the top PC explained a large proportion of the total variance, up from 70%, it should be used to reduce the complexity of the data with small loss of information. Thus, the use of PCA was effective in reducing the number of milk quality traits, resulting in more efficient and precise analysis and interpretation of the data.

Correlations between original traits and principal components often help the interpretation of such components, thereby unravelling the complex multivariate dataset structure. The cluster average might guide technical assistance, aiming at specific advice. The scatter plots of PC1, PC2 and PC3 revealed the dispersion of farmers across the spectrum of nutritional, hygienic and milk yield traits (Figure 1 and 2).

Farm 18 might be used, with technical assistance, as a model of herd strategies to improve the average milk quality. It is important to highlight that herd management practices carried out by farmers drive milk nutritional quality, especially actions related to feed and hygienic-sanitary management as well as mating selection and genetic composition of the animals. The characteristics of this farm can be published in booklets, which can be distributed by regional technical assistants to producers. This may encourage neighboring producers to use similar production techniques.

In the cases of farms 53, 15, 07, and 33, technical assistance may focus on actions required to improve FC for this group of farmers. The stage of lactation, season, heat stress, diet composition and nutritional management may partially explain the reduction in FC. Feeding large amounts of concentrate may cause rumen acidosis and impact on overall cow health as well as reducing milk fat synthesis (Barducci et al., 2015). Hence, a greater number of feeding events or a total mixed diet may provide an adequate rumen environment for milk fat synthesis.

Bovine genetic composition may be used as a supplementary tool to improve milk fat content through selection of animals with high genetic values for milk fat content (e.g., Jersey; Bodenmuller Filho et al., 2010).

In the cases of producers 50 and 70, hygienic sanitary management needs improvement in order to increase the microbiological and chemical milk composition produced by these groups of animals.

Among the actions to reduce SCC, it was worth mentioning the correct management of animal waste, care when driving cows to milking centers, mastitis detection through California Mastitis Test (CMT) testing, preparation of the udder for milking, post-milking teat disinfection, keeping animals standing after milking, treating ‘dry’ cows, and discarding animals with recurring cases of mastitis. In any control program, special attention should be given to labor training because success depends on a highly skilled workforce, which encompasses care that goes beyond common milking procedures.

Caloric stress may decrease the protein content of milk by the reduction of dry matter intake. Food management practices, such as providing food in the cooler hours of the day, increasing feeding frequency, and access to water might also be helpful (Nakamura et al., 2012).

Producer 63 presented a good volume of milk with low TBC values, although protein and lactose levels could be improved. Probably, the solid milk content of this producer was lower due to the higher volume of milk generated. Animals specialized in production tend to have a lower percentage of solid content. Positive emphasis on hygiene practices adopted by the producer during milking might be used as a model by technicians to apply on properties that present high TBC, for instance producers 22 and 16.

The group of farmers 18, 15, and 33 produced milk with satisfactory protein, lactose, and defatted dry extract content, but these producers need to improve milk yield and TBC values (Figure 2). Industry and dairy technicians need to direct their actions to encourage such farmers to increase the amount of milk produced and guide them to apply efficient hygienic procedures during milking. The hygienic practices of producers 63, 65, and 66 might help technicians to create a hygienic procedure pipeline.

Producers 56 and 15 presented good values of DDE, PROT, and LC, but milk production needs improvement. Producers 74, 61, and 65 produced good volume of milk, but did not stand out in terms of their nutritional value. Producers 50 and 70 presented the worst production volume and poor quality of milk in relation to nutritional factors. It was also noted that there were no producers who simultaneously excelled both in production volume and quality of milk. Through these notes, technical assistants can direct their actions to each specific situation and thus improve the entire milk production set-up.

At the end of the PC1-positive and PC3-positive quadrants producers presented milk with good values for solid content and good values of milk storage ^{temp}erature (0 to 4°C) and high values for TBC. Producer 15 yielded milk with good nutritional values but could improve with respect to sanitary management during milking. Producers 18 and 58 can serve as positive benchmarks for technical assistance to devise management strategies for producers with higher TBC values, such as producers 9 and 14.

It can be noted that most of the producer groups in the central region of Figure 6, there being no producer that stands out in the positive ends of PC2 and which was also negative in PC3.

This indicates that producers can greatly improve production and hygienic-sanitary quality. The group of producers 1, 9, 5, 22, and 14 presented low production volume and low hygienic milk quality.

Analyzing all the graphs simultaneously, we observe the behavior of the producers regarding the volume produced, and the nutritional and hygienic qualities during the rainy and dry periods. It was verified that producer 18 presented the best milk quality in relation to nutritional and hygienic parameters. Producers 50 and 70 presented milk of inferior quality with respect to these latter characteristics.

The paths to be tracked by different producers in the quest for the best quality of milk produced are distinct and depend on their position in the intersecting planes involving the PC1, PC2, and PC3 axes. It should be emphasized that the differentiation of technical advice and the possible strategies of action to improve milk quality for different groups of producers should be based on the most important variables for the construction of the first main components. The other variables should be considered, but generally for all groups of producers.

Conclusion

Using PCA makes it possible to simplify the view of the dairy industry extent, and facilitates orientation of technical assistance, having referential within the studied populations. Among the evaluated cases, producers 50 and 70 exhibited a negative relationship with milk quality during the two periods, and producer 18 presented the best nutritional and hygienic quality milk over the dry period as well as in the rainy season, being able to serve as a recipient for field days.

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