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# Cow's functional traits and physiological status and their relation with milk yield and milk quality in a compost bedded pack barn system

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**ABSTRACT** - We aimed to determine the relationship of milk yield, milk composition, and somatic cell score (SCS) with functional traits (lameness score, udder cleanliness score, udder depth, and teat end hyperkeratosis score) and physiological status (parity and days in milk (DIM) of cows housed in compost bedded pack barn system (CBP) using multivariate analysis. Data were collected in September and October of 2017 and evaluated using factor and cluster analysis. Four factors were formed, with the first factor showing the traits responsible for altering the lactose content in milk, the second factor comprising the effect of DIM, the third factor representing the teat end hyperkeratosis score, and the fourth factor demonstrating the udder cleanliness. The cluster analysis formed three clusters. Clusters 2 and 3 differed for protein and lactose content, SCS, parity, DIM, lameness score, and udder depth. Cluster 1 differed from the others, especially due to the high milk yield. Cow conformation traits and physiological status affected milk yield and composition in CBP. There is an association between udder depth, parity, and SCS with negative impact on lactose content. Parity is also associated with higher milk yield and teat end hyperkeratosis score. Cows with lower lameness score presented dirtier udders due to increased movements on the feed parlor and water troughs areas. Special attention to the improvement of the udder conformation traits, as well as management practices that reduce hyperkeratosis, are necessary to keep high yielding, healthy, and cows with higher longevity. The cleanliness in the feed parlor and water troughs area is very important to preserve milk quality and healthy cows.

**Keywords:** lameness score, somatic cell count, teat end hyperkeratosis, udder cleanliness score, udder depth

## Introduction

All housing systems for dairy cattle aim at improving comfort for lactating cows, allowing expression of their natural behavior, maintaining hygiene and health, and generally improving living conditions of lactating cows and farmers (Bewley et al., 2017). The compost bedded pack barn (CBP) is a type of confinement system that allows animals to move freely inside the stall, free of bays or partitions (Janni et al., 2007). In this system, feces and urine are disposed and incorporated into the material that composes the compost bed. Through a 6-24 month of composting process, the compost bed turns into

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a semi-solid fertilizer (Janni et al., 2007; Black et al., 2014). Different materials can be used as carbon source in the compost bed such as wood sawdust, wheat straw, soybean straw, flax seed, rice straw, coffee husks, and peanut shells (Shane et al., 2010b). This system requires regular bedding addition, which means a new layer of material, and requires compost bed tilling at least twice a day to incorporate animal waste and to allow entry of air into the deeper layers (Black et al., 2013). This procedure allows greater microbial aerobic activity, heating and drying the compost bed, which provides a dry and fresh surface for the cows to lie down (Shane et al., 2010a).

In the last few years, the CBP has been widely used in Brazil, especially in the south region, aiming to improve cattle comfort and longevity and to reduce somatic cell count (SCC) in milk (Black et al., 2013), and thus improving milk yield (Ofner-Schröck et al., 2015; Pilatti and Vieira, 2017). However, because it is a new housing system in Brazil, it still presents some challenges regarding the correct management of the compost bed, which directly and indirectly interferes in milk quality (Pilatti and Vieira, 2017). These challenges are mostly related to the lack of new bedding material, failure to till bed for as much time as necessary, and environmental issues such as high humidity. Improper compost bed management can increase bed humidity and decrease its temperature (Endres, 2009). These issues predispose the occurrence of digital lesions, as well as greater udder dirtiness by the adhesion of bedding material to the udder (Klaas et al., 2010; Fávero et al., 2015; Albino et al., 2018), which can increase SCC (Albino et al., 2018) and put mammary gland health at risk (Black et al., 2014).

Physiological status may also influence the rise of SCC level in milk, such as later days in milk (DIM), through greater desquamation of mammary gland epithelial cells (Malik et al., 2018). A greater parity number is also related to the rise of SCC level since the udder tends to become more pendulous with increasing age, which can increase the risk of traumas during locomotion as well as more dirtiness (Pérez-Cabal and Alenda, 2002; Cardozo et al., 2015). Another factor related to a higher SCC level is an increasing teat end hyperkeratosis score, which may lead to a reduced ability of closing the teat sphincter, facilitating the entry of pathogenic microorganisms into the mammary gland (Cardozo et al., 2015).

Further studies on CBP systems in Brazil are necessary, especially for the variables related to functional traits and the physiological status of lactating cows, milk quality, and the management of this confinement system. Therefore, the use of multivariate analysis is critical to evaluate multifactorial effects (Mele et al., 2016) aiming to simplify the available information (Todde et al., 2016). Therefore, the objective of our study was to determine the association among milk yield, milk composition, SCC, functional traits (lameness score, udder cleanliness score, udder depth, and teat end hyperkeratosis score), and physiological status (parity and DIM) of each cow in a CBP applying a factor and a cluster analysis procedure.

## Material and Methods

The experiment was carried out on a dairy farm located in Bom Retiro, Santa Catarina (27°47'50" S, 49°29'21" W, 890 m altitude), south of Brazil, in a subtropical humid climate region, type Cfb (C = humid sub-tropical, f = oceanic climate, without dry season, and b = with temperate summer), according to the Köppen classification (Peel, 2007; Alvares et al., 2013). The farm housed approximately 280 lactating cows, with a daily production of approximately 9,000 L of milk. The herd was composed mainly of Holstein cows (about 60% of the cows) and different "blood" levels of crossbred Holstein × Simmental cows (Figure 1).

Lactating cows kept in the CBP were divided into three groups according to their production level; group 1 included cows in early lactation and with high milk yield, with an average of 45 L/milk/day; group 2 included cows in mid lactation, producing an average of 33 L/milk/day; and group 3 included cows in late lactation, producing an average of 22 L/milk/day. Cows were fed a total mixed ration combining corn silage, ryegrass (fresh and silage), and concentrates. The diet offered to the cows was calculated to provide 100% of the nutritional requirement (NRC, 2001), in which the proportion of each ingredient was adjusted according to the milk yield of each group. Cows were mechanically milked three times a

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day (05:00, 13:30, and 20:30 h), and the individual cow milk yield (MY) was electronically recorded (DeLaval® electronically milk-recording system). Cows had access to the feed parlor right after milking, for about 2 h and 30 min (about 7.5 h/day), three times a day.

Before the beginning of milking, pre-dipping procedures were performed, by removing the first three milk jets from each teat in a strip cup with black background to diagnose changes on milk appearance such as presence of blood, watery appearance, clots, flakes, pus etc., which indicates mastitis. A disinfectant solution (Biofoam™, DeLaval) was applied in each teat right after this test, and 30 s later, each teat was dried with a disposable wipe. Immediately after milking, the post-dipping procedure was performed by applying an iodine-based solution (Della Barrier®, DeLaval).

The bedding material used for the CBP system was composed of wooden sawdust. Weekly, as a routine, a new layer of material was added to the bed. The bedding material was mixed twice a day (morning and afternoon) by using a soil cultivator that reached around 30 cm beneath ground level. Average bedding temperature was of 25° C, measured at a depth of 20 cm, and moisture content yielded 62% in September and October 2017. We evaluated the bed temperature and humidity on the same day we performed the other evaluations. For the evaluation of the bed temperature, we used a thermometer and measured the bed temperature 20 cm below the surface at six different places in each of the three divisions of the CBP, totaling 18 evaluations. Subsequently, we used the mean temperature of all measurements. To estimate the moisture content of the compost bed, we also took samples at 18 different points in the CBP. We mixed the individual samples and prepared a composed sample. The composite sample was dried in a forced-air oven at 55 °C for 72 h to determine the humidity. During the same period, the average air temperature and humidity reached 13.4-14.7 °C and 80-82%, respectively (EPAGRI/CIRAM, 2019). The average pack area in the CBP provided at least a space of 10.5 m$^2$ per lactating cow (Figure 2).

The individual cow milk samples of all lactating cows were taken once a month in 40-mL bottles containing Bronopol. Samples were sent to a laboratory participating in the Milk Quality Program of Brazil for milk composition and SCC analysis. All analyses were performed by using automatic equipment. The SCC test was performed by flow cytometry (Soma Count FC), and milk composition was determined by an infrared technique (Dairy Spec FT Bentley®).

Data collection and animal evaluations were carried out once monthly during September and October 2017. Data of breed, parity, and DIM were collected from the farm records. On the same day when



**Figure 1 -** Crossbred Holstein × Simmental cows (A) and purebred Holstein cows (B) of the farm where the research was carried out.

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the milk samples were taken, we performed the evaluation of teat end hyperkeratosis score, udder cleanliness score, udder depth, and lameness score.

Teat end hyperkeratosis score was visually classified using a scale of 1 to 4 (1 - teat end without ring formation, 2 - teat end with small ring formation, 3 - teat end with rough ring formation, 4 - teat end with a very rough ring; Figure 3) according to the methodology described by Mein et al. (2001). The scoring was performed after milking and before post-dipping procedures. After the evaluation of teat end hyperkeratosis score, the average hyperkeratosis score of each cow (average among the four teats) was calculated.

Udder cleanliness scoring was performed using a scale from 1 (clean) to 4 (very dirty) according to the model proposed by Ruegg (2002) before pre-dipping procedures. The udder depth was defined as the distance of the udder floor to the line of hock using a scale from 1 to 3 (1 = udder floor above the line of hock, 2 = udder floor on hock line, 3 = udder floor below the line of the hock; Figure 4) as described by Coentrão et al. (2008). The lameness score was recorded two times: the first one during the exit of the milking parlor, while animals were in locomotion, and the second one, at the feeding parlor, while cows were standing. A scale from 1 to 5 points was applied, with 1 for cows with no locomotion problems, and 5 for cows with serious locomotion problems, according to Sprecher et al. (1997).



**Figure 2 -** Outside view (A) and inside view (B) of the compost bedded pack barn with the lactating cows of the dairy farm where the research was carried out.
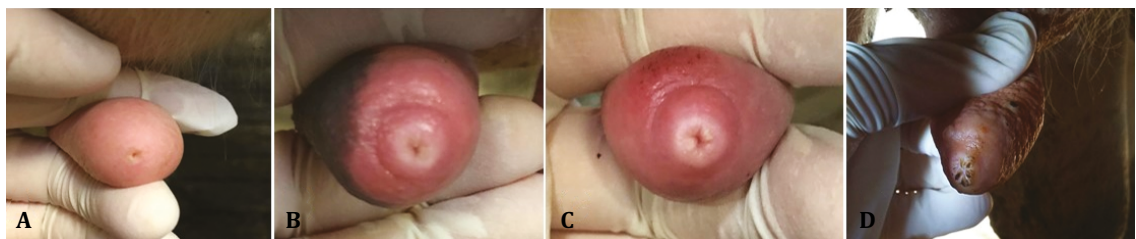


**Figure 3 -** Teat end hyperkeratosis score, in which 1 - teat end without ring formation (A), 2 - teat end with small ring formation (B), 3 - teat end with rough ring formation (C), 4 - teat end with a very rough ring (D).

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Variables evaluated were individual cow milk yield (kg/cow/day), fat, protein and lactose contents, SCC, DIM, parity, teat end hyperkeratosis, lameness score, udder depth, and udder cleanliness. Outliers or potential errors from milk sampling were removed by data standardization. Records between 8 and 305 days of lactation, milk yields between 10 and 60 L per day, fat contents between 1.5 and 5.6%, protein contents between 2.0 and 5.3%, and lactose contents between 3.0 and 5.2% remained in the data set. In addition, cows were classified into first, second, and third or higher parities. To obtain normality, SCC data were transformed to somatic cell score (SCS) by the equation $SCS = \log_2 (SCC/100) + 3$ (Ali and Shook, 1980). Because the effect of the genetic group was not significant, we did not include it in the final results. All cows, independent of the genetic group, were kept and managed under the same conditions.

Data were evaluated using multivariate analysis technique (factor and cluster analysis) using the SAS® statistical software (SAS Institute 2002). Data were previously standardized by PROC STANDARD so that all variables would equally contribute to the analysis to avoid that variables with a larger numerical scale would have a greater effect than those with a smaller scale. The Factor analysis (PROC FACTOR) served as a tool to evaluate the relationships among the variables aiming at reducing the original set of variables to a smaller number of (significant) factors. To verify the suitability of the model, Kaiser-Meyer-Olkin (KMO) statistics was used. Factor loads higher than 0.40 were considered



**Figure 4 -** Udder depth scores as the distance of the udder floor from the hock line, in which 1 = udder floor above the hock line (A), 2 = udder floor on hock line (B), and 3 = udder floor below the hock line (C).

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significant, and Promax rotation was used. The number of factors was defined by the eigenvalue, using all eigenvalues higher than 1. A cluster analysis was performed by PROC FASTCLUS to calculate clusters for cows with (more or less) similar characteristics and distinct differences among each other. Ward's hierarchical method was applied by using the Euclidean distance to estimate standardized means for each cluster followed by a back transformation to the original means.

A subsequent discriminant analysis (Proc DISCRIM) was performed to re-classify the data (cows) correctly for each cluster. Since a considerable proportion of data (cows) were wrongfully distributed within their respective clusters, we chose to use a non-parametric discriminant analysis using the NPAR method combined with the k-nearest neighbor (KNN) algorithm, which classified all data within the clusters. Within the discriminant analysis, the STEPWISE method using the STEPDISC procedure was used to select the variables responsible for cluster differentiation. In addition, the canonical discriminant analysis (CANDISC procedure) was used to graphically demonstrate the distances within and between clusters.

Finally, the assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were tested to compare the means in the clusters formed by the cluster analysis. Since the variables did not meet at least one of the assumptions, presenting a lack of normality or homogeneity of variance and/or both, we chose to perform the analysis of variance by the GLIMMIX procedure, which considers the type of distribution of the response variable for the comparison of means. If these results were significant, the least squares mean values were compared by the Tukey-Kramer test considering the 5% significance level.

## Results

The descriptive analysis shows the variability of the collected data as arithmetic means and the respective standard deviations including minimum and maximum (Table 1). The average SCC of 346.50 cells × 1,000/mL is below the value recommended by the Normative Instruction no. 76 (Brasil, 2018).

**Table 1 -** Descriptive statistics and standard deviation (SD) of mean milk yield and composition, somatic cell count (SCC), and individual characteristics of lactating cows in a compost barn confinement system

| Variable | N | Minimum | Average | Maximum | SD |
|---|---|---|---|---|---|
| Milk yield (kg cow$^{-1}$ day$^{-1}$) | 504 | 8.60 | 34.91 | 62.70 | 9.86 |
| Total dry extract (g kg$^{-1}$) | 504 | 96 | 122.5 | 156.0 | 7.8 |
| Defatted dry extract | 504 | 70.4 | 86.6 | 97.0 | 3.8 |
| Fat (g kg$^{-1}$) | 504 | 20.0 | 35.9 | 68.0 | 5.8 |
| Protein (g kg$^{-1}$) | 504 | 23.1 | 30.6 | 40.0 | 2.7 |
| Lactose (g kg$^{-1}$) | 504 | 33.0 | 45.9 | 51.1 | 2.5 |
| SCC (cells × 1000/mL) | 504 | 4.00 | 346.50 | 9,999.00 | 763.15 |
| Somatic cell score | 504 | 1.32 | 3.09 | 9.64 | 2.16 |
| Days in milk (days) | 504 | 8 | 155.56 | 395 | 96.35 |
| Parity | 504 | 1 | 2.22 | 3.00 | 0.86 |
| Udder depth[1] | 474 | 1 | 1.50 | 3.00 | 0.77 |
| Teat end hyperkeratosis[2] | 473 | 1 | 1.61 | 3.50 | 0.68 |
| Lameness score[3] | 504 | 1 | 1.88 | 5.00 | 0.77 |
| Udder cleanliness score[4] | 504 | 1 | 2.53 | 4.00 | 0.92 |

N - number of observations.
[1] 1 - udder floor above the line of hock, 2 - udder floor on hock line, 3 - udder floor below the line of the hock.
[2] 1 to 4 score: 1 - teat end without ring formation to 4 - teat end with a very rough ring.
[3] 1 to 5 score: 1 for cows with no locomotion problems to 5 for cows with serious locomotion problems.
[4] 1 to 5 score: 1 - clean to 4 - very dirty.

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Factor analysis regarding the variables from individual cow milk yield (kg/cow/day), fat, protein, lactose contents, SCS, functional traits, physiological status represented by DIM, parity, teat end hyperkeratosis, lameness score, udder depth, and udder cleanliness expressed 57.6% of the total variance in the first four factors, with a KMO of 0.579 (Table 2). Higher communalities show the importance of the variables within the study. The first factor shows the parameters responsible for altering the lactose content, as demonstrated by a negative relationship between SCS, udder depth, and parity with lactose content. The negative relationship between these variables is represented by the opposition of their factor loading, for example, high SCS (0.716) with low lactose content (−0.736), as shown for the first factor. The second factor comprises the effect of DIM, represented by the positive relation between DIM and milk fat as well as protein content. The third factor represents teat end hyperkeratosis score. It combines variables that affect the hyperkeratosis score, resulting from an unfavorable positive relationship between parity and milk yield and teat end hyperkeratosis score. The negative relationship among cows with the highest lameness score (more locomotion problems) and lower udder cleanliness score (udders less dirty) is defined by the fourth factor.

The canonical discriminant analysis graphically demonstrates the Euclidean distances used for the separation between and within clusters (Figure 5). The STEPWISE method identified the variables responsible for the differentiation of clusters, comprising the final model ($P<0.0001$) by order of relevance. These variables were parity, milk yield, lactose content, DIM, udder depth, SCS, teat end hyperkeratosis score, protein content, lameness score, udder cleanliness score, and fat content (Table 3). All variables recorded were considered in the stepwise analysis. The partial $R^2$ explains the impact of each variable for the differentiation of cows among the three clusters. A proportion of 69.7% of the distance among the clusters is explained by parity, followed by milk yield and lactose content.

The cluster analysis formed three clusters with similar characteristics within them and distinct differences among clusters defined by the Euclidian distance (Table 4). There were differences among the three clusters for lactose content, SCS, DIM, lameness score, and udder depth, while fat content did not differ among clusters. Clusters 2 and 3 did not differ for milk yield, udder cleanliness score, and hyperkeratosis scores, but differed for protein and lactose content, SCS, parity, DIM, lameness score, and udder depth. Cluster 3 is represented by younger cows with intermediate

**Table 2 -** Factor loads, eigenvalue, variance percentage, and communalities of each variable regarding the evaluation of the inter-relation of milk yield and composition, somatic cell score (SCS), and individual characteristics of lactating cows in a compost barn confinement system

| Variable | Factor[1] | | | | Communality |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| SCS | 0.716 | 0.102 | −0.062 | −0.023 | 51.3 |
| Udder depth[2] | 0.517 | −0.002 | 0.313 | 0.296 | 70.4 |
| Lactose (g kg$^{-1}$) | −0.736 | −0.208 | 0.117 | 0.089 | 50.2 |
| Protein (g kg$^{-1}$) | 0.147 | 0.850 | 0.036 | −0.045 | 57.7 |
| Days in milk (days) | 0.195 | 0.773 | −0.051 | −0.120 | 73.2 |
| Fat (g kg$^{-1}$) | −0.298 | 0.451 | 0.308 | 0.401 | 63.6 |
| Milk yield (kg cow$^{-1}$ day$^{-1}$) | −0.064 | −0.265 | 0.730 | −0.317 | 55.8 |
| Teat end hyperkeratosis[3] | −0.167 | 0.271 | 0.617 | −0.122 | 45.2 |
| Parity | 0.458 | −0.070 | 0.606 | 0.131 | 65.9 |
| Lameness score[4] | 0.208 | −0.167 | −0.116 | 0.723 | 44.7 |
| Udder cleanliness[5] | 0.206 | 0.004 | 0.372 | −0.562 | 43.3 |
| % Variance | 20.3 | 15.7 | 11.4 | 10.2 | |

[1] Factors formed by FACTOR analysis.
[2] 1 - udder floor above the line of hock, 2 - udder floor on hock line, 3 - udder floor below the line of the hock.
[3] 1 to 4 score: 1 - teat end without ring formation to 4 - teat end with a very rough ring.
[4] 1 to 5 score: 1 for cows with no locomotion problems to 5 for cows with serious locomotion problems.
[5] 1 to 5 score: 1 - clean to 4 - very dirty.

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DIM (average of 146), shallow udder, and lower lameness score than cows of cluster 2, while cows of cluster 2 present a higher parity and DIM as well deeper udder and higher lameness score, producing milk with higher SCS and, therefore, lower lactose content. Cluster 1 is different from the others, especially due to high milk yield combined with low protein content and fewer DIM, representing cows with higher parity (similar to cluster 2) and higher teat end hyperkeratosis and cleanliness scores, while lactose content, SCS, udder depth, and lameness score are intermediate in comparison with clusters 2 and 3.
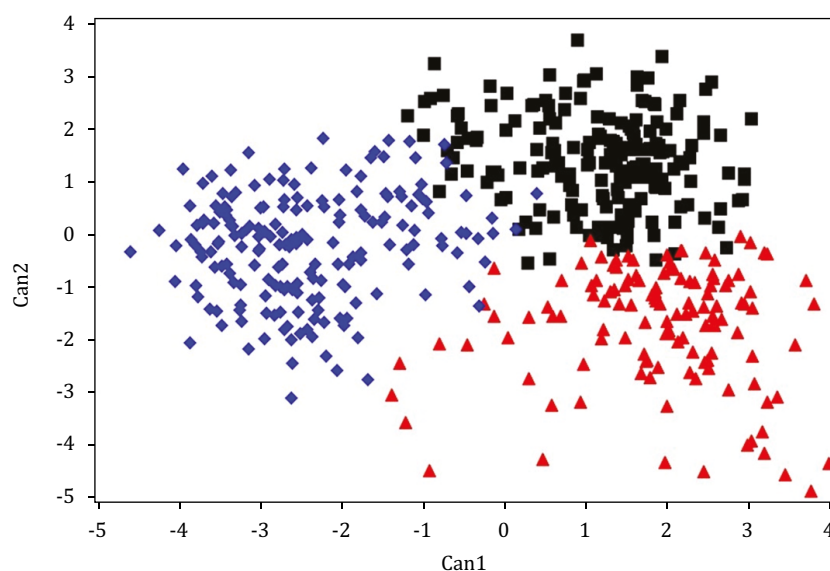


Cluster 1 (■), cluster 2 (▲), cluster 3 (♦).

**Figure 5 -** Canonical discriminant analysis demonstrates the Euclidean distances between and within the clusters formed by the variables milk yield and composition, somatic cell score (SCS), and individual characteristics of lactating cows in compost barn confinement system.

**Table 3 -** Discriminant analysis of the variables that determine the differentiation of the clusters and their respective statistical parameters for the variables that represent the inter-relation of milk yield and composition, somatic cell score (SCS), and individual characteristics of lactating cows in a compost barn confinement system

| Variable | Partial $R^2$ | P>F | P<Lambda | P>ASCC |
|---|---|---|---|---|
| Parity | 0.697 | <0.0001 | <0.0001 | <0.0001 |
| Milk yield (kg cow$^{-1}$ day$^{-1}$) | 0.393 | <0.0001 | <0.0001 | <0.0001 |
| Lactose (g kg$^{-1}$) | 0.219 | <0.0001 | <0.0001 | <0.0001 |
| Days in milk (days) | 0.163 | <0.0001 | <0.0001 | <0.0001 |
| Udder depth[1] | 0.108 | <0.0001 | <0.0001 | <0.0001 |
| SCS (log2) | 0.078 | <0.0001 | <0.0001 | <0.0001 |
| Teat end hyperkeratosys[2] | 0.044 | <0.0001 | <0.0001 | <0.0001 |
| Protein (g kg$^{-1}$) | 0.042 | <0.0001 | <0.0001 | <0.0001 |
| Lameness score[3] | 0.025 | = 0.0020 | <0.0001 | <0.0001 |
| Udder cleanliness[4] | 0.018 | = 0.0126 | <0.0001 | <0.0001 |
| Fat (g kg$^{-1}$) | 0.014 | = 0.0333 | <0.0001 | <0.0001 |

ASCC - Average Squared Canonical Correlation.
[1] 1 - udder floor above the line of hock, 2 - udder floor on hock line, 3 - udder floor below the line of the hock.
[2] 1 to 4 score: 1 - teat end without ring formation to 4 - teat end with a very rough ring.
[3] 1 to 5 score: 1 for cows with no locomotion problems to 5 for cows with serious locomotion problems.
[4] 1 to 5 score: 1 - clean to 4 - very dirty.

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**Table 4 -** Clusters formed by the variables regarding to the evaluation of the inter-relation of milk yield and composition, somatic cell score (SCS), and individual characteristics of lactating cows in a compost barn confinement system

| Variable | Cluster[1] | | | P |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Parity | 2.77a | 2.79a | 1.30b | <0.0001 |
| Days in milk | 114.34c | 230.20a | 146.41b | <0.0001 |
| Milk yield (kg cow$^{-1}$ day$^{-1}$) | 43.36a | 28.96b | 30.51b | <0.0001 |
| Fat (g kg$^{-1}$) | 36.2 | 36.7 | 35.2 | = 0.0574 |
| Protein (g kg$^{-1}$) | 29.6b | 32.7a | 30.1b | <0.0001 |
| Lactose (g kg$^{-1}$) | 46.3b | 43.6c | 47.1a | <0.0001 |
| SCS (log2) | 2.85b | 4.77a | 2.20c | <0.0001 |
| Udder cleanliness[2] | 2.79a | 2.43b | 2.33b | <0.0001 |
| Lameness score[3] | 1.91b | 2.13a | 1.68c | <0.0001 |
| Udder depth[4] | 1.62b | 2.15a | 1.00c | <0.0001 |
| Teat end hyperkeratosys[5] | 1.83a | 1.57b | 1.41b | <0.0001 |
| Number of observations | 188 | 127 | 189 | |

[1] Clusters formed by the cluster analysis; Arithmetic means in the same row followed by different letters are different by Tukey test at 5% significance level.
[2] 1 to 5 score: 1 - clean to 4 - very dirty.
[3] 1 to 5 score: 1 for cows with no locomotion problems to 5 for cows with serious locomotion problems.
[4] 1 - udder floor above the line of hock, 2 - udder floor on hock line, 3 - udder floor below the line of the hock.
[5] 1 to 4 score: 1 - teat end without ring formation to 4 - teat end with a very rough ring.

## Discussion

The physiological traits of milk yield and composition of the lactating cows at the CBP system matched all the assumptions of milk quality recommended for the Brazilian dairy farmers (Brasil, 2018). The CBP system can have a positive effect on milk quality, especially regarding indicators of mammary gland health (Tables 2 and 4). The CBP potentially reduces SCS (Black et al., 2013; Black et al., 2014), which relies on the appropriate management of the compost bed, tilling it at least two times a day and regularly adding new carbon-source materials (Endres, 2009). On the other hand, an improper bed management can increase udder dirtiness by the adherence of bedding material to the udder (Fávero et al., 2015; Albino et al., 2018). The cleanliness of the lactating cows indicates the capacity of the CBP in keeping the udder clean resulting in a lower incidence of clinical and subclinical mastitis (Eckelkamp et al., 2016a). It is worth emphasizing that, at the time when the study was carried out, weather conditions were also challenging for the good quality of the compost bed, because of low air temperature combined with high air humidity. The combination of these two factors may help to explain why the compost bed temperature was low (around 25 °C) and the humidity high (62%).

Considering the effects of mastitis on mammary gland health, and thus its effect on milk composition, the factors related to the system that affect mastitis, represented here by the SCS, also affect milk composition. Under the conditions of this study, it can be verified that the variables of cleanliness and lameness scores do influence SCS and, indirectly, the milk composition (Table 4). A higher cleanliness score is related to a higher risk of development of mastitis due to a higher exposure of the mammary gland to contaminants (Albino et al., 2018). The lameness score is also related to greater udder dirtiness. Cows with locomotion problems show longer lying times in humid environments (Eckelkamp et al., 2016b; Mostafa and Mahran, 2016), especially under improper management of the compost bed, which can increase humidity and decrease the bed temperature (Endres, 2009). In addition, locomotion problems decrease cow immunity due to stress caused by pain (Eicher et al., 2013), leading to an increased occurrence of mastitis.

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In our study, lactating cows with lower lameness scores (animals with less locomotion problems) show greater udder cleanliness scores (dirtier udders), meaning that more mobile cows have dirtier udders. That is shown by the cleanliness factor (Table 2). It can be concluded from cluster 1 (Table 4) that cows at early lactation in combination with higher milk yield present greater udder dirtiness. The lower lameness score (1) allows cows to move more and better. That is important especially for high yielding cows with rising nutrient requirements at early lactation stages. Therefore, these animals need to increase their movement in the feed parlor area to select sites with greater amounts of feed. That way, they can meet their physiological needs of higher nutrient intake to sustain higher milk production (Nielsen et al., 2011). Cows that moved more consequently increased udder dirtiness occurrence, due to increased contact with feces and urine accumulated in the feed parlor area (DeVries et al., 2012). Furthermore, in our study, cows with lameness problems lie down immediately after reaching the compost bed, remaining cleaner compared with cows with lower lameness scores, due to greater movement of cows in water troughs area with strong accumulation of manure (Mostafa and Mahran, 2016).

In relation to udder cleanliness, however, it is observed that cows in CBP with deeper udders have better cleanliness scores than cows with intermediate deep udders, which can be visualized in the clusters formed by cluster analysis (Table 4). In this regard, clusters 2 and 3 present the best udder cleanliness score when compared with cluster 1, as cows from cluster 2 present deeper udders. However, due to an increased parity, the udder tends to become more pendular, which may increase traumas during locomotion as well as dirtiness and, thus, the SCS (Pérez-Cabal and Alenda, 2002; Cardozo et al., 2015).

Higher milk yield is related to higher parity and higher teat end hyperkeratosis score, relations that were observed in the factor represented by teat end hyperkeratosis score of the factor analysis (Table 2) and cluster 1 of the cluster analysis (Table 4). The correlation of production and teat end hyperkeratosis is evident in cluster 1, in which cows with higher hyperkeratosis scores are those with higher milk yields. The same relation was observed by Neijenhuis et al. (2001). It can be explained by the fact that multiparous cows have higher milk yields (Hristov et al., 2013), which require a longer milking time. Hyperkeratosis is also related to DIM, which affects milk yield and milking time (Cerqueira et al., 2018). Blood circulation is partially interrupted by the pressure exerted by the milking machine on the teat skin. This increases local pressure and can form small cracks, leading to small deposits of keratin, causing greater resistance of teat to milking. These keratin deposits also reach the teat sphincter causing sphincter closing failure after milking and facilitating the entry of microorganisms into the mammary gland (Mein et al., 2003).

The negative relations of SCS, udder depth, and parity with lactose content in milk can be demonstrated with the factor lactose content (Table 2) and cluster 2 in the cluster analysis (Table 4). Cows with higher parity numbers have deeper udders, higher SCS, and thus lower lactose contents, opposite to the third cluster, comprised of cows with lower parity numbers and shallow udders, higher lactose contents, and lower SCS. In the first cluster, cows presented intermediary characteristics in relation to the second and third clusters. Alessio et al. (2016) found a negative relation between parity and SCS with lactose content, evaluating data of 73 dairy herds in Santa Catarina, Brazil.

Cows with higher parity present deeper udders due to a weakening of the suspensory ligaments and an increase in udder size with the advancement of lactations. Therefore, udder tends to get closer to the floor, being exposed to pathogens and increasing the risk of new and chronical intramammary infections, which also increase SCS in milk (Ruegg and Pantoja, 2013; Cardozo et al., 2015). Cluster 2, however, is comprised of cows with deeper and cleaner udders in CBP, a factor that can be related to the good management of the compost bed.

The reduction of the lactose content in milk with high SCS is a consequence of the lesions in the secretory tissue of the mammary gland that lead to a reduction in the synthesis of lactose (Coelho et al., 2014; Alessio et al., 2016). Another factor that causes lactose reduction is its passage to the blood. Lesions in the tight junctions caused by the endotoxins released by the microorganisms and inflammatory mediators

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can increase the vascular permeability, raising lactose levels in the blood and urine (Stelwagen et al., 1998). Lactose is used as a substrate by pathogenic bacteria (Blum et al., 2008; Alessio et al., 2016). The reduction in lactose levels can also be a consequence of the increase in parity, regardless of the SCS levels (Miglior et al., 2007).

The positive relation of DIM with fat content and especially with the milk protein content can be demonstrated by the factor effect of DIM within the factorial analysis (Table 2), and with cluster 2 for the cluster analysis (Table 4). An increase in the milk protein content was observed with advancing DIM, while the fat content did not present differences for the clusters formed. This observation can be related to a lower milk yield at late lactation combined with a higher concentration of milk solids components (Miglior et al., 2007; Smith et al., 2013).

According to the literature, the use of the CBP system enables better housing conditions, promoting an increase in the welfare of lactating cows. The housing system is one of the factors that have an impact on milk and solids production, once improvements to the welfare of lactating cows increase milk and solids yield after the reduction or elimination of stressors that negatively influence milk yield and quality. The lameness score, for example, might be improved by a reduction of digital lesions, and a better udder cleanliness score would lead to an improved milk quality (Fávero et al., 2015; Albino et al., 2018). The last one, apart from facilitating milking procedure, is also related to improvements of the immunity of lactating cows, reducing the SCS as a variable that has a significant economic impact on the dairy production chain (Black et al., 2013; Ruegg and Pantoja, 2013; Black et al., 2014; Cardozo et al., 2015; Eckelkamp et al., 2016a). Therefore, the CBP has a high potential to provide comfort and welfare to dairy cows. It is necessary, however, to perform a well-organized management of the compost bed (Pilatte and Vieira, 2017).

## Conclusions

Udder conformation and physiological status influence milk quality in a compost bedded pack barn housing system, through the association of depth udders and udder health, older cows with higher somatic cell score, and the consequent lower lactose content. As higher parity is also associated with higher milk yield and teat end hyperkeratosis score, special attention to the improvement of the udder conformation traits, as well as management practices that reduce hyperkeratosis, are necessary to keep high yielding, healthy, and cows with higher longevity.

As cows without locomotion problems have dirtier udders in compost barn confinement system because of their greater movement, the cleanliness in the feed parlor and water troughs area is very important to preserve milk quality and healthy cows.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: R. Kappes and D.A. Knob. Data curation: R. Kappes, D.A. Knob and W.B. Rodrigues. Formal analysis: D.A. Knob, A. Thaler Neto and D.R.M. Alessio. Methodology: R. Kappes and D.A. Knob. Software: D.A. Knob and D.R.M. Alessio. Supervision: R. Bonotto. Writing-original draft: R. Kappes. Writing-review & editing: D.A. Knob, A. Thaler Neto, D.R.M. Alessio and A.M. Scholz.

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