Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v43i1.52604

ANIMAL PRODUCTION

Influence of meteorological elements on behavioral responses of gir cows and effects on milk quality

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ABSTRACT. This study aimed to analyze the principal components of the meteorological variables, physiological and behavioral response of cows subjected to different cooling times and their influence on milk quality, in the dry and rainfall periods, and to establish multiple regression models for milk quality. The data used in the study came from an experiment conducted in the Agreste Region of Pernambuco. The pre-milking cooling time was 10, 20, 30 min. and the control (without cooling). Sixteen multiparous lactating Gir cows were selected. Data were analyzed by principal component analysis and a multiple regression analysis was applied to determine milk quality. There was a strong relationship between somatic cell count (SCC) and activity of the animal in the shade for dry, and lying for rainfall, with increased SCC in cow milk. It was possible to establish two multiple regression models to determine milk quality in dry and rainfall periods. According to the principal component analysis, the cooling time to meet the thermal requirement of the animals was 20 min., regardless of the season and milking shift.

Keywords: animal activity; somatic cells; cooling; principal components.

Received on March 10, 2020.
Accepted on January 15, 2021.

Introduction

Animal stress due to high temperatures affects both milk production and quality. This phenomenon often occurs in tropical, subtropical and temperate regions (Kadzere, Murphy, Silanikove, & Maltz, 2002; West, 2003; Liu et al., 2017). For Holstein dairy cows, the ideal temperature should be between 24 and 26°C, where temperatures above or below this range may cause interference with animal health, performance, behavior and qualitative depreciation of milk (Miranda & Freitas, 2009; Cruz, Monteiro, Guimarães, Antunes, and Nascimento, 2016).

Thus, improvements in the thermal conditioning of cows, seeking the best possible comfort for these animals has been commonly investigated. Waiting pens or pre-milking rooms are critical points in the production units, because in these environments the animals are exposed to possible heat stress events that fluctuate in intensity and duration due to the adopted management.

Recent research shows that the use of evaporative adiabatic cooling systems, which consists of the combination of forced ventilation and air humidification through low pressure nebulizers, has provided better comfort conditions to cows (Avendaño-Reyes et al., 2012; Chen, Schütze, & Tucker, 2015; Porto, D'Emilio, & Cascone, 2017).

Among the phenomena affecting the comfort of dairy cows, the effect of meteorological elements (solar radiation, air temperature, relative humidity and wind speed) are the most important and may act as stressors (Porto et al., 2017). However, the climate of the place is the factor that determines the use or not of cooling systems, with emphasis on meeting the thermal needs of animals, such as Ortiz, Smith, Bradford, Harner, and Oddy (2010a; 2010b) and Ortiz et al. (2015), which studied the need for adaptation of the thermal environment in the comfort of Holstein cows in arid region (Temperature > 30°C) and found that the use of cooling systems were essential for the thermal comfort of these animals and improvements in milk productivity.

Explaining the effect of meteorological elements and the behavioral responses of cows within this microclimate, associated with milk quality, is complex, as it involves a large set of variables, since conventional statistical analyses can capture the effect of only one or two variables. Nevertheless, Macciotta, Cecchinato, Mele, and Bittante (2012) state that the use of multivariate statistics is feasible, as this technique can explain more than two variables involved.

In multivariate statistics, total variance values greater than 70% indicate a level of adequacy of statistics, classified as well accepted (Kaiser, 1958). Studies such as by Gabbi et al. (2018), who evaluated total digestible nutrient levels on milk yield, composition and quality and Tian et al. (2016) who conducted an integrated metabolomic study of milk from heat-stressed lactating cows, using multivariate statistics, the authors observed a total variance of 87.22 and 86.1%, respectively. Zhang, Zhu, Jiang, and Mao (2017) compared ruminal metabolites in dairy cows fed low-concentrate and high-concentrate diets and obtained a total variance of 46.40 and 64.30%, respectively.

In this context, the objective of this study was to analyze the principal components of the meteorological variables, physiological and behavioral response of dairy cows subjected to different cooling times and their influence on milk quality in the dry and rainfall periods, and to establish multiple regression models for milk quality.

Material and methods

The data used in the study came from an experiment conducted on a dairy farm in the dry period (February to March) and rainfall period (July to August) of 2009 (Figure 1). Located in the Agreste Mesoregion, Ipojuca Valley Microregion, State of Pernambuco (8° 36' 34.82" S and 36° 37' 33.09" W; 755 m).

The average rainfall of the region is 620.3 mm, with an average temperature of 22.8 in dry period and 20.3°C in rainfall period (*Agência Pernambucana de Águas e Climas* [APAC], 2009; *Instituto Nacional de Meteorologia* [INMET], 2019). According to the Köppen climate classification, the climate is characterized as Bsh - semiarid.

The study lasted 56 days for each season, totaling 112 days, divided into 4 phases of 14 days. The first seven days of each experimental phase were used to adapt the animals to cooling conditioning at pre-milking. After adaptation of the animals, was recorded meteorological variables at pre-milking, behavioral responses and the determination of milk quality. The effect of cooling was evaluated based on three time periods of permanence of animals at pre-milking, 10, 20, 30 min. and control (without cooling).

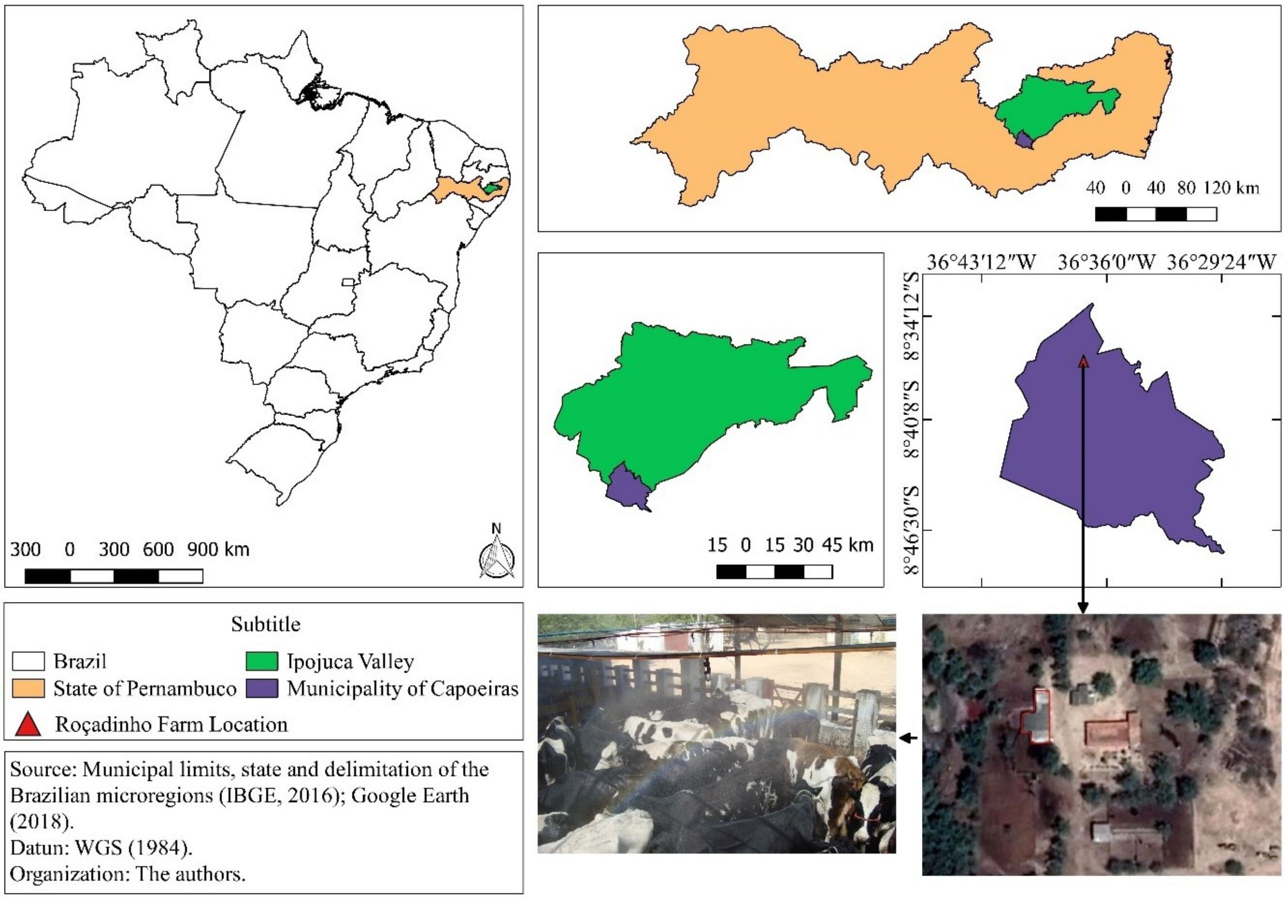

Figure 1. Location of the farm in the municipality of Capoeiras, State of Pernambuco, Brazil.

Sixteen multiparous lactating Gir cows with average weight of 500 kg and average milk production of 18 kg day^{-1} were randomly distributed in 4 sets (S1, S2, S3 and S4), with 4 experimental phases (P1, P2, P3 and P4) and 3 cooling times, T1 (10 min. cooling at pre-milking), T2 (cooling for 20 min. at pre-milking), T3 (cooling for 30 min. at pre-milking) and control (no cooling at pre-milking).

Air temperature - T (°C) and relative air humidity - RH (%) were recorded using HOBO Pro Dataloggers HB8 dataloggers (Onset Computer Corporation Bourne, MA, USA). The sensors were positioned at the geometric center of the pre-milking, 2.5 m from the floor and 1.5 m in the outer environment (reference), in a meteorological shelter.

The wind speed - Ws (m s^{-1}) was recorded by a propeller anemometer (AZ Instrument®/model 8908). The records were performed in the external and internal environments of pre-milking, during the period of stay of animals at pre-milking.

The management of the animals in the milking occurred in a confined system. The milking time in the morning was between 5 and 7 hour and in the afternoon was from 13 to 15 hour. After milking, the animals had access to an open resting area with natural shading (trees).

Behavioral data were recorded by means of planned observations at significant intervals. Activities of eating, drinking, ruminating, walking, lying down and standing were recorded. The records were made using the focal method at 10 min. intervals, from 7 to 13 hour and from 15 to 17 hour, done twice each week of data recording, according to the methodology established by Almeida et al. (2013).

The somatic cell count in milk was made in two collections for each phase, in the morning and afternoon, with individual milk samples of each animal, in their respective treatments. Subsequently, the samples were analyzed using the Flow Cytometry technique, used to count, examine and classify microscopic particles suspended in the flowing liquid medium. This analysis was carried out in the laboratory of the Dairy Herd Management Program of the Northeast region (Progene) of the Federal Rural University of Pernambuco (UFRPE).

Rectal temperature (RT, °C) were measured with the aid of a digital veterinary thermometer (scale between 20 and 50°C), introduced into the animal rectum, for 1 min. to stabilize and obtain the value of temperature.

Was applied a principal component analysis for 13 variables for dry and rainfall periods, totaling a set of 26 variables: air temperature (T), relative humidity (RH), wind speed (Ws), somatic cell count (SCC multiplied by 1000), rectal temperature (RT), animal activity in the shade, sun, ruminating, walking, lying, standing, eating and drinking.

Based on the principal components, a covariance matrix was obtained for extracting the eigenvalues that originate the eigenvectors. To identify the variables that showed correlation, the Kaiser criterion was used, considering the eigenvalues above 1, which generate components with relevant amount of information contained in the original data (Kaiser, 1958).

Based on the Principal Component Analysis, the main predictor variables influencing milk quality were determined and a multiple regression analysis was applied to determine a model for the dry period and one for the rainfall period. The model fit was performed using the Analysis of Variance (ANOVA), adopting the P-value of less than 0.05, to adjust the predictor and model variables.

Results and discussion

Morning shift

Table 1 lists the principal components of environmental variables, rectal temperature, behavioral parameters and milk somatic cell count for the morning shift in both seasons. The principal components PC1 and PC2 presented eigenvalues greater than 1, according to the criteria established by Kaiser (1958), with values of 14.334 and 8.575, respectively, for the dry and rainfall periods.

PC1 and PC2 showed a cumulative total variance of 88.10%. Similar results were observed by Gabbi et al. (2018), in which they related total digestible nutrient levels, experimental period, milk yield, composition and quality, in Jersey, Holstein and Jersey x Holstein dairy breeds, and obtained a cumulative PC2 result of 87.24%.

The principal component of each variable in the dry showed somatic cell count (SCC) strongly influenced by air temperature (T), rectal temperature (RT), activity of the animal standing in the shade. The variables relative humidity (RH), wind speed (Ws), activity in the sun, rumination, walking, lying down, eating and drinking were inversely proportional to SCC.

Table 1. Principal components of environmental variables, rectal temperature, behavioral parameters and somatic cell count in milk from dairy cows in the morning shift in dry and rainfall periods.

Variable	Principal Component Dry Period		Principal Component Rainfall Period	
	*PC1	PC2	PC1	PC2
[1]T	-0.251	0.104	-0.207	0.209
[2]RH	0.251	-0.104	0.222	0.186
[3]Ws	0.193	-0.219	0.200	-0.211
[4]SCC	-0.122	-0.067	-0.212	0.168
[5]RT	-0.214	-0.192	-0.119	-0.298
Shade	-0.130	0.281	0.207	0.170
Sun	0.056	-0.331	-0.191	-0.207
Ruminating	0.239	-0.056	-0.262	0.020
Walking	0.196	-0.216	0.099	0.304
Lying	0.227	-0.172	-0.219	-0.189
Stand	-0.234	0.124	0.223	0.090
Eating	0.173	0.177	-0.104	-0.311
Drinking	0.124	0.069	0.227	0.160
Eigenvalue	14.334	8.575	14.334	8.575
Proportion	0.551	0.330	0.551	0.330
Cumulative	0.551	0.881	0.551	0.881

[1]T: air temperature; [2]RH: relative humidity; [3]Ws: wind speed; [4]SCC: somatic cell count; [5]RT: rectal temperature; *PC: principal component.

It was verified the influence of the activity of the animal in the shade with the SCC in the dry. Thus, it can be stated that the greater activity of the animal in the shade, causes increase in SCC, even though it is a countermeasure of the animal to prevent heat stress by avoiding direct solar radiation, it can be exposed to contact with the soil and manure (soil moistened by urine, feces, among others), which in turn has a significant amount of bacteria, which can cause an increase in SCC (Alhussien & Dang, 2018).

In the rainfall period, SCC was strongly influenced by air temperature (T), rectal temperature (RT), animal activity in the sun, rumination, lying down and eating. Relative humidity (RH), wind speed (Ws), activity in the shade, standing and drinking were inversely proportional to SCC. With mild temperatures in the rainfall in the morning shift, it was found that the activity of the animal in the shade was not a significant factor for the increase in SCC, but the activity of the lying animal, with the udder in direct contact with the moist soil, promoting the accumulation of bacteria.

Alhussien and Dang (2018) evaluated the impact of different seasons on somatic cell count, cortisol and neutrophil functionality in milk of three Indian native cattle breeds (Tharparkar, Gir and Sahiwal) and stated that SCC is strongly influenced by higher temperatures, because the higher the air temperature, the higher the SCC. However, in our findings, the air temperature for both seasons was within or near the comfort zone for the animal in the morning shift. Given this, the activity of the animal in the shade and lying, in dry and rainfall periods, is the most plausible explanation for the increase in SCC.

Also, according to Alhussien and Dang (2018) the phagocytic activity of the animals in three climatic conditions (rainfall period, hot and dry and hot and humid) showed lower activity in the hot and humid climate. Phagocytic activity is the body's immune response, and its reduction results in a higher incidence of bacterial infections. Thus, the highest SCC values observed by the authors were in this hot humid period, and it is safe to state that the higher humidity is one of the agents responsible for the increased SCC.

Figure 2 illustrates the principal component analysis for environmental variables, rectal temperature, somatic cell count and behavioral parameters for dry and rainfall periods (Figure 2A) and evaporative adiabatic cooling systems at times (0, 10, 20 and 30 min.; Figure 2B) in the morning shift.

In rainfall, the activity of the animal in the shade was not a factor contributing to the increase in SCC, but the activity of the lying animal, which had a direct relation with SCC, since practically all places in this season are more likely to be humid, not only shaded areas, unlike the dry period.

Anderson et al. (2013) evaluated the efficiency of mister system stationary fans (CTRL) with the FlipFan Dairy Cooling System (FLFN) which employ fans and misters that follow the shadow, thus providing longer cooling times for animals, and observed that cows cooled by FLFN spent more time lying down throughout the day. However, according to the authors, flooring under high humidity may be a promising agent for increasing SCC. In the present study, the animals that received longer cooling time in rainfall were longer lying down (Figure 2A and B), implying greater contact of cow udder with humid places, with possible concentration of bacteria.

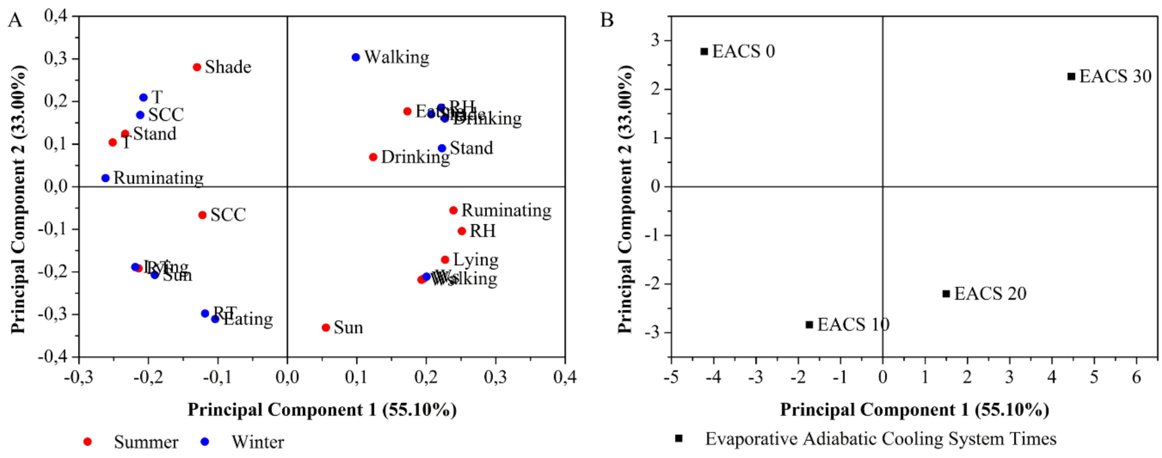

Figure 2. Principal components for environmental variables, rectal temperature, behavioral parameters and milk somatic cell count in the morning shift in dry and rainfall periods (A); Principal components of the time of operation of the evaporative adiabatic cooling system in the morning (B). T: air temperature; RH: relative humidity; Ws: wind speed; RT: rectal temperature; SCC: somatic cell count; EACS: evaporative adiabatic cooling system (0, 10, 20 and 30 min.).

It was also found that the use of evaporative adiabatic cooling system in rainfall was beneficial for milk quality, reducing the SCC. Almeida Neto, Pandorfi, Almeida, and Guiselini (2014) examined the effect of different evaporative adiabatic cooling times in rainfall in a semiarid region and did not observe changes in milk quality using the cooling system.

The lowest activity of ruminating and, consequently, eating occurred at high temperatures, as T is inversely proportional to the activities of rumination and eating (Figure 2A). Corroborating Avendaño-Reyes et al. (2012) and Soriani, Panella, and Calamari (2013), who observed a similar relationship, in which high ambient temperatures reduce rumination, ruminal contractions and thus inhibit the animal appetite.

The same was observed by Abeni and Galli (2017), who evaluated the rumination time and diet digestibility of Holstein dairy cows exposed to hot environment. Also, according to the authors, animals subjected to high temperatures spent more time standing. Similar results were observed in the present study for the control, where the animals spent more time standing in the dry period and less time in rumination, which shows that the animals were under thermal stress (Figure 2A and B).

In the rainfall period for PC2, there was a strong relationship between the activity of the walking animal, when associated with the evaporative adiabatic cooling system for 30 min. and with the control, which is explained by two premises; the animal was in optimal comfort (EACS 30) and was in search of food, or the animal was under thermal stress (no cooling) and increased walking activity for heat dissipation or search for water, being the first more plausible because the activity of eating and ruminating is opposite to walking for rainfall, which in turn drinking activity is related to walking (Figure 2A and B). The rectal temperature is opposite to the activity of the walking animal, which also indicates a stress condition. Therefore, the animal was under thermal stress, assuming the behavioral indicator walking in rainfall, corroborating De, Kumar, Saxena, Thirumurugan, and Naqvi (2017) and Abeni and Galli (2017), who observed that the greater activity of the walking animal indicates a condition of thermal stress.

Evaluating the principal component 1 (PC1), it is observed that application of EACS 20 and EACS 30 did not differ in the improvement of animal comfort, in which Ws and RH are located in the same vector of both EACS times (20 and 30 min.) for both seasons. Thus, the cooling time of 20 min. would be the most recommended, assuming a reduction in electricity and water consumption (Figure 2A and B).

Afternoon shift

Table 2 lists the principal components of the environmental variables, rectal temperature, behavioral parameters and milk somatic cell count for the afternoon shift in both seasons. PC1 and PC2 presented eigenvalue greater than 1, according to the criteria established by Kaiser (1958), being 16.112 and 7.287, respectively, for the dry and rainfall periods.

PC1 and PC2 were retained for interpreation, considering that they presented total cumulative variance of 90.00%. Macciotta, Vicario, and Cappio-Borlino (2006) and Yilmaz, Eyduran, Kaygisiz, and Javed (2011) applying multivariate analysis on data of milk production of Simmental and Brown Swiss cows, respectively, the authors obtained a total variance of 80% in the production of Simmental cows and 82.8% for the production of Brown Swiss cows.

Analyzing the influence of each variable in the seasons studied, the variables that influenced the somatic cell count (SCC) in dry were air temperature (T), rectal temperature (RT), animal activity in the shade (Sh), and standing (P) and for the rainfall, were air temperature (T), rectal temperature (RT), activity of the animal in the sun, ruminating and lying down (Table 2). The variables T and RT were those that showed a positive direct correlation with the SCC, both in the morning (Table 1) and afternoon (Table 2) for both seasons.

The same trend of SCC increase in both dry and rainfall periods can be observed in the afternoon shift. Shade was one of the factors that influenced the increase in SCC in the dry period, while in rainfall, the activity of the lying animal had the greatest influence (Table 1 and 2). However, Almeida et al. (2013) observed no difference in somatic cell count and no relationship of this variable with the activity of the animal in the shade or lying in the afternoon.

Wildridge et al. (2018) state that there is a delay in the effect of environmental variables on milk composition and quality from 1 to 2 days for lactating cows. Given this, in the afternoon, when there are higher temperatures, the stress imposed to animals can contribute to the increase in SCC in a time scale of up to two days later.

Figure 3 illustrates the principal component analysis for environmental variables, rectal temperature, somatic cell count and behavioral parameters for dry and rainfall periods (Figure 3A) and evaporative adiabatic cooling systems at times 10, 20, 30 min. and control (Figure 3B) in the afternoon shift.

Table2. Principal components of environmental variables, rectal temperature, behavioral parameters and somatic cell count in milk from dairy cows in the afternoon shift in dry and rainfall periods.

Variable	Principal Component Dry Period		Principal Component Rainfall Period	
	*PC1	PC2	PC1	PC2
[1]T	-0.245	0.071	-0.246	0.058
[2]RH	0.243	-0.083	0.247	-0.004
[3]Ws	0.205	-0.204	0.209	-0.184
[4]SCC	-0.149	-0.253	-0.220	0.148
[5]RT	-0.237	-0.096	-0.233	0.085
Shade	-0.145	0.276	0.172	0.224
Sun	0.082	-0.345	-0.154	-0.260
Ruminating	0.223	-0.013	-0.245	-0.028
Walking	0.207	-0.197	0.066	0.343
Lying	0.228	-0.142	-0.187	-0.243
Stand	-0.226	0.088	0.205	0.135
Eating	0.137	0.226	-0.066	-0.354
Drinking	0.120	0.092	0.194	0.216
Eigenvalue	16.112	7.287	16.112	7.287
Proportion	0.620	0.280	0.620	0.280
Cumulative	0.620	0.900	0.620	0.900

[1]T: air temperature; [2]RH: relative humidity; [3]Ws: wind speed; [4]SCC: somatic cell count; [5]RT: rectal temperature; *PC: principal component.

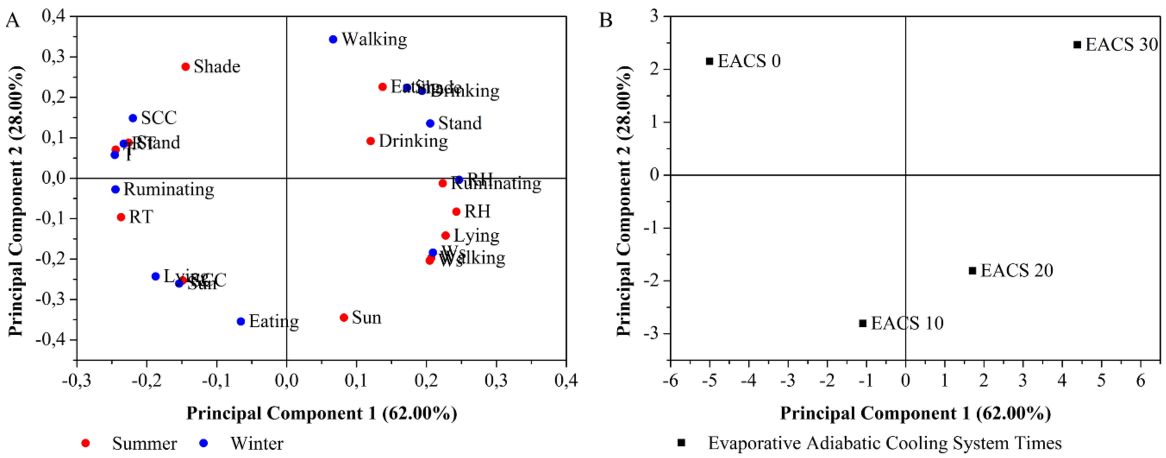

Figure 3. Principal components for environmental variables, rectal temperature, behavioral parameters and milk somatic cell count in the afternoon shift in dry and rainfall periods (A); Principal components of the time of operation of the evaporative adiabatic cooling system in the afternoon (B). T: air temperature; RH: relative humidity; Ws: wind speed; RT: rectal temperature; SCC: somatic cell count; EACS: evaporative adiabatic cooling system (0, 10, 20 and 30 min.).

The activity of the animal standing in the afternoon (Figure 3A) for animals without cooling (Figure 3B) in dry, had a direct relationship with T, with increased SCC, decreasing milk quality. However, in rainfall this influence cannot be observed due to lower rainfall temperatures. Abeni and Galli (2017), monitored the activity of lactating Holstein cows and their rumination time for early detection of heat stress in a region of Italy, in the present study it can be observed the same influence, animals under thermal stress presented higher standing activity in the search for shade and water.

The same situation was reported by Allen, Hall, Collier, and Smith (2015), in which the increase in body temperature provided an increase in the time standing, as well as the results of Brzozowska, Łukaszewicz, Sender, Kolasińska, and Oprządek (2014), in which cows presented the highest number of steps per day during the dry months.

As observed in Figure 2, the operation of the evaporative adiabatic cooling system for 20 min. was sufficient for improvements in animal comfort. The same was observed for the afternoon shift (Figure 3).

Regression model

According to the principal component analysis applied in the dry period, it was possible to establish a model for SCC determination through the multiple regression analysis, with the main variables that influenced the SCC. However, regression models were generated as the predictor variables showed a p-value greater than 0.05, discarding those variables that did not fit the model. The model that best fits is shown below:

$$SCC = -7487 + 195.7 \times T + 42.2 \times RH - 23.4 \times Sh$$

$$R^2 = 0.5127$$

where: SCC: Somatic cell count (multiplied by 1,000); T: Air temperature (°C); RH: Relative humidity (%); Sh: Animal activity in the shade (%).

Table 3 shows the analysis of variance for the model obtained in the dry period. With respect to this generated model, the p-value was less than 0.05 for all predictor variables, and this model can be considered adequate.

In the rainfall period, it was also possible to establish a model for the determination of SCC by multiple regression analysis, with the main variables that influenced the SCC. The same discard of predictor variables was made for the rainfall, generating regression models as the predictor variables had a P-value greater than 0.05. The model that best fits is shown below:

$$SCC = 596 - 47.0 \times T + 59.2 \times Ly$$

$$R^2 = 0.5610$$

where: SCC: Somatic cell count (multiplied by 1,000); T: Air temperature (°C); Ly: Activity of the lying animal (%).

Table 4 lists the analysis of variance for the model obtained in the rainfall period. Predictor variables as well as the regression model were significant, and the p-value was less than 0.05 for the model and all predictor variables, and the model can be considered adequate.

Table 3. Analysis of variance for the multiple regression model of the dry period.

SV	DF	SQ	QM	Fvalue	p-value
Regression	3	657667	219222	9.820**	0.000
T	1	372390	372390	16.680**	0.000
RH	1	261536	261536	11.710**	0.002
Sh	1	80191	80191	3.590ns	0.050
Residual	28	625174	22328		
Total	31				

T: air temperature (°C); RH: relative humidity (%); Sh: activity of the animal in the shade (%); SV: source of variation; DF: degrees of freedom; SQ: sum of squares; QM: mean square; *Significant at 5% probability; **Significant at 1% probability; nsNon-significant.

Table 4. Analysis of variance for the multiple regression model of the rainfall period.

SV	DF	SQ	QM	Fvalue	p-value
Regression	2	61207	30603	7.67**	0.007
T	1	46271	46271	11.59**	0.005
Ly	1	26185	26185	6.56*	0.025
Residual	12	47901	3992		
Total	14	109108			

T: air temperature (°C); Ly: activity of the lying animal (%); SV: source of variation; DF: degrees of freedom; SQ: sum of squares; QM: mean square; *Significant at 5% probability; **Significant at 1% probability; nsNon-significant.

Conclusion

The application of the Principal Component Analysis (PCA) on environmental variables, behavioral parameters and milk quality of lactating cows was efficient to identify their effects on somatic cell count.

For the management adopted with the animals, in the region of the study, the increase in SCC is related to the activity of the animal in the shade (dry) and lying down (rainfall), however, it is necessary to conduct further studies to accurately state these findings.

Principal component analysis showed that sufficient time of operation of the evaporative adiabatic cooling system in dry and rainfall for animal comfort is 20 min. on both milking shifts.

Principal Component Analysis allowed to extract the main predictor variables influencing milk quality and to establish mathematical models for the dry and rainfall periods, with emphasis on milk quality through somatic cell count.

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