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ANIMAL SCIENCE

Influence of production level, number, and stage of lactation on milk quality in compost barn systems

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Abstract: This study evaluated the influence of milk production, number of lactations, and days in milk (DIM) on the quality and composition of milk from dairy cows housed in a compost barn (CB) system. The study was carried out using a six-year database, counting 31,268 observations from 2,037 cows of European breeds. Multiparous cows showed higher fat and protein production. Lactose showed high levels for primiparous and the initial stage of lactation (4.65%) and was negatively influenced by somatic cell count (SCC). Milk urea nitrogen was higher (14.01%) from 106 to 205 days in milk, and the other components were higher at >305 days. Therefore, the solids content was higher in the first and second lactations due to the high contents of lactose, fat, and milk protein, but lactose was reduced over lactations. In contrast, high DIM increased SCC and concentrated solids due to lower milk production. The effect of milk production, stage, and lactation order on the composition and milk quality of herds housed in CB showed the same pattern as in other production systems.

Key words: Compost bedded pack barn, confinement, dairy cows, lactation physiology.

INTRODUCTION

Rearing cows in compost barns (CB) has become common on dairy farms and has been seen as a promising system (Fernández et al. 2020, Llonch et al. 2021, Emanuelson et al. 2022). It is partially due to an increase in milk production, welfare, lifespan, better use of the land area, efficient use of labor, and improved quality of life for farmers (Barberg et al. 2007, Mota et al. 2017, Vilela et al. 2017, Piovesan & Oliveira 2020, Vieira et al. 2021).

Brazil and the United States lead the ranking of the most scientific productions about compost barn systems (Silva et al. 2022). In Brazil, studies have focused on CB characteristics (Oliveira et al. 2019, Radavelli et al. 2020a, b, Guesine et al. 2023), healthy, welfare, and animal behavior (Pilatti & Vieira 2017, Costa et al. 2018, Mota et al. 2019, Piovesan & Oliveira 2020, Yameogo et al. 2021), microclimate and heat stress (Vieira et al. 2021, Frigeri et al. 2023), milk composition (Weber et al. 2020, Nogara et al. 2021), mastitis, and udder health (Fonseca et al. 2023, Freu et al. 2023).

On the other hand, questions related to the bed material used in CB and how the bed could affect the comfort and production of cows have been lesser researched (Damasceno et al. 2020, Valente et al. 2020). Moreover, there is scarce information about how cow characteristics (milk production, period of lactation, and number of lactations) affect milk quality and composition in CB. These cow characteristics have a significant impact, especially on the udder health of cows housed on CB and the dairy activity profitability (Breitenbach 2018, Biasato et al. 2019, Marcondes et al. 2020). Thus, our objective was to analyze the influence of milk production, number of lactations, and days in milk (DIM) on the quality and composition of milk from dairy cows housed in a CB system, using a six-year database.

MATERIALS AND METHODS

This study is characterized as an observational descriptive research, a type of longitudinal retrospective study based on data obtained by consulting the records of a database.

The dataset contained information on the composition and quality of milk from four different herds, 208±94 cows, respectively, located in the municipality of Castro/PR, Brazil (24°47'32" South and 50°00'42" West, at 996 meters above sea level, humid subtropical climate according to the Köppen classification, and mean temperature of 17 °C, Alvares et al. 2014). The data were provided by the Paraná Association of Cattle Breeders of the Holstein Breed (APCBRH) of Curitiba/PR and comprised 60 months of evaluation, between 2016 and 2021. The year 2019 contained the least amount of information relating to these herds. All farms had similar breeding conditions, where the animals were confined in a CB system with sawdust and shaving bedding. Dairy cows belonged to breed groups of European origin, with a predominance of Holstein animals. The animals received a total mixed diet, consisting of corn silage, commercial concentrate, wet brewery residue, pre-dried oats and ryegrass, soybean hulls, corn meal, minerals, additives, and water.

The analyzed milk variables consisted of fat (%), protein (%), lactose (%), milk urea nitrogen (MUN, mg/dL), and SCC (cells/mL), together with the productive level of the herds, DIM, and number of lactations. Table I shows the

Table I. Descriptive statistics of the distribution of data regarding milk production.

Variable	N[1]	Min[2]	Median	Mean	Max[3]
Lactation					
1	10,566	5.90	33.40	33.45	70.00
2	8,749	6.00	38.20	38.00	74.50
3	6,025	7.20	39.30	39.07	79.00
> 3	5,928	5.20	38.65	38.59	79.20
Milk Production					
< 25 liters	4,110	5.20	21.25	20.24	24.90
25 a 40 liters	15,743	25.00	33.20	32.98	40.00
> 40 liters	11,415	40.10	46.50	47.98	79.20
DIM[4]					
≤ 105	9,224	5.20	42.40	42.46	79.00
106 a 205	9,189	5.90	39.00	39.20	79.20
206 a 305	8,176	6.10	33.10	33.27	65.10
> 305	4,679	5.50	26.80	26.99	64.70

[1]Number of observations, [2] minimum values, [3]maximum values, [4] days in milk.

descriptive statistics of the data. An analysis via infrared spectrophotometry was used to determine milk fat, protein, lactose, and MUN (Bentley model 2000, Bentley Instruments Inc., Chaska, MN, USA). SCC was determined using an electronic counter (Somacount 500, Bentley Instruments Inc., Chaska, MN, USA).

After tabulating all data, the initial sample included 46,423 observations from 2,200 cows. However, some exclusions were made to eliminate biased effects, which could influence the final results. This cut-off point was defined based on the 90th percentile of the coefficient of variation of the data and, therefore, the 10% with the highest variation in the number of cows tested was excluded.

Some extreme data were categorized for these exclusions, such as very advanced lactations (> 7 lactations), milk production (from < 5 to > 80 liters daily), days in milk (< 5 to ≥ 500 days), milk fat (< 2.00 to ≥ 6.00%), protein (< 2.00 to ≥ 6.00%), lactose (< 3.00 to ≥ 6.00%), total solids (TS) (< 8.00 to ≥ 16.00%), MUN (< 7.00 to ≥ 25.00 mg/dL), and SCC (< 1,000 to ≥ 5,000,000 cells/mL). A total of 31,268 observations from 2,037 cows were left after all the exclusions (Figure 1).

Categorizations were made for DIM, number of lactations, and milk production (MP). Lactations were considered as 1, 2, 3, and > 3, while MP was categorized as < 25 liters, 25 to 40 liters, and > 40 liters, and DIM as ≤ 105 days, 106 to 205 days, 206 to 305 days, and > 305 days. A mixed generalized linear model was used for data analysis. The number of lactations, milk production categories, and DIM were included as fixed components for modeling, and the cows were considered as repeated measures of random effects for the same animal analyzed in different months. Only the model for SCC used a lognormal distribution, whereas a normal distribution model was used for the other

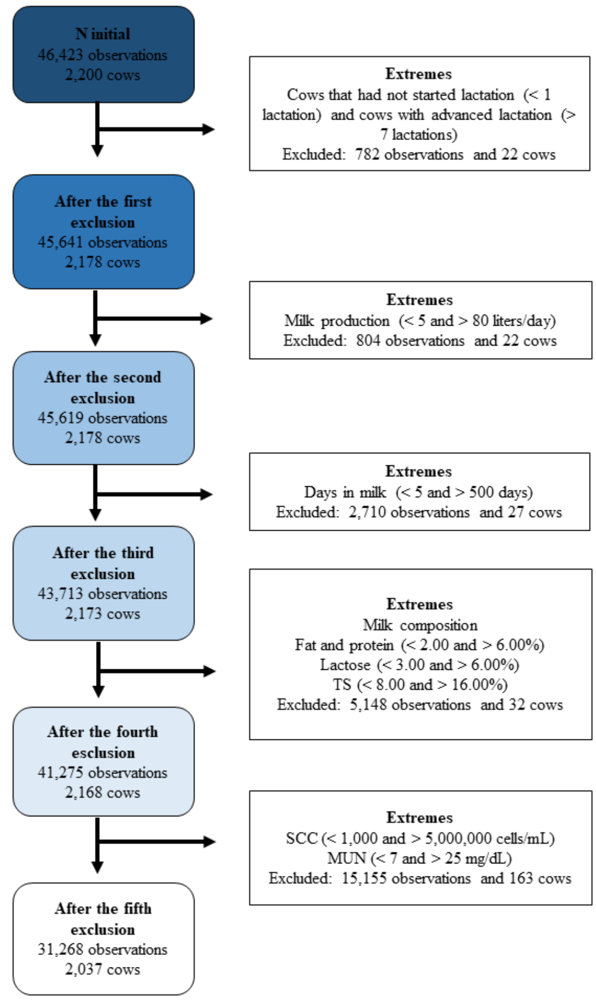

Figure 1. Data distribution and exclusion of extreme data from the analysis.

variables. The general statistical model used is shown below:

$$y_{ijklm} = \mu + Lact_i + MP_j + DIM_k + Cow_l + \varepsilon_{ijklm}$$

where y_{ijklm} is the measure of the response variable for the n-th observation, μ is the constant common to all observations, $Lact_i$ is the fixed effect of the number of lactations with = 4, MP_j is the fixed effect of the milk production category with = 3, DIM_k is the fixed effect of days in lactation with = 4, Cow_l is the random effect of repeated measurement of the cow over the months, ε_{ijklm} is the random error associated with the observation.

All the data were analyzed using PROC GLIMMIX of the SAS University Edition (SAS Institute 2012), considering statistically significant differences at the level of < 0.05 (5%) probability.

RESULTS

Fat

No statistical difference was observed in the fat content among the second and third lactations (3.65 and 3.64%, respectively), with the highest levels of this component being verified in these lactations compared to the first (3.59%) and over three lactations (3.58%) (Table II). Furthermore, the highest fat content (3.95%) was found in milk productions < 25 liters and > 305 DIM (3.79%).

Protein

The milk protein content presented a statistical difference among the studied lactations (p<0.0001), with a higher value in the second lactation (3.41%) and lower values in cows with more than three lactations (3.30%) (Table III). Milk protein showed a similar pattern to that of the fat component, being higher in productions < 25 liters (3.53%) and with > 305 DIM (3.54%).

Lactose

The lactose content presented a difference among lactations, in addition to decreases of 5.07% between the first lactation and above three lactations. The highest levels of lactose (4.66% and 4.64%, respectively) were observed in milk productions > 40 liters and ≤ 105 DIM (Table IV).

Table II. Influence of lactation, milk production and DIM variables on milk fat content.

Fat (%)				
Variable	**N[1]**	**Mean %**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	3.59 b	0.031	
2	8,749	3.65 a	0.032	
3	6,025	3.64 a	0.032	
> 3	5,928	3.58 b	0.032	
Milk Production				<0.0001
< 25 liters	4,110	3.95 a	0.031	
25 to 40 liters	15,743	3.62 b	0.032	
> 40 liters	11,415	3.26 c	0.033	
DIM[2]				<0.0001
≤ 105	9,224	3.50 c	0.032	
106 to 205	9,189	3.51 c	0.032	
206 to 305	8,176	3.66 b	0.032	
> 305	4,679	3.79 a	0.032	

[1]Number of observations, [2]days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

Table III. Influence of lactation, milk production and DIM variables on milk protein content.

Protein (%)				
Variable	**N[1]**	**Mean %**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	3.36 c	0.010	
2	8,749	3.41 a	0.010	
3	6,025	3.38 b	0.010	
> 3	5,928	3.30 d	0.010	
Milk Production				<0.0001
< 25 liters	4,110	3.53 a	0.010	
25 to 40 liters	15,743	3.37 b	0.010	
> 40 liters	11,415	3.19 c	0.010	
DIM[2]				<0.0001
≤ 105	9,224	3.16 d	0.010	
106 to 205	9,189	3.33 c	0.010	
206 to 305	8,176	3.43 b	0.010	
> 305	4,679	3.54 a	0.011	

[1]Number of observations, [2] days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

Table IV. Influence of the variables lactation, milk production and DIM on the lactose content of milk.

Lactose (%)				
Variable	**N[1]**	**Mean %**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	4.73 a	0.005	
2	8,749	4.59 b	0.005	
3	6,025	4.53 c	0.005	
> 3	5,928	4.49 d	0.005	
Milk Production				<0.0001
< 25 litros	4,110	4.49 c	0.005	
25 to 40 litros	15,743	4.61 b	0.005	
> 40 litros	11,415	4.66 a	0.005	
DIM[2]				<0.0001
≤ 105	9,224	4.64 a	0.005	
106 to 205	9,189	4.62 b	0.005	
206 to 305	8,176	4.57 c	0.005	
> 305	4,679	4.50 d	0.005	

[1]Number of observations, [2] days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

Total solids

The total solids content of milk presented no difference between the first and second lactations (12.65 and 12.62%, respectively) and decreased in productions > 25 liters. The DIM period that provided the highest solids content was > 305 DIM (12.80%) (Table V).

Milk urea nitrogen

MUN was higher in the first lactation (14.08 mg/dL) compared to the other lactations, showing a drop of 5.82%. Lower levels of MUN were observed in productions < 25 liters (13.28 mg/dL). However, MUN was higher between 106 and 205 DIM (14.00 mg/dL) (Table VI).

SCC

SCC was higher in cows with a higher number of lactations (>3) and with > 305 DIM (174,013 and 117,300 cells/mL, respectively). Cows with productions > 40 liters also had a higher mean SCC (159,465 cells/mL) than the other production categories (Table VII).

DISCUSSION

Milk fat and protein contents were higher in multiparous cows. These components and the produced volume were also higher in multiparous cows (4.14%, 3.18%, and 22.47 liters, respectively) than in primiparous cows (3.82%, 3.01%, and 19.26 liters, respectively), according to data from Sitkowska (2008).

Reductions of 8.35% in fat content were observed in daily milk productions above 25 liters. It occurs through the dilution effect, favored by the increase in milk production (Galvão Júnior et al. 2010). The milk fat content is less expressive in the Holstein breed due to its significant milk production (Ludovico et al. 2019). The higher the production level of the animal, the lower the percentage of milk fat (>

Table V. Influence of lactation, milk production and DIM variables on milk total solids.

Total Solids (%)				
Variable	**N[1]**	**Mean %**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	12.65 a	0.037	
2	8,749	12.62 a	0.037	
3	6,025	12.51 b	0.038	
> 3	5,928	12.32 c	0.038	
Milk Production				<0.0001
< 25 liters	4,110	12.94 a	0.037	
25 to 40 liters	15,743	12.57 b	0.037	
> 40 liters	11,415	12.06 c	0.038	
DIM[2]				<0.0001
≤ 105	9,224	12.25 d	0.037	
106 to 205	9,189	12.42 c	0.037	
206 to 305	8,176	12.63 b	0.037	
> 305	4,679	12.80 a	0.038	

[1]Number of observations, [2]days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

25 liters = 3.11%), and the lower the daily milk production (< 15 liters) and the higher the DIM (> 316 days), the higher the concentration of total solids, fat, and protein (Cabral et al. 2016), which is not beneficial. A high number of lactations is associated with higher milk production (Kappes et al. 2020), but an inverse effect occurs when the value exceeds five lactations (Zafalon et al. 2005).

The lactose content showed a decrease of 3.01% as the DIM progressed and 5.07% when the number of lactations increased in the evaluated herds of the European breed. Sabek et al. (2021) reported that the increase in the number of lactations of cows and the DIM favor changes in milk characteristics, including a reduction in the lactose content. Lactose has a negative correlation with SCC and the number of lactations of animals, and the increase in SCC led to a reduction in the lactose content and, consequently, lower milk production. Kappes et al. (2020) observed similar data in the CB system, in which lactose was affected by the number of lactations, SCC, and udder depth.

Milk lactose is an important osmotic regulator, as it is related to water in the mammary gland, thus contributing to the produced milk volume. In the present study, the highest lactose content (4.66%) was found in milk productions above 40 liters. This fact justifies the high correlation (0.98) between lactose and milk production (Miglior et al. 2007). The same authors also verified a moderate to high magnitude of heritability (0.48 to 0.51) for this component.

Factors such as DIM, milk production, number of lactations, animal age, and breed contribute to the variation in lactose content (Galvão Júnior et al. 2010). According to Ludovico et al. (2019), Holstein cows have a higher lactose content (4.56%) compared to Jersey (4.47%) and Girolando (4.52%) animals. Regarding the

Tabele VI. Influence of lactation, milk production and DIM variables on milk urea nitrogen.

Milk Urea Nitrogen (mg/dL)				
Variable	**N[1]**	**Mean %**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	14.08 a	0.230	
2	8,749	13.74 b	0.230	
3	6,025	13.51 c	0.230	
> 3	5,928	13.26 d	0.230	
Milk Production				<0.0001
< 25 liters	4,110	13.28 b	0.228	
25 to 40 liters	15,743	13.83 a	0.229	
> 40 liters	11,415	13.83 a	0.232	
DIM[2]				<0.0001
≤ 105	9,224	13.40 c	0.230	
106 to 205	9,189	14.00 a	0.230	
206 to 305	8,176	13.74 b	0.230	
> 305	4,679	13.44 c	0.231	

[1]Number of observations, [2]days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

Girolando genotypes (1/2 HG, 5/8 HG, and ¾ HG), the lactose content in milk was higher as the presence of Holstein genes increased (Ludovico et al. 2019). The comparison of data from 32 ½ blood Brown Swiss and Holstein cows in the initial third of lactation showed no variation in the lactose content between the genetic groups, with an average content of 4.61% (Deitos et al. 2011). These data reinforce that milk lactose has low variability (Kaskous 2018). Lactose is the component that most contribute to the total solids content (> 4.3%) (Santos & Fonseca 2019) and, possibly for this reason, it favored the higher levels of total solids during the first and second lactations (12.65 and 12.62%, respectively).

Quantifying solids content is important to assess the nutritional quality of milk. Some components are used in payment programs and/or quality bonuses in the dairy industry (Cabral et al. 2016) to stimulate specialization in dairy activity and reach higher levels of competitiveness in this sector (Monteiro Junior et al. 2021). Auldist et al. (2007) also highlighted the influence of extensive lactations (> 16 months) on the reduction in solids content in milk, as losses were smaller from 10 to 16 months. However, we found in our study that high levels of total solids (TS) were observed in DIM above 305 days (12.80%), possibly due to the lower milk production of cows in this period of lactation, which leads to TS concentration.

Urea production in the liver via the urea cycle comes from the excess protein in the diet of dairy cows (or the lack of synchronism in the rumen environment due to low dietary starch content), which reaches other tissues such as

Table VII. **Influence of lactation, milk production and DIM variables on milk SCC.**

Somatic cells count (cells/mL)				
Variable	**N[1]**	**Mean cells/mL**	**Standard error**	**p-value**
Lactation				<0.0001
1	10,566	54,480 d	0.037	
2	8,749	71,214 c	0.037	
3	6,025	105,510 b	0.038	
> 3	5,928	174,013 a	0.038	
Milk Production				<0.0001
< 25 liters	4,110	92,000 b	0.036	
25 to 40 liters	15,743	52,852 c	0.037	
> 40 liters	11,415	159,465 a	0.040	
DIM[2]				<0.0001
≤ 105	9,224	73,325 d	0.038	
106 to 205	9,189	84,903 c	0.037	
206 to 305	8,176	97,548 b	0.037	
> 305	4,679	117,300 a	0.039	

[1]Number of observations, [2] days in milk.
Means with the same letters in the columns, for each variable, do not differ from each other at the 5% level.

the mammary gland via blood circulation, which can be measured in the milk (Televičius et al. 2021, Vlizlo et al. 2021, Wang et al. 2021). In the present study, a 4.14% increase was observed in MUN from daily productions of 25 liters, a fact that can be explained because MUN is positively associated with milk production (Doska et al. 2012). MUN is a component of milk that reflects the nutritional management of the herd, being a direct indicator of protein intake and metabolism, that is, the nutritional condition of the cow, energy, and protein balance, in addition to indicating the occurrence of metabolic disorders (Eicher et al. 1999, Vlizlo et al. 2021).

There is a negative association between the pregnancy rate and MUN levels in bulk milk, with MUN values above 19 mg/dL promoting reductions in reproductive performance (Televičius et al. 2021). Almeida et al. (2021) studied three dairy herds housed in free stalls in southern Brazil and found that individual cow MUN concentration (not from bulk tank milk) should not exceed 15.5 mg/dL before conception, as it can negatively impact future milk production and cow fertility. Considering this limit, we can state that the analyzed herds had adequate MUN levels (13.26 to 14.08 mg/dL).

The highest MUN levels in milk were positively associated with the first lactation (14.08%), followed by a decrease of 5.82% in cows with more than three lactations. High MUN levels were also observed in the first lactation (16.16 mg/dL) in dairy herds in the state of Paraná, Brazil (Doska et al. 2012), probably due to the oversupply of protein for this category. However, changes in MUN may be a consequence of the physiological state of the animals, as high MUN levels were still verified in both the first and second lactations even providing the same feed (single diet) to the cows in a tie-stall system (Sabek et al. 2021). Additionally, the same authors also verified that the highest MUN levels (17.01 and 16.95 mg/dL) were found between the DIM of 101 to 200 and 201 to 305 days, respectively.

Younger cows in the herd had better mammary gland health, as they are temporarily less exposed to environmental and contagious risks than multiparous cows. For this reason, primiparous and early-lactating cows showed lower SCC values (54,480 and 73,325 cells/mL, respectively) in this study, which indicates good management during the dry period. The number of lactations influences the increase in milk SCC, as 764 (with ≥ 3 lactations) out of 2,657 animals, that is, 28.75% of the cows, had an intramammary infection (Souza et al. 2009). Cows with a higher number of lactations are more likely to have subclinical mastitis verified by their increased individual SCC (Schunig 2021).

High SCC (considering cows with SCC ≤ 200,000 cells/mL as healthy) is an indicator that inflammation is occurring in the mammary gland (Dohoo & Leslie 1991, Botton et al. 2019, Schunig 2021), which may progress to clinical or subclinical mastitis. Subclinical mastitis can increase the total protein content of milk, even without a quality improvement (Zafalon et al. 2008). In the primiparous cows, losses from SCC were lower because the animals had less contact with pathogens that cause mastitis. The animals are more exposed and more susceptible to infection with advancing lactations and increasing age (Magalhães et al. 2006). The seasons of the year also affect this variable, as there is a higher incidence of mastitis in the summer and fall (SCC 404,000 and 438,000 cells/mL, respectively) than in the spring and winter (341,000 and 308,000 cells/mL, respectively), considering the CB system (Weber et al. 2020).

In addition, high SCC values from cows with extensive lactations contribute to lower lactose content (4.36%) (Kappes et al. 2020). This is due to the damage caused to milk-secreting cells, decreasing lactose synthesis and, consequently, lowering milk and total solids production (Harmon 1994, Coelho et al. 2014, Alessio et al. 2016, Ludovico et al. 2019). Thus, the percentage of lactose in the milk has a negative correlation with SCC, ranging from 0.41 to 0.49 (Eckstein et al. 2014, Silva et al. 2018, Silva & Antunes 2018). The reduction in lactose in cases of mastitis occurs because this component is consumed by bacteria, forming lactic acid and leading to casein instability (Santos & Fonseca 2019).

The influence of the number of lactations and DIM on SCC has already been reported in the literature. A low mean SCC during the first lactation can be attributed to the health of the mammary gland in primiparous cows (Cabral et al. 2016). The higher the number of lactations, the greater its risk of becoming a source of infection and disease transmission within the herd (Zafalon et al. 2005). Additionally, negative effects on SCC are also observed in cows with advanced DIM (Schunig 2021). Dias et al. (2017) also reported that number of lactations and DIM influence milk production and composition.

Information regarding management and nutrition is scarce although the database has a considerable number of observations. Additionally, the literature lacks studies demonstrating the effect of milk production, DIM, and number of lactation on the pattern of milk quality and composition in CB, as well as the incidence and prevalence of mastitis in these herds. Thus, further discussions on this topic are still necessary. In CB, monitoring the temperature of the bed is essential to control its humidity, as 31% of the variation in the tank components is explained by variables related to the compost barn (Nogara et al. 2021).

CONCLUSION

The effect of milk production and stage and number of lactation on the composition and milk quality of herds housed in CB shows the same pattern as in other production systems. Milk production affected total milk fat, protein, and solids as a function of the dilution effect in productions above 40 liters per day and with advanced DIM, with higher levels of total solids being observed during the first and second lactations of dairy cows. Both, lactose and MUN, were higher in primiparous cows. Although milk SCC tends to increase as the number of lactations increases due to the higher exposure to mastitis-causing agents, compromised milk quality and composition are only verified after five lactations. Thus, the good health of the mammary gland could be verified in this study, as the highest mean of SCC obtained was 159,465 cells/mL, which characterizes a healthy cow. These data reinforce that the analyzed farms manage to obtain quality milk, adding potential bonuses for quality to their remuneration.

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KFN was responsible for writing the manuscript, MB analyzed the statistical data, JAH contributed by providing the data for the research, and MZ helped by reviewing the manuscript.