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Characterization and relationship between bulk tank milk composition and compost bedded variables from dairy barns in Rio Grande do Sul state, Brazil

Karise Fernanda NOGARA[1,*], **Marcos BUSANELLO**[2], **Ione Maria Pereira HAYGERT-VELHO**[3], **Maity ZOPOLLATTO**[1], **Karen Dal Magro FRIGERI**[4], **Paulo Sergio Gois ALMEIDA**[3]

[1]Department of Animal Science, Federal University of Paraná, Curitiba, Paraná, Brazil

[2]Department of Animal Science, College of Agriculture "Luiz de Queiroz"/University of São Paulo - ESALQ/USP, Piracicaba, São Paulo, Brazil

[3]Department of Animal Science and Biological Sciences, Federal University of Santa Maria, Palmeira das Missões, Rio Grande do Sul, Brazil

[4]Federal University of Technology-Paraná, Dois Vizinhos, Paraná, Brazil

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Abstract: Studies with compost bedded pack dairy barn system (CBDB) are still recent in Brazil. Thus, we used principal component and canonical correlation analysis to characterize and verify the relationship between compost bedded variables (CB) and bulk tank milk variables (BTM). Data are from 8 dairy farms comprising August to October 2018, in the northwest region of Rio Grande do Sul state, Brazil. Results indicated a heterogeneous BTM composition and CB management among dairy farms, besides a strong relationship between the two sets of variables (1st pair, $r_c = 0.972$; $p = 0.0253$). Linear combination of CB variables explained 31.2% of the BTM variation. Protein (– 0.53) and total bacterial count (TBC) (0.91) were the principal variables in BTM set, while compost bedded TBC (0.51), temperature at surface (– 2.28) and 20 cm depth (1.83), barn spacing per cow (– 0.53), and pH value (– 0.55) were the principal variables in CB set. A significant effect of CB management on BTM was found where bedding temperature should be the principal variable for monitoring.

Key words: Composition, compost barn, dairy cattle, milk quality, multivariate statistical analysis

1. Introduction

Brazil ranks fourth in the world ranking of milk-producing countries, with an increasing production in recent years[1]. Several advances in the production system have occurred over the years, which allowed to boost and improve milk production in the country, but it also needs to provide working conditions for the people to make production feasible [1]. Recently, dairy farmers in some regions of Brazil (mainly South and Southeast) started to use a confined farming system known as compost bedded pack dairy barn (CBDB). This system is characterized by a composting barn with a large resting area with a bed that allows the free movement of the animals, improving its health, longevity, and productivity. Generally, the bed in the CBDB is composed of sawdust or shavings being turned at least twice a day and being separated from the feeding area [2, 3].

Despite CBDB is a recent adopted system for dairy cows rearing in the world, various studies have been developed involving their use [4, 5, 6]. Results indicate that CBDB allows an increase in milk production (29.3 kg before adoption vs. 30.7 kg after CBDB adoption) [7], better working conditions for the farmers, greater profitability, greater comfort offered to cows by the housing system, decreased somatic cell count (SCC), and problems with legs and hooves [8]. Managements such as monitoring the moisture of the compost bedded (CB) by turning and also using fans are essential to maintain a soft and dry space for cows, ensuring the health of the mammary gland [9,4].

A study evidenced a significant reduction in bulk tank SCC and mastitis incidence after CBDB adoption on Minnesota, USA dairy farms dairy farms (n = 12 herds) [2]. Studies also report the effects of variables related to CB quality in CBDB on the bacterial population of the CB and the milk quality [5,10]. Higher CB temperatures are related to a decrease in the bacterial population, especially of *Klebsiella spp*. and *Streptococcus spp*. [5]. In addition, it

[1] FAO – Food and Agriculture Organization of the United Nations (2020). Dairy market review: Overview of global dairy market developments in 2019 [online]. Website: http://www.fao.org/3/ca8341en/CA8341EN.pdf [accessed 15 September 2020]

* Correspondence: nogara.karise@gmail.com

is known that CB with high bacterial loads can result in an increase in total bacterial count (TBC) [10] and SCC in the bulk tank milk (BTM).

In Brazil, studies with CBDB are still few and recent [11-15]. In the Rio Grande do Sul state, several dairy farmers in the northwest region have adopted the CBDB system [16,17]. Although there are some research with the CBDB, dairy farmers are facing difficulties in handling the CB due to the lack of information on the subject for the region because that system has been implemented recently [15,17]. The lack of raw material for replacement of the CB is one of the greatest difficulties faced by farmers, which directly interferes in the management of the CB and can negatively affect animal response and milk quality, verified by the high levels of SCC and TBC [15,17,18,].

Besides the CBDB being a recent topic under study, most of the studies mentioned above made use of univariate statistical techniques for their assessments. Few are the studies using multivariate approaches in the animal science area, representing less than 2% of the total [19]. Considering the importance of the interrelation between variables in a productive system, we have some studies in Brazil with milk production that made use of multivariate techniques, which have been shown to be more interesting to explore the data [20-22]. Within the multivariate techniques, the principal component analysis (PCA) allows to characterize individuals/observations based on a linear combination of the variables, while the canonical correlation analysis (CCA) allows to verify the relationship between two sets of variables and the strength of this relationship [23].

Therefore, knowing the importance of conducting more studies with CBDB, especially in Brazil and using multivariate techniques, we aim to 1) characterize BTM composition and CB management in CBDB through PCA and 2) evaluate the influence of variables related to the CB of CBDB on the BTM composition from 8 dairy farms located in the northwest of Rio Grande do Sul, Brazil using CCA. We hypothesized that CB variables such as temperature on the surface, at 10 cm, and 20 cm depth, pH value, compost bedded total bacterial count (cbTBC), moisture, barn space per cow (BSC), and dirt score of the cows significantly affect the BTM composition in that region.

2. Material and methods

2.1. Study design and data collection

The study was designed as an observational cross-sectional study, which represents an analysis at a specific point in time. The STROBE statement was used as a guideline for the conduction and reports of this study [24]. It comprised data from August to October 2018 of 8 dairy farms located in the northwest of Rio Grande do Sul state, Brazil. A nonprobabilistic sampling method called "convenience sampling" was used to select the farms for the study, where the farms were selected in order of appearance according to their convenient accessibility [25]. The climate of that region is classified as a humid subtropical or temperate climate (Cfa type) [26], which presented a mean temperature of 15.8 °C and precipitation of 148.1 mm in the studied period[2]. All the farms reared their cows at a CBDB and were visited once a month. Those dairy farms had a median of 49 lactating dairy cows in the studied period (interquartile range = 45 dairy cows). All the cows were of the Holstein purebred or Holstein × Jersey crosses. The dairy farms were selected for convenience from contacts with known farmers who agreed to participate in the study.

Bulk tank milk composition (fat, protein, SCC, and TBC) and variables related to the CB (moisture, temperature, cbTBC, and pH) were collected. Also, the cows' dirt score and the BSC were measured and included in the CB set of variables. All the evaluations were made once a month and did not affect the routine of the farms, being only the observation of the cows, collection of production data, and CB sampling. Daily milk production per cow was not provided by the farmers because they had no equipment to measure it at the farm.

In each visit the CB temperature was measured with a digital meter 4×1 (2Vintens, digital model). That measuring was performed in nine areas on the dairy barn, divided into quadrants on surface and covering the depths 10 cm and 20 cm, as performed by Albino et al. (2017)[5]. After that, the CB samples of each quadrant were collected, homogenized, and stored in plastic bags for posterior measurement of pH value, moisture, and cbTBC at the laboratory.

The cows' dirt score was measured in all lactating cows of the dairy herds, considering the adherence of materials and manure in the teat and udder of the cows as described by Schreiner and Ruegg (2002) [27]. Briefly, dirt score measured as score 1 = teat and udder completely clean, score 2 = teat and udder are a bit dirty, score 3 = teat and udder with a median dirty, and score 4 = teat and udder with high and well adhered dirty. The median of cows' dirt score was used as a measure of the herd in each month evaluated. The BSC was measured each month considering the barn area (not CB area) and the number of cows inside that for each month. Milk samples were collected by the dairy company that purchased the raw milk from the dairy farms for posterior laboratorial analysis.

2.2. Sample analysis

The compost bedded pH analysis was held at the bromatological laboratory from Federal University of Santa Maria – Campus Palmeira das Missões where the

[2] INMET - Instituto Nacional de Meteorologia (2020). Banco de dados meteorológicos [online]. Website: https://bdmep.inmet.gov.br/ [accessed 10 June 2020]

samples were diluted in distilled water in the proportion of 1:5, the mixture was homogenized by shaking and remained resting for 15 min, after that the pH measuring was done using a digital pHmeter. The CB moisture was determined by drying the samples in an oven with forced air ventilation (55 °C) for up to 72 h and determined by gravimetry. The cbTBC was determined from plate cultivation at the laboratory of the veterinary hospital from UNIJUÍ University, where 10 g of compost bedded material was weighed and diluted at level 10^{-1} in a 90 mL of a saline solution (85%). After that, 1% of polysorbate (Tween 80) was added to the mixture and then it remained resting for 15 min, and so, the mixture was homogenized by shaking. In the sequence, 1 mL of the mixture was transferred to six tubes containing 9 mL of the saline solution until it reaches the appropriate dilution (10^{-1} to 10^{-6}). So, 0.1 mL of the dilution was transferred to a surface of three Petri plates containing a nutrient agar culture medium using a micropipette. The plates with the material remained resting for 2 min, and, after that, they were inverted and incubated at 35° C for 24 to 48 h. The bacterial count was obtained by multiplying the mean number of colonies found in the plates by the chosen dilution.

As mentioned above, milk samples were collected and sent for analysis by the dairy company that purchased the raw milk of the farms. Milk samples were sent to the Laboratory of Dairy Herds Services of the University of Passo Fundo, Rio Grande do Sul state. Fat and protein milk contents were determined by near-infrared reflectance spectroscopy (NIRS, Bentley 2000, Bentley Instruments, USA). The SCC and TBC were determined by flow cytometry (Somacount 300, Bentley Instruments,USA).

2.3. Statistical analysis

Descriptive statistics were performed to verify the coherence of the data (minimum, median, quartile range, and maximum). The SCC, TBC, and cbTBC were log-transformed because they did not present normal distribution based on the Shapiro–Wilk's test. Linear models were performed for all variables considering the fixed effects of dairy farm and month of the year to obtain the residuals of the variables, which are the values without the possible effects of farm management and month of the year [28]. Variables were separated into sets as follows: BTM (fat, protein, TBC, and SCC) and CB variables (BSC, cbTBC, moisture, the temperature at 0, 10, and 20 cm depth, and pH value). Following, residual Kendall's correlation was performed between all the variables to verify the relations among then using the nontransformed data (within the set and between sets).

Kendall's correlation analysis also allowed the verification of problems with multicollinearity for the next analyses, which were the principal component analysis (PCA) and canonical correlation analysis (CCA). Multicollinearity was also checked with the variance inflation factor (VIF), where VIF = 1 indicates that variables are uncorrelated, a VIF between 1 and 5 indicates moderate correlation, and a VIF between 5 and 10 indicates a high degree of correlation [29]. Based on it, the temperature at 10 cm was excluded from the PCA and CCA because it presented very high correlation coefficients ($r > 0.8$) with the temperature at 0 and 20 cm.

The PCA was performed to characterize the dairy farms regarding the BTM and CB variables. Medians were calculated for each variable and dairy farm for posterior use on the PCA considering all the period of study. *Biplot* graphs [30], eigenvalues, and eigenvectors of each principal components (PC) were presented. Most influent variables within the PCs were considered when they presented eigenvectors > 0.45 or < –0.45. Separate PCA were performed for BTM and CB variables because the assumption of observations number ≥ variables number.

For the CCA, multivariate normality was verified using Mardia's test [31, 32] for the two sets of variables, and no problems were found out for skewness and kurtosis. After that, the CCA was performed between the sets of BTM and CB variables. The CCA was performed to verify the multivariate correlation between the groups BTM and CB. The canonical correlation coefficient was calculated for each pair of canonical variables (CV), besides the squared canonical correlation coefficient and redundancy index. Canonical loadings were also presented to indicate the importance of the original variables into each CV. Most influent variables within the CVs were considered when they presented canonical loadings > 0.45 or < – 0.45.

All the analyses were performed using SAS University Edition software[3]. Descriptive statistics were performed using SAS PROC MEANS, while linear model and residuals calculation were done using SAS PROC GLM, Kendall's correlation analysis was performed using SAS PROC CORR, PCAs were performed using SAS PROC PRINCOMP, Mardia's test was performed using a macro %Multinorm, and the CCA was performed using SAS PROC CANCORR. Statistical significance was considered at the level of 0.05 (5%) of probability.

3. Results

3.1. Descriptive statistics and Kendall's correlations

Descriptive statistics are presented in Table 1. Briefly, the median fat content in milk was 3.61%, while protein content was 3.22%. The TBC and SCC presented a median

[3] SAS Institute Inc 2015. SAS® OnDemand for Academics: User's Guide. [online]. Website: https://odamid-usw2.oda.sas.com/SASStudio/ [accessed 12 June 2020]

Table 1. Descriptive statistics for the bulk tank milk composition and compost bedded variables for the farms in the studied period.

Variables[a]	N°	Minimum	Median	Quartile Range	Maximum
Bulk tank milk composition					
Fat (%)	24	3.36	3.61	0.30	3.98
Protein (%)	24	3.01	3.22	0.12	3.37
TBC (cfu mL^{-1})	24	12.00×10^3	52.75×10^3	176.00×10^3	575.00×10^3
SCC (cells mL^{-1})	24	121.50×10^3	641.00×10^3	455.00×10^3	1163.50×10^3
Compost bedded variables					
BSC (m^2)	24	11.90	18.27	8.46	32.31
cbTBC (cfu g^{-1})	24	200.00×10^3	3000.00×10^3	1125.00×10^4	7000.00×10^4
Dirt score (1 to 5)	24	1.00	1.00	0.00	3.00
Moisture (%)	24	43.80	55.75	11.13	67.75
T0 cm (°C)	24	14.00	27.76	8.75	41.48
T10 cm (°C)	24	17.08	32.06	12.59	48.59
T20 cm (°C)	24	18.71	34.56	15.65	54.07
pH	24	6.13	9.20	0.47	9.74

[a] TBC – total bacterial count (log-transformed), SCC – somatic cell count (log-transformed), BSC – barn space per cow, cbTBC – compost bedded total bacterial count (log-transformed), T0 – compost bedded temperature at 0 cm (surface), T10 – compost bedded temperature at 10 cm depth, and T20 – compost bedded temperature at 20 cm depth.

of 52750 cfu mL^{-1} and 641000 cells mL^{-1}, respectively. For the CB variables, the median BSC was 18.27 m^2, the median moisture was 55.75%, the median temperature varied from 27.76 to 34.56 °C from 0 to 20 cm. The median cbTBC was 3000×10^3 cfu g^{-1}, while the median dirt score was 1, and the median pH value was 9.20.

Kendall's correlation analysis was performed for the variables of BTM composition, CB variables, and between the two sets of variables. A moderate correlation coefficient was found only between protein and fat content (r = 0.52), with very weak correlations for the other variables ($-0.20 < r < 0.20$) (Table 2). For the compost bedded variables, high correlation coefficients were found for the temperatures at 0, 10, and 20 cm each other ($r \geq 0.80$) (Table 3). Moderate correlation coefficients were found between moisture with the temperature at 20 cm (r = – 0.45), besides among BSC and temperature measures (r > 0.40) (Table 3). Also, moderate to weak correlations were found for dirt score with moisture (r = 0.40), cbTBC (r = – 0.36), and pH (r = 0.35); moisture with BSC (r = – 0.37), temperature at 10 cm (r = – 0.32), and pH (r = 0.33); and also, between cbTBC and pH (r = – 0.35) (Table 3).

Considering the correlations among the variables in the two sets, fat content presented moderate correlation coefficients with BSC and temperature measures (except at 20 cm) (r > 0.40), besides moderate to weak correlation coefficients with cbTBC and temperature at 20 cm (r > 0.30) (Table 4). Protein content presented high correlation coefficients with temperature measures and BSC (r > 0.60) (Table 4). The TBC presented only moderate to weak correlation coefficients with cbTBC (r > 0.30) and temperature measures (r < – 0.30), while SCC also presented only moderate to weak coefficient correlations with dirt score (r < – 0.30) and cbTBC (r > 0.30) (Table 4). The other coefficients were very weak ($-0.30 < r < 0.30$).

Table 2. Residuals Kendall's correlation coefficients for the bulk tank milk composition variables each other.

Variables[a]	Fat	Protein	TBC	SCC
Fat (%)	1			
Protein (%)	0.517	1		
TBC (cfu mL^{-1})	0.086	– 0.162	1	
SCC (cells mL^{-1})	0.001	0.015	0.124	1

[a] TBC – total bacterial count (log-transformed) and SCC – somatic cell count (log-transformed).

3.2. Principal component analyses

Only the 1st and 2nd PCs were presented from PCAs. For BTM variables, the 1st PC presented an eigenvalue of 2.21 and explained 55.52% of data variation, while the 2nd PC

Table 3. Residuals Kendall's correlation coefficients for the compost bedded variables each other.

Variables[a]	Dirt score	Moisture	BSC	cbTBC	T0 cm	T10 cm	T20 cm	pH value
Dirt score (1 to 5)	1							
Moisture (%)	0.395	1						
BSC (m^2)	- 0.096	–0.367	1					
cbTBC (cfu g^{-1})	–0.356	–0.219	0.319	1				
T0 cm (°C)	–0.096	–0.250	0.483	0.269	1			
T10 cm (°C)	–0.182	–0.317	0.417	0.202	0.933	1		
T20 cm (°C)	–0.224	–0.450	0.483	0.202	0.800	0.867	1	
pH	0.353	0.333	–0.100	–0.353	–0.150	–0.150	–0.150	1

[a] BSC – barn space per cow, cbTBC – compost bedded total bacterial count (log-transformed), T0 – compost bedded temperature at 0 cm (surface), T10 – compost bedded temperature at 10 cm depth, and T20 – compost bedded temperature at 20 cm depth.

Table 4. Residuals Kendall's correlation coefficients for the bulk tank milk composition with compost bedded variables.

Variables[a]	Fat (%)	Protein (%)	TBC (cfu mL^{-1})	SCC (cells mL^{-1})
Dirt score (1 to 5)	0.224	– 0.224	0.036	– 0.331
Moisture (%)	– 0.100	– 0.250	0.143	– 0.200
BSC (m^2)	0.533	0.617	0.200	0.200
cbTBC (cfu g^{-1})	0.319	0.269	0.375	0.387
T0 cm (°C)	0.517	0.800	– 0.314	– 0.050
T10 cm (°C)	0.450	0.800	– 0.391	– 0.050
T20 cm (°C)	0.383	0.733	– 0.314	– 0.017
pH value	– 0.067	– 0.217	– 0.067	– 0.033

[a] TBC – total bacterial count (log-transformed), SCC – somatic cell count (log-transformed), BSC – barn space per cow, cbTBC – compost bedded total bacterial count (log-transformed), T0 – compost bedded temperature at 0 cm (surface), T10 – compost bedded temperature at 10 cm depth, and T20 – compost bedded temperature at 20 cm depth.

presented an eigenvalue of 1.18 and explained 29.57% of data variation, totalizing 85.09% of data variation jointly (Table 5). Fat (0.64), protein (0.59), and SCC (0.49) content in milk were the most important variables in the 1st PC, while TBC (0.87) was the most important in the 2nd PC (Table 5). So, the 1st PC was named "high fat, protein, and SCC contents", and the 2nd PC was named "high TBC content" (Figure 1A).

For CB variables, the 1st PC presented an eigenvalue of 3.19 and explained 45.61% of data variation, while the 2nd PC presented an eigenvalue of 1.64 and explained 23.45% of data variation, totalizing 69.07% of data variation jointly (Table 5). Moisture (– 0.50) and temperatures at 0 cm (0.51) and 20 cm depth (0.52) were the most important variables in the 1st PC, while dirt score (– 0.57), BSC (0.49), and pH value (0.57) were the most important in the 2nd PC (Table 5). So, the 1st PC was named "Compost bedded temperature × moisture" and the 2nd PC was named "Dirt score × pH and BSC" (Figure 1B).

Biplot graphs help us to understand the characteristics of the dairy farms regarding bulk tank milk and compost bedded variables (Figure 1A and Figure 1B). For example, farmer A is around the farmers' mean for the BTM and CB variables, while the farmer G is around the farmers' mean for the CB variables but presented high SCC, protein, and fat content in milk. Farmer B had a high bulk tank TBC, a high cow's dirt score, and CB moisture, besides low CB temperature, pH value, and BSC. Farmers C and H had high SCC, protein, and fat content in milk with high CB moisture and low temperature, although farmer C also presented low TBC. The farmer D also had low TBC, SCC, protein, and fat content in milk but with high CB

Table 5. Principal components, eigenvalues and eigenvectors for the bulk tank milk and compost bedded variables.

Bulk tank milk variables[a]				
Principal component (PC)	Eigenvectors	Eigenvalue	Proportion	Cumulative
1st PC	PC1 = **+ 0.64x1 + 0.59x2** – 0.03x3 + **0.49x4**	2.21	55.52	55.52
2nd PC	PC2 = – 0.18x1 – 0.13x2 **+ 0.87x3** + 0.43x4	1.18	29.57	85.09
Compost bedded variables[b]				
Principal component (PC)	Eigenvectors	Eigenvalue	Proportion	Cumulative
1st PC	PC1 = – 0.29x1 **– 0.50x2** – 0.27x3 + 0.12x4 **+ 0.51x5 + 0.52x6** – 0.21x7	3.19	45.61	45.61
2nd PC	PC2 = **– 0.57x1** + 0.06x2 **+ 0.49x3** – 0.17×4 + 0.09x5 + 0.20x6 **+ 0.57x7**	1.64	23.45	69.07

[a] For bulk tank milk variables: x1 = bulk tank milk fat concentration (%), x2 = bulk tank milk protein concentration (%), x3 = bulk tank milk total bacterial count (cels mL^{-1}), x4 = bulk tank milk somatic cell count (cfu mL^{-1});

[b] For compost bedded variables: x1 = dirty score (1 to 5), x2 = moisture (%), x3 = barn space per cow (m^2), x4 = compost bedded total bacterial count (cfu g^{-1}), x5 = compost bedded temperature at 0 cm (surface) (°C), x6 = compost bedded temperature at 20 cm depth (℃), x7 = pH; in bold the most important variables composing the principal components based on their eigenvectors.

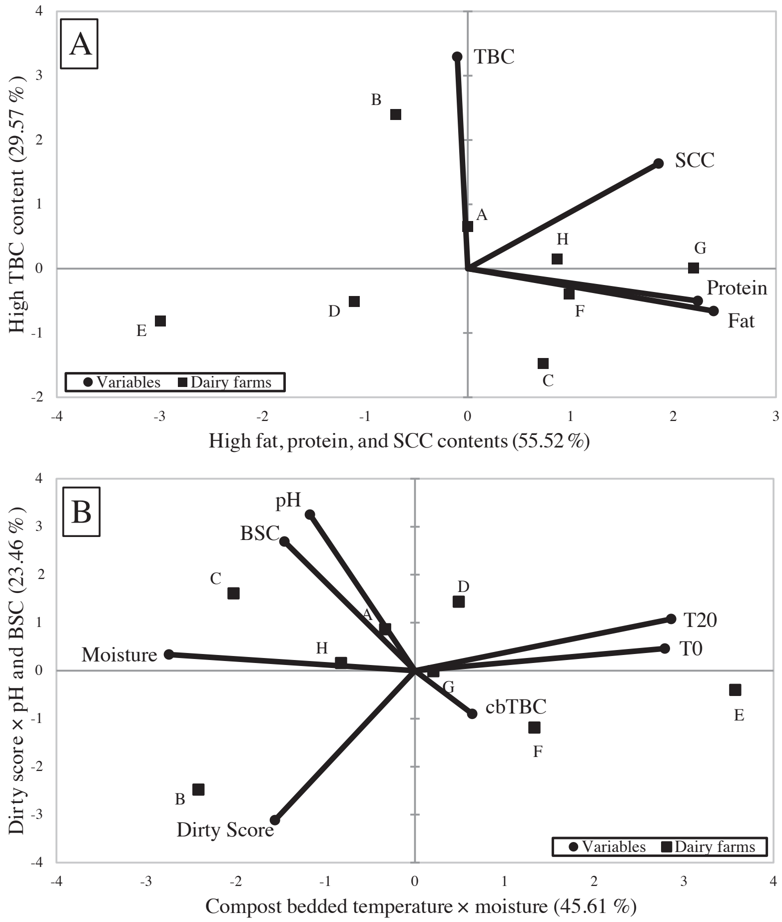

Figure 1. Biplot characterizing the 8 dairy farms regarding the bulk tank milk (A) and compost bedded variables (B) in the northwest of Rio Grande do Sul, Brazil. (a) protein (%), fat (%), TBC – total bacterial count (cfu mL^{-1}), SCC – somatic cell count (cells mL^{-1}), BSC – barn space per cow (m^2), cbTBC – compost bedded total bacterial count (cfu g^{-1}), T0 – compost bedded temperature at 0 cm (surface) (℃), T20 – compost bedded temperature at 20 cm depth (°C). Letters from A to H represent the dairy farms.

temperature and low moisture. The farmer E had low SCC, protein, and fat content in milk, with high CB temperature and low moisture. Farmer F had high SCC, protein, and fat content in milk but associated with high cbTBC and cow's dirt score with low CB pH value and BSC.

3.3. Canonical correlation analysis

Following, the CCA between the two sets of variables indicated a significant relationship for the 1st pair of CV (p = 0.0253), being the others not significant (Table 6). The 1st pair of CV explained 66% of data variation and presented a very high canonical correlation coefficient (r_c = 0.972) and squared canonical correlation coefficient (R_c^2 = 0.946), presenting an eigenvalue of 17.35. The canonical loadings of the BTM variables indicate that protein (– 0.53) and TBC (0.91) were the most important variables in their CV, while the canonical loadings for the CB variables indicate that BSC (– 0.53), cbTBC (0.51), temperature at surface (– 2.28), temperature at 20 cm depth (1.83), and pH value (– 0.55) were the most important variables in their CV (Table 6). The redundancy index (R_i) indicates that 31% of the variation in BTM variables was explained by the CB variables for the 1st pair of CV.

4. Discussion

In this study, we aimed to characterize the milk production in CBDB based on the BTM composition and the variables related to the CB. Also, we aimed to verify the multivariate relationship between these sets of variables to understand how the CB variables can affect the BTM composition. Few high correlations were found using Kendall's correlation within and between sets of variables, and the most important correlations were weak or moderate. For BTM variables, there are many studies in the literature using simple (bivariate) correlation [21,33,34], but we only found a moderate correlation between milk protein and fat content, while others found correlations among lactose content, TBC, and SCC [20,33]. Studies evaluating the relationships among CB variables with each other and with BTM variables are more scarce [4,7], where we found basically a strong correlation between CB temperature and milk protein content. Although widely used, simple correlation has often been misused. In general, the studies do not exclude possible confounding factors (such as treatments) for the correlation analysis, which can affect the results and alter the true correlation

Table 6. Canonical correlation analysis on bulk tank milk composition (y) vs. compost bedded variables (x).

Canonical variables	Standardized canonical variation combination[a]	Eigenvalue	Canonical correlation (r_c)	Squared canonical correlation (R_c^2)	Redundancy index (R_i)	Proportion	p-value
1	U1= + 0.36y1 **– 0.53y2 + 0.91y3** + 0.13y4	17.35	0.972	0.946	0.312	0.66	0.0253
	V1= + 0.43x1 + 0.37x2 **– 0.53x3 + 0.51x4 – 2.28x5 + 1.83x6 – 0.55x7**						
2	U2= + 0.98y1 – 1.02y2 – 0.86y3 + 0.98y4	6.49	0.931	0.867	0.100	0.25	0.1337
	V2= + 0.08x1 + 0.22x2 – 0.50x3 + 0.63x4 + 0.38x5 + 0.16x6 + 0.76x7						
3	U3= + 0.07y1 + 1.03y2 + 0.08y3 – 0.17y4	2.02	0.818	0.669	0.303	0.08	0.4512
	V3= + 0.05x1 – 0.69x2 + 0.40x3 + 0.55x4 + 2.61x5 – 3.41x6 + 0.28x7						
4	U4= – 0.95y1 + 0.12y2 – 0.79y3 + 1.46y4	0.18	0.390	0.152	0.016	0.01	0.8599
	V4= – 0.58x1 + 0.88x2 – 0.17x3 + 0.25x4 – 3.58x5 + 3.50x6 + 0.31x7						

[a] Also can be called as canonical loadings, U1 to U4: the first to fourth canonical dependent variables, and V1 to V4 are the first to fourth canonical independent variables; y1 = bulk tank milk fat concentration (%), y2 = bulk tank milk protein concentration (%), y3 = bulk tank milk total bacterial count (cels mL^{-1}, log-transformed), y4 = bulk tank milk somatic cell count (cfu mL^{-1}, log-transformed), x1 = dirty score (1 to 5), x2 = moisture (%), x3 = barn space per cow (m^2), x4 = compost bedded total bacterial count (cfu g^{-1}, log-transformed), x5 = compost bedded temperature at 0 cm (surface) (°C), x6 = compost bedded temperature at 20 cm depth (°C), x7 = pH; in bold the most important variables composing the 1st pair of canonical variables based on their canonical loadings.

coefficients [28]. In addition, the correlations *p*-value is affected by the number of observations and tends to be significant the larger the database [21]. Thus, the results of simple correlations demonstrate that using a multivariate approach to study the relationship between CB variables and the BTM variables is a more appropriate approach.

The PCA characterized dairy farmers in regard to BTM composition and CB management. Two PCA were used separately for the BTM and CB variables due to the number of dairy farms lesser than the number of response variables, where the ideal would be to perform a PCA with all variables together. The PCA for the BTM variables indicated that all of them (fat, protein, SCC, and TBC) were important for dairy farms characterization, being these results similar to those from Bodenmüller Filho et al. (2010) [35]. The PCA for CB variables presented temperature, cow's dirt score, BSC, and pH value as the most important variables for dairy farms characterization. Although PCA is a known statistical technique, studies using it to assess the characteristic of CB in CBDB are scarce.

Using the PCA, we can differentiate the dairy farms that properly managed the CB from those that still have management problems, besides checking the differences in their BTM. One of the most notable problems on those dairy farms was the use of the ventilation system. Most of the dairy farms activated the ventilation system only after turning the CB to help remove moisture, while dairy farm B did not even have a ventilation system, which was an aggravating factor for the proper CB management. Such a situation became even more critical when the BSC was reduced, thus contributing to the greater compaction of the CB due to the high moisture content and low temperatures, like the cases of dairy farms B and H. Efficient ventilation systems are important to remove moisture from the CB and also help in the heat dissipation from cows, improving their comfort [18].

High median TBC value of the database (52750 cfu mL^{-1}) can be a result of the problems that the dairy farms face in relation to CB management. Dairy farm B obtained the highest TBC values in milk, followed by high cow's dirt score, possibly due to the higher humidity and low temperature of their CB. However, some dairy farmers were able to manage properly the CB, showing good results in the BTM composition, as well as for the CB variables, as in the cases of dairy farms D and E. Dairy farm E had good CB management with high temperatures in the CB and low humidity, possibly due to the ventilation being activated every 5 min, contributing to the drying of the CB. However, other dairy farmers were unable to manage properly the CB, resulting in problems related to controlling the humidity and dirtiness of the cows.

Our initial hypothesis was that the CB variables would significantly affect the BTM variables, which was evidenced by the results from CCA. The CCA showed the effect of cbTBC, BSC, CB temperature and pH value mainly on the bulk tank TBC, indicating that high bacterial loads in the CB can result in high bacterial load in the BTM [10, 36]. The CB temperatures at 0 and 20 cm depth were the most important variables within its CV based on their canonical loadings and are directly linked to the fermentation process, being also related to the compost bedded pH value and cbTBC [37].

In CBDB, the increase in bulk tank TBC can occur due to the difficulty of CB management, which can contribute to bringing dirt from the teat to the milking equipment, and consequently to the BTM if the hygiene and cleaning process of the teat occurs inefficiently. The TBC can be controlled through a hygiene routine during the milking process. Failures during the cleaning of the milking equipment (teat cup, piping, cooling tanks, and others), predipping, and cooling of the milk can contribute to their increase [38, 39]. High milk quality can be obtained in CBDB by maintaining an adequate routine for CB management and clean cows [6]. Some issues must be monitored to achieve success in CBDB, such as the type of bedding material and control of its moisture, ventilation system to help the CB drying, frequency of CB turning, and BSC [18]. However, it is evident that monitoring the CB temperature is very important to maintain an adequate fermentation process, with the bulk tank TBC being a possible indicator of good CB management. Good CB management with controlled temperature and moisture is crucial to avoid bedding compaction and growth of the pathogenic microbial population [2,7]. High cbTBC are undesirable because they are associated with the presence of microorganisms at the teat ends and increased rates of clinical and subclinical mastitis [5,7].

While the effect of CB variables on bulk tank TBC is easier to explain and understand, the effect on protein content may be less direct. The milk protein content generally varies depending on cow's nutrition. However, the occurrence of mastitis due to high cbTBC can lead to increased protein concentration in milk due to reduced cow production [40-42]. Although we did not find an effect of the CB variables on bulk tank SCC, it was high (median = 641000 cells mL^{-1}) for the dairy farms studied, which indicates a problem with latent mastitis and a high occurrence of that disease on those dairy farms.

Such results demonstrate the challenges that these dairy farmers face to maintain adequate conditions of the CB in the studied region. However, in general, their cows were clean (dirt score 1), which indicates that the CB had a clean surface, few exposed manure, adequate incorporation of manure and controlled moisture. Correct CB management results in cleaner cows (legs, udders, and teats) and influences their health [43]. The literature mentions ideal CB temperature values between 43 and 60 °C at 10 to 20 cm depth, with moisture between 40 and 60% [6,44], and different pH values ranging from 8.45 to 9.20 [37,45,46]. The medians CB pH and moisture were within the indicated by the literature in our study, however, the temperature was below the recommended, which may indicate an inadequate fermentation process. The CB pH value and temperature are factors of great influence within the CBDB together with moisture, organic matter, carbon:nitrogen ratio, bedding area, water holding capacity, bedding density, barn width, frequency of turning, surface temperature, and ventilation system [18]. Animal stocking should be in accordance with barn dimensions [44], however, it is recommended that 7.4 to 12.5 m^2 cow^{-1} or even 15 m^2 cow^{-1} is available [6,37]. Animal welfare can be favored with more space per cow housed and the presence of soft beds [47]. Also, the larger the BSC and the higher the CB temperature, the lower the cbTBC, which may contribute to a lower occurrence of mastitis [7,48].

Dirtier animals tend to have bedding/manure residues on their hairs, udder, and teats. High CB temperatures and adequate moisture are conditions where composting works efficiently, reducing the pathogenic microbial population, providing better animal hygiene, as well as reducing the occurrence of mastitis and bulk tank SCC [7,8]. The improvement in milk quality is achieved through actions carried out by the dairy farmer including sanitary procedures, management, feeding, and also the genetic potential of the animals [35, 49]. Thus, we can say that CBDB are heterogeneous in the northwest of Rio Grande do Sul, mainly due to the management that can influence both the CB and BTM variables [18].

Finally, although the data used for this study concentrate data of only 3 months, it is from eight dairy farms that represent the reality of different dairy farms that use CBDB in the northwest region of the Rio Grande do Sul state, Brazil. According to the evaluation done for a short period of time, care is needed when extrapolating the results of this study. Further studies using multivariate analyses over a longer period of time are necessary to better understand the influence of variables related to CB with the BTM variables. Future research on this topic may include the identification of the bacterial population of the CB (which was not done in this study), checking if it correlates with high levels of bulk tank SCC and TBC, incidences of mastitis in the CBDB, other aspects related to CB management, as the material used, when the CB replacement is made, etc.

5. Conclusion

We evidenced heterogeneity in the BTM composition and CB characteristics in the dairy farms in the northwest of Rio Grande do Sul, Brazil, with some farms presenting high milk quality and CB management, while others presenting the contrary. Also, we found that CB characteristics strongly influence milk quality parameters and that 31% of the variation in BTM variables is explained by the CB variables. Protein and TBC content in BTM are the variables most affected mainly by the barn space per cow, cbTBC, CB temperature (at 0 and 20 cm depth), and pH value. The CB temperature is the most important variable for monitoring, while the bulk tank TBC may be an indicator of good CB management.

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Conflict of interest

The authors declare that there is no conflict of interest.

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