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Effect of shading on milk production and quality in Girolando cows

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Abstract

The aim of this study was to evaluate the effect of shading on milk production and milk quality in Girolando cows. A total of 16 Girolando cows were used, divided into two groups for two treatments: the first group remained in a paddock without shade and the second in a paddock with shade. Every 15 days, each group of animals underwent one of the treatments, totaling 120 days of experiment. The 15 days of permanence in each treatment was divided into two periods: 7 days for adaptation and 8 days for sample collection. During the period of data collection, the following variables were measured: the temperature and relative humidity of the air in the paddocks and the milking room; evaluation of the temperature and humidity index; respiratory rate and rectal and superficial temperatures of the skin and udder of the animals; and digital infrared thermographic imaging. Additionally, milk samples were collected for physicochemical evaluation and weighed to assess the daily production of the animals. Descriptive evaluations of the behavior of the animals were carried out, aiming to evaluate the thermal comfort. A cross-over experimental design was used, with an interval between the two treatments. Subsequently, analysis of variance, Pearson's correlation, and principal component analysis were performed, and the thermographic images were processed in FLIR Tools software to ensure greater precision in relation to surface temperatures. The period of the year during which the animals are kept in sun and shade can affect the average, maximum, and minimum temperatures, the surface temperature of the skin, as well as the temperature and humidity index. It is concluded that, in animals that were subjected to both shaded and unshaded areas, there were correlations among milk constituents and between physiological and environmental variables, without any effect on milk production.

Keywords: race Girolando; temperature; thermographic images; milk quality; moisture.

Practical Application: The implementation of shaded areas in paddocks has been shown to improve the thermal comfort of Girolando cows, alleviating heat stress without affecting milk production. Thermographic imaging has proven to be a useful tool for monitoring physiological and environmental parameters.

1 INTRODUCTION

The climate acts on the animal, which is constantly trying to adapt to environmental conditions in the search for well-being. Cattle in tropical climates, especially those raised on pasture, are exposed to the sun and other inclement weather for several hours a day and become susceptible to a permanent state of stress (Deitenbach et al., 2008).

Heat stress negatively affects milk production, decreasing milk yield and causing a significant impact on the economic potential of dairy farmers' activities (Cruz et al., 2011). Dikmen and Hansen (2009) define heat stress as the sum of forces external to the animal that act to shift body temperature away from a certain point, caused mainly by environmental factors such as temperature, humidity, solar radiation, and wind speed.

Whenever the ambient temperature is higher than the thermoneutral zone, cows are affected (Atrian & Shahryar, 2012). Dairy cows are homeothermic animals that have a thermal comfort zone with minimal energy expenditure to maintain body temperature. When the temperature reaches values that exceed the thermal comfort zone, it results in thermal heat stress, which harms animal welfare (Silva et al., 2006).

Changes in behavioral and physiological responses occur in animals to facilitate heat loss and reduce heat production to maintain body temperature. Because of the high heat load, animals change their behavior, resulting in increased use of shade, less time lying down, and more time near water (Silva et al., 2016).

Shade is extremely important and beneficial for cattle physiologically, behaviorally, and in terms of production (Kendall

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et al., 2006). Under summer conditions, cows with access to shade have decreased respiratory rate and core body temperature compared to cattle without shade (Blackshaw & Blackshaw, 1994). When access to shade is provided, cattle readily use it to alleviate the negative effects of increased heat load (Schütz et al., 2010). During grazing, cows seek shade when the ambient temperature and solar radiation increase (Kendall et al., 2006).

In the assessment of animal welfare, thermal comfort indices have been developed to characterize two or more bioclimatic variables and assess the environments to which animals are exposed (Nascimento et al., 2013).

However, cows' responses to heat stress affect feed intake, water metabolism, electrolyte imbalance (sodium, potassium, and chloride), and variations in blood components, factors that directly affect animal production and physiology (Atrian & Shahryar, 2012). Increased respiration and transpiration are classified as evaporative cooling techniques and the main means of dissipating excess heat under conditions of thermal heat stress. An increased body temperature indicates the cow's inability to completely dissipate the heat load (Staples & Thacher, 2011). Moreover, behavioral changes occur with increased water consumption, decreased rumination, decreased daytime grazing, and increased nighttime grazing (Schirmann et al., 2012).

In order to assess the effects of heat stress, various concepts and non-destructive and non-invasive equipment can be useful for acquiring data without directly influencing the organisms, avoiding stress alterations. Infrared thermography is one of the most effective alternatives for achieving this goal since it does not come into contact with the animals and, consequently, there are no contraindications for its use in any species (Leão et al., 2015).

Environmental variables can cause behavioral and physiological changes, resulting in low production and reproductive rates. Alternations to mitigate the effect of thermal heat stress and to promote animal welfare are essential to be adopted according to the environmental conditions of each region, always evaluating the cost/benefit ratio (Atrian & Shahryar, 2012).

With this in mind, the aim of this study was to describe the effects of heat stress on milk production, the influence of sun and shade treatments, the temperature and humidity index (THI) in assessing the animals' thermal comfort, physiological variables, and climatic factors, as well as the use of infrared thermography as an effective tool for assessing variations in body temperature, in order to observe the effects of heat stress.

1.1 Relevance of the work

This study highlights the critical role of shading in improving the thermal comfort and welfare of Girolando cows, especially in hot climates. By identifying correlations between environmental conditions, physiological responses, and milk quality components, it provides valuable insights for sustainable dairy farming. The integration of thermographic imaging further underscores its relevance as a non-invasive tool for monitoring animal health, promoting ethical and efficient management practices in the dairy industry.

2 MATERIAL AND METHODS

The project was submitted to the Ethics Committee for the Use of Animals (CEUA) of the Instituto Federal Goiano and was approved under opinion no. 6454200519.

2.1 Project description

The research was conducted at the Federal Institute of Goiás – Rio Verde Campus. The experiment was carried out over two periods, totaling 120 days. In the first 45 days of the experiment, 16 Girolando cows weighing an average of 450 kg were used, divided into two groups of eight animals. The first group remained in an unshaded paddock (50,000 m2), totally exposed to the sun (Treatment 1), and the second group remained in a paddock with natural shade (25,000 m2) (Treatment 2) (Figure 1). During this period, the animals' diet consisted of Brachiaria brizantha with access to the paddocks and commercial concentrate supplied after milking at a rate of 3 kg per animal per day.

During the other 75 days of the experiment, 16 Girolando cows weighing an average of 450 kg were divided into two groups of eight animals. The first group remained in an unshaded paddock (approximately 5,000 m2), totally exposed to the sun (Treatment 1), and the second group remained in a shaded paddock (approximately 5,000 m2), made up of a shade cloth providing 80% shade (4.5 m2 per animal) and natural shade made up of trees (Treatment 2). During this period, the animals were fed a diet of corn silage and commercial concentrate, provided together in a trough twice daily, at 35 kg of silage and 3 kg of concentrate per animal per day.

The paddocks had feeding troughs and water. Every 15 days, each group of animals underwent one of the treatments, i.e., the animals that remained in Treatment 1 were transferred to Treatment 2 after 15 days, as was the group of animals that remained in Treatment 2 for 15 days. This alternation took place for 120 days until the end of the experiment.

During the data collection period, the temperature and relative humidity (RH) of the air in the paddocks where the animals were kept and the milking parlor were measured; the Temperature and Humidity Index (ITU), respiratory rate, and

Figure 1. Girolando cows kept in a paddock with shading (A) and without shading (B).

rectal temperature and surface temperature of the animals' skin and udder, as well as digital infrared thermography imaging, were evaluated. In addition, milk samples were collected for physicochemical evaluation and weighed to assess the animals' daily production. The animals' behavior was also evaluated in order to assess thermal comfort.

2.2 Evaluation of temperature and relative humidity in the paddocks and milking parlor

During the experimental period, the air temperature and humidity in the confinement paddocks and inside the milking parlor were measured using an Akso® model AK 28 digital thermo-hygrometer, with a humidity range of 20–95% and a temperature range of −10°C to 60°C. The temperatures and RH of the paddocks and milking parlor were measured at 8 a.m. and 2 p.m., the time period of the two daily milkings, and the maximum and minimum temperatures and RH of the day were recorded.

2.3 Evaluation of the temperature and humidity index

The maximum and minimum temperatures and humidity data were used to calculate the UTI using the formula proposed by Kelly (1971) (Equation 1):

$$\mathbf{ITU} = (\mathbf{1.8} \times \boldsymbol{T} + \mathbf{32}) - (\mathbf{0.55} - \mathbf{0.0055} \times \mathbf{UR})\ \mathbf{x}\ (\mathbf{1.8} \times \mathbf{T} - \mathbf{26}) \quad (1)$$

Where:

T: Temperature; and

RH: Relative humidity.

2.4 Assessment of the animals' respiratory rate, rectal temperature, and skin surface temperature

During each data collection period (8 days), respiratory rate and rectal temperature were collected on days 1, 3, and 5. Before the afternoon milking, at 1 pm, the animals' flank movements were observed for 30 s, with the animals in the pasture. The observed values were multiplied by two to obtain the number of respiratory movements per minute.

The animals' rectal temperature was taken by inserting an INCOTERM® model 6900.02 digital clinical thermometer into the rectum until the device beeped, obtaining the temperature in degree Celsius (°C). The temperature was measured at 3 pm, after the second milking of the day, with 48-h intervals between measurements.

The surface temperature of the skin was measured during the (8 days) data collection period at milking time. An infrared thermometer was positioned 0.30 m from the animal, pointing at the last rib. It was then directed at the skin of the udder where there were no signs of superficial veins and arteries.

2.5 Infrared thermography

Digital infrared thermographic photos were taken with a FLIR® C2 thermographic camera once for each collection period (8 days), at 2 pm. The photos were taken while the animals were grazing, focusing on the eye, neck, abdomen (rib), and udder regions to assess body temperature.

The emitting core of the thermographic camera was directed at a right angle and explored at a distance of 1 m from them. The thermographic icons (graphs describing the temperatures) were processed using software (FLIR Tools 2.1® – E40), applying polygons to measure the temperatures of the desired areas.

2.6 Collection of fresh milk samples to evaluate production and physicochemical quality

Fresh milk samples were taken. The samples represented the individual daily milk production of each animal (two-thirds in the morning milking + one-thirds in the afternoon milking) with the aid of individual meters attached to the clusters of liners. At the bottom of the meters, there is a valve that was set to the stirring function for 30 s before the samples were taken, thus homogenizing the milk correctly. The valve was then set to empty, and the milk was transferred from the meter to the collection bottles.

The milk samples were collected aseptically in plastic bottles of approximately 40 mL containing Bronopol® preservative for analysis of chemical composition, urea, casein, and somatic cell count (SCC). After collection, the milk samples were identified and packed in isothermal boxes containing ice and sent to the Milk Quality Laboratory of the Food Research Center of the Veterinary and Zootechnical School of the Federal University of Goiás for electronic evaluation.

The chemical composition of the milk was analyzed in terms of fat, protein, lactose, defatted dry extract (DDE), and total dry extract (TDE), which were determined using the analytical principle based on the differential absorption of infrared waves by the milk components, using the Milkoscan 4000 equipment (Foss Electric A/S. Hillerod, Denmark). The samples were previously heated in a water bath at 40°C for 15 min to dissolve the fat. The urea and casein contents were determined by the differential absorption of infrared waves, transformed by Fourier transform infrared spectroscopy (FTIR), using the Lactoscope equipment (Delta Instruments), and the results were expressed in mg per dL and percentage (%), respectively.

SCC analysis was carried out according to the analytical principle based on flow cytometry using the Fossomatic 5000 Basic (Foss Electric A/S. Hillerod, Denmark). Before analysis, the milk samples were previously heated in a water bath at 40°C for 15 min to dissolve the fat. The results were expressed in CS per mL.

The daily milk production of each animal was measured using the meters attached to the sets of liners, expressed in kg of milk per animal per day.

2.7 Evaluation of behavioral parameters

Daily observations were made of the animals in order to analyze their behavior in the sun and shade. The visual observations took place with the animals in the paddock before the afternoon milking at 1 pm. Visual assessments were carried out by just two people throughout the data collection period.

A spreadsheet was used to fill in the following categories and definitions for assessing the thermal comfort of the anials, adapted from Ferreira (2010): grazing animal: animal with its mouth close to the ground or grasping forage, being able to move slowly forward, but with its mouth below or at the top level of the pasture; standing ruminating: animal chewing, with dorsoventral and laterolateral jaw movements; in station; ruminating lying down: animal chewing, with dorsoventral and laterolateral jaw movements; sternal decubitus position; standing idle: animal in station without chewing movements; standing still; lying down: animal in the sternal or lateral decubitus position without chewing movements; lying still; walking: animal moving with its head above the top level of the pasture; moving without grasping food or chewing; drinking: animal with its lips submerged in water with throat movements characteristic of water ingestion; and mineralizing: animal licking or ingesting mineral supplements.

2.8 Scanning electron microscopy of milk

A sample pool of 2 L of milk from the animals in each treatment was collected every 15 days for scanning electron microscopy (SEM). The milk samples were freeze-dried, defatted, and sent to the High-Resolution Microscopy Multiuser Laboratory at the Physics Institute of the Federal University of Goiás for evaluation.

A Jeol® model JSM-6610 scanning electron microscope was used to evaluate the SEM, equipped with energy dispersive X-ray spectroscopy (EDS) Thermo Scientific NSS Spectral Imaging. The results are presented as images.

2.9 Statistical analysis

A cross-over design was used, with a 7-day adaptation interval between the two treatments and 8 days of data collection. The data were initially subjected to exploratory residual analysis.

Subsequently, analysis of variance, Pearson's correlation, and principal component analysis were carried out, using the ExpDes.pt, Hmisc, and FactoMine R and Factoextra packages, respectively. The data were analyzed using the computer program R. To interpret the correlation coefficient, the following classification was used: $r < .30$, low correlation; $r = .30–.70$, moderate correlation; and $r > .70$, high correlation.

The thermographic images were processed using FLIR Tools software to ensure greater precision and specificity in relation to the surface temperatures that were to be evaluated. Different processing methods were used, such as demarcating the region of interest and determining the average, maximum, and minimum temperatures. The circle measurement tool was used for the eye region, the line measurement for the neck region, and the square measurement for the abdomen and udder regions.

3 RESULTS AND DISCUSSION

The highest UTI was observed in animals kept in the sun, followed by animals in the shade and milking parlor (Table 1).

The UTI result of 75 for the animals kept in the sun indicates that they were in a stressful environment, which could be mild or moderate, while the UTI of 71 for the animals kept in the shade indicates that they were in normal climatic conditions without heat stress, as described by Armstrong (1994). Most researchers report that the UTI for dairy cattle above 72 results in significant negative effects, as do lower values. Johnson et al. (1962) reported a significant decline in milk production with UTIs above 70. Ravagnolo and Misztal (2002) indicated 68 as the upper critical UTI. Other authors have shown that variations in UTI values can have negative consequences on milk quality variables, even when UTI values are lower than those considered critical (Giustini et al., 2007).

The highest temperatures were observed in the afternoon in the unshaded paddock. The temperature at the time of measurement and the maximum temperature were higher when compared to the milking parlor and the shaded paddock; however, the minimum temperature in the milking parlor and the RH were higher in the shaded paddock.

Corroborating the results of this study, and taking into account that the animals were of the Girolando breed, Rocha et al. (2012) described that crossbred animals have a thermoneutrality zone of between 5°C and 31°C. The temperatures at the time of this study were higher but did not affect milk production.

Table 1. Average values and standard errors of the daily temperature and humidity index, ambient temperature at the time of measurement, maximum and minimum temperatures, and relative humidity in the milking parlor of animals kept in the shade and in the sun at different times of the day.

Period	**Variables**	**Treatment**			**CV (%)**
		Milking	**Sun**	**Shadow**	
Diary	ITU	68 c ± 0.25	75 a ± 0.42	71 b ± 0.33	6.30
Morning	Moment (°C)	19.61 b ± 0.43	26.09 a ± 0.69	19.52 b ± 0.90	27.02
	Maximum (°C)	33.77 c ± 0.54	47.53 a ± 0.63	40.38 b ± 0.68	12.05
	Minimum (°C)	14.21 a ± 1.15	9.63 b ± 0.73	10.75 b ± 0.77	64.06
	RH (%)	74.36 a ± 1.95	54.89 b ± 2.70	76.63 a ± 2.11	25.62
Afternoon	Moment (°C)	29.28 b ± 0.53	35.96 a ± 0.85	31.44 b ± 0.61	13.90
	Maximum (°C)	34.45 c ± 1.39	46.38 a ± 0.82	40.90 b ± 0.65	15.80
	Minimum (°C)	13.85 a ± 1.02	9.58 b ± 0.57	10.43 b ± 0.83	51.05
	RH (%)	37.90 a ± 2.19	27.60 c ± 2.52	32.90 b ± 2.59	52.07

Different letters in the line differ at the 5% probability level. CV: coefficient of variation; ITU: temperature and humidity index; RH: relative humidity.

In terms of behavior, the animals that were in the sun spent part of their time in the trough and grazing, with only a few lying down near the trough. As such, the animals preferred to feed, and because they were in the sun, it was expected that the feeding time would decrease, but most of the time spent standing was to facilitate heat exchange. Matarazzo et al. (2007) observed that more time spent standing indicates a situation of heat stress and cows try to keep more of their body surface area exposed. The fact that the animal remains standing when it is idle or ruminating can indicate thermal discomfort (Kendall et al., 2006).

During the research, the animals remained in the shaded paddock, which was made up of natural trees and shade clothes. Most of the time, they lay under the shade of the trees ruminating, and only a few grazed. Reinforcing what was observed in this study, Leão (1996) reported that animals seek the shade of trees rather than man-made structures. The shade offered by trees, whether isolated or in groups, is more effective, not only because it reduces the incidence of direct solar radiation but also because it reduces air temperature through the evaporation of leaves. In this way, the animals seek shade and reduce their activities during the hottest hours of the day by lying down in the resting area (Blackshaw & Blackshaw, 1994).

The standing or lying position of the cows can indicate whether the animal is in a comfortable situation. The animals in the shaded paddock in this study preferred to lie down during rumination time, indicating that they were in thermal comfort. According to Fraser and Broom (1990), cattle have four basic activities: movement, grazing, rumination, and idleness, and during idleness and rumination, the animals prefer to lie down.

In the present study, cows in the sun and shade did not suffer variations in milk yield, protein, casein, urea, lactose, electrostatic discharge (ESD), and SCC; however, rectal temperature, respiratory rate, milk fat content, and total suspended solids (TSS) did differ (Table 2).

Animals in the sun and shade had a daily production of 9.13 kg and 8.92 kg of milk, respectively, indicating that there was no impact on milk production. At low levels of milk production (for example, below 25 kg of milk per day), there is little impact from heat stress (Collier et al., 2019). According to Aguiar and Bacarri Junior (2003), medium- and high-production cows are more affected by high air temperatures, especially when associated with high humidity and intense solar radiation.

However, reduced dry matter intake is only responsible for 35–50% of the limitation in milk production; the remaining reduction may be attributed to changes in the endocrine profile, energy metabolism, and post-absorptive nutrient partitioning in heat-stressed cows Heat-stressed cows appear to have a reduced ability to mobilize adipose tissue and therefore rely on glucose as an energy source for peripheral tissue (Rhoads et al., 2009; Wheelock et al., 2010).

In the present study, rectal temperature and respiratory rate indicated that animals in both sun and shade treatments were not experiencing heat stress. This corroborates Marins et al. (2020), who reported that animals with a respiratory rate of 45–65 movements per minute and a rectal temperature of 38.4°C–38.6°C maintain normal appetite, reproduction, and production without stress. Also, according to Pires and Campos (2004), animals with a rectal temperature of between 38°C and 39.1°C have controlled heat stress and show no signs of altered food consumption, reproduction, or production. The respiratory rate and rectal temperature data observed indicate that in the environmental conditions to which the animals were subjected, the latent forms of heat loss were sufficient to maintain the rectal temperature within the limits considered normal.

Rectal temperature results within the normal thermal comfort range indicate that average UTI values between 71 and 75 did not cause stress to the animals. These results are in line with Lemerle and Goddard (1986), who reported an increase in rectal temperature for UTIs above 80.

Within the thermoneutral zone, a cow's body has a respiratory rate of around 23 respiratory movements per minute and a rectal temperature of around 38.3°C. In cases of severe stress, the respiratory rate can be close to 90 respiratory movements per minute and the rectal temperature at 40.1°C (Tavares & Salman, 2020). Roman-Ponce et al. (1977) reported that cows

Table 2. Mean values and standard errors of production, rectal temperature, respiratory rate, fat, protein, lactose, total dry extract, defatted dry extract, urea, casein, somatic cell count, and logarithm of the somatic cell count of milk from Girolando cows raised in sun and shade.

Parameters	Treatment		CV (%)
	Sun	Shadow	
Production (kg of milk per day)	9.13 ± 0.13	8.92 ± 0.131	29.18
Rectal temperature (°C)	38.25 a ± 0.07	37.99 b ± 0.04	2.15
Respiratory rate	59.44 a ± 1.09	49.94 b ± 0.98	25.08
Fat (g/100 g)	3.43 b ± 0.05	3.65 a ± 0.03	16.14
Protein (g/100 g)	3.26 ± 0.03	3.28 ± 0.04	11.19
Casein (g/100 g)	2.52 ± 0.01	2.55 ± 0.03	12.50
Urea (mg/dL)	10.00 ± 0.17	9.63 ± 0.18	28.12
Lactose (g/100 g)	4.27 ± 0.02	4.25 ± 0.04	5.80
TSS (g/100 g)	11.85 b ± 0.04	12.15 a ± 0.02	7.18
ESD (g/100 g)	8.43 ± 0.06	8.41 ± 0.03	8.39
SCC (SC/mL)	553.185 ± 42.422	597.829 ± 54.530	146.77

Different lowercase letters in the row differ at the 5% level, according to the F test. °C: Degree Celsius; CV: coefficient of variation; SC: somatic cell; SCC: somatic cell count; TSS: total soluble solids; ESD: estimated standard deviation.

receiving shade had lower respiratory rates (54 and 82 breaths per minute, respectively) and lower rectal temperatures (38.9°C and 39.4°C, respectively), and produced 10% more milk than cows without access to shade.

With regard to the physicochemical composition of the milk, the animals kept in natural shade produced milk with higher levels of fat and TSS, while the animals in the sun had lower levels of fat and TSS (Table 2).

The reduction in the fat and TSS levels of the animals kept in the sunny paddock may be related to the reduction in forage consumption due to heat stress. According to Bouraoui et al. (2002), a reduction in forage consumption can result in an inadequate level of fiber in the diet to maintain normal rumen functions. Total milk solids levels vary throughout the year, increasing in cold seasons and decreasing in hot seasons (Ponsano et al., 1999). In addition, the reduction in milk protein also seems to be attributable to restricted feed consumption (Kamiya et al., 2005).

Milk fat content decreases in heat-stressed cows due to a reduction in short-chain fatty acids, which constitute the majority of fatty acids in milk, and an increase in long-chain fatty acids. This can be explained by the lower consumption of forage and roughage by lactating cows, resulting in reduced acetic acid production and changes in the acetate/propionate ratio, thereby altering the composition of milk (Bernabucci & Calamari, 1998; Collier, 1985). Explaining the reduction in the fat content of animals kept in sunny paddocks, when they are under thermal stress from the heat, as already mentioned, whether mild or moderate, they reduce their consumption of dry matter and, consequently, the milk solids content varies.

In the present study, when the UTI value was 75 for the animals kept in the sun, the fat value was 3.43 g/100 g, and when the UTI was 71 for the animals in the shade, the fat value was 3.65 g/100 g. Therefore, as the UTI increased, the percentage of fat decreased. Abeni et al. (1993) reported that when the UTI was higher than 75, lower milk fat values were observed. When the UTI was less than 75, the milk fat content averaged 3.46 g/100 g, and when the UTI was 75 or more, the milk fat content averaged 3.17 g/100 g. Data on changes in protein and fat content as a result of thermal heat stress vary, with authors reporting a reduction, increase, or no change in the components (Knapp & Grummer, 1991; Lacetera et al., 2003; Roman-Ponce et al., 1977).

Similar to this study, Barbosa et al. (2004) found higher levels of fat in the milk of animals kept in the shade, as well as higher levels of SCC compared to those kept in the sun. The increased SCC was attributed to animals crowding under the shade, which increased the levels of environmental pathogens. Almeida et al. (2013), in a study evaluating the milk quality of mixed-breed cows with and without thermal comfort, observed that the average values of the milk components, fat, protein, lactose, total solids, and SCC showed no difference in the samples from the different treatments.

Table 3 shows the results of the skin and udder temperatures of cows raised in the sun and shade during the different periods.

It was observed that the cows' skin and udder temperatures varied due to the effect of the time of day, both in the shade and in the sun, but the highest temperature was in the udder. When evaluating the skin temperature parameter, there was variation between the treatments only in the morning, with the sun treatment resulting in 29.08°C and the shade 30.45°C.

The average skin temperatures in the sun and shade were 29.08°C and 35.17°C, respectively. According to Collier et al. (2006), for all heat exchange processes to occur normally, the skin surface of lactating cows must have temperatures below 35°C. According to Baccari Júnior (2001), cattle dissipate heat to the environment through their skin using the mechanisms of radiation, conduction, and convection, physical processes known as sensible heat loss. And, udder skin temperature increases during milking (Janeczek et al., 1995; Paulrud et al., 2005). Immediately after milking, the udder skin becomes highly sensitive to hypothermia (Janeczek et al., 1995).

The temperatures measured by the thermograph (average, maximum, and minimum) of the abdomen, eye, udder, and neck of the animals in the sun were higher compared to the animals in the shade. Differences were found for the average, maximum, and minimum abdomen; average eye; average, maximum, and minimum udder; and average neck (Table 4).

The animals were thermographed in the paddocks before afternoon milking, so the animals in the sun were exposed to higher temperatures compared to those in the shade, and the animals with black spots had higher temperatures as observed. However, depending on the region of the body and metabolic reactions, skin temperature can vary independently (Barnabé et al. 2015; Nikkhah, 2015; Stelletta et al., 2012). Remember that in the interaction between the external environment and the animal's body, the skin and hair act as a buffer layer (Jodkowska, 2000).

The highest average temperatures were observed in the abdomen (shade 36.8°C and sun 39.4°C). Body surface temperature

Table 3. Mean values and standard error for skin temperature and udder temperature of Girolando cows reared under sun and shade in the morning and afternoon.

Variables	Treatment			CV (%)
	Period	Sun	Shadow	
Skin (°C)	Morning	29.08 b ± 0.06	30.45 a ± 0.07	5.25
	Afternoon	35.15 ± 0.08	35.17 ± 0.09	4.75
Udder (°C)	Morning	31.90 ± 0.09	32.01 ± 0.10	6.95
	Afternoon	35.45 ± 0.08	35.52 ± 0.09	3.45

Different lowercase letters in the same row differ at the 5% level, according to the F test. CV: Coefficient of variation.

is influenced by tissue blood flow, metabolism, and evaporation through sweat. When measuring temperature, external factors (ambient temperature, RH, and wind action) must be taken into account (Barnabé et al., 2015; Nikkhah, 2015).

Navarini et al. (2009), in an experiment carried out in the municipality of Diamante Do Oeste, state of Paraná, evaluated the surface temperature of the back of Nelore females using infrared thermography, finding average values for three UTI values: 35.2°C with a UTI of 76, 34.3°C with a UTI of 78, and 34.7°C with a UTI of 80.

The average eye temperatures of animals kept in the shade were 36.6°C and in the sun 38.1°C. Considering the animal's standing and lying postures, heat loss by convection through direct contact with the ground is less likely for the head region (Jeelani et al., 2019). The ocular temperature is pointed out in the literature as the best point for observing stress using thermographic images due to the high level of vascularization of the posterior edge of the eyelid and changes in blood flow due to the action of the sympathetic system (Stewart et al., 2007). Therefore, the eye region is less likely to be influenced by secondary factors (e.g., contact with the ground while resting and immersion in water by parts of the body while lying down, which can fluctuate skin temperature). A smaller surface area of the head region contributes to less heat loss through sweat compared to other body regions (Jeelani et al., 2019).

Queiros et al. (2013), in a study measuring the surface temperature of mixed-breed cows by measuring the temperature of the eye using infrared thermography, observed an average of 37.8°C in the afternoon.

According to Johnson et al. (2011), the thermographic camera can measure eye temperature, which indicates the internal temperature of the animals. Therefore, environmental variables such as solar incidence and wind speed, as well as the distance from the camera, can negatively influence the results of eye temperature measurements (Church et al., 2014).

In this study, the average udder temperatures varied (shade 36.4°C and sun 37.3°C), similar to that found by Daltro (2014), which was 37.04°C, suggesting that the high temperature on the udder surface can be considered a heat sink due to the high blood demand.

Arcaro Júnior et al. (2005) evaluated the physiological parameters of Holstein cows raised in the municipality of Nova Odessa – SP, under a UTI of 73, considered critical, and observed changes in the rectal temperature (37.8°C), respiratory rate (48 movements per minute), as well as skin temperatures of the head (31.2°C), back (33.5°C), and mammary gland (32.8°C).

The average neck temperatures varied (shade 36.3°C and sun 37°C). Silva (2000) reported that, due to differences in the metabolic activity of the various tissues, the temperature is not homogeneous throughout the body and varies according to the anatomical region.

Figure 2 shows infrared thermography images of animals kept in a paddock exposed to the sun and a paddock with natural and artificial shade. The temperatures are described in each image.

Figure 3 shows the results of the correlation between the environmental and milk physicochemical variables and the thermographic temperatures of Girolando cows raised in the sun and shade.

Variables with similar behavior are grouped together, and circles that are not visible are points where the correlation is not significant. Red circles indicate a negative correlation, and the larger the circle and the stronger the color, the more intense the correlation. The blue circles are positive correlations; the thermographic temperatures formed a block of high positive correlations.

The middle abdomen had a high positive correlation with the middle udder ($r = .83$). Mazocco et al. (2017) found a positive correlation between the flank and udder ($r = .74$), showing that the surface temperature of the udder and flank varies with the ambient temperature.

The strong negative correlations observed in Figure 3 include afternoon milking temperature with afternoon milking humidity, average morning temperature with average morning humidity, maximum morning milking with minimum morning milking, average afternoon temperature with average afternoon humidity,

Table 4. Maximum, minimum, and average values for the temperature of the abdomen, eye, udder, and neck of Girolando cows raised in the sun and shade.

Variables	Treatments		*p*-value
	Sun	Shadow	
Maximum abdomen	42.7 ±1.04	40.4 ± 1.19	.0158
Minimum abdomen	35.9 ± 0.631	33.7 ± 0.733	.0006
Middle abdomen	39.4 ± 0.779	36.8 ± 0.894	.0006
Maximum eye	40.8 ± 0.913	39.4 ± 1.032	.0761
Small eye	36.3 ± 0.577	33.6 ± 0.629	2.528
Middle eye	38.1 ± 0.574	36.6 ± 0.635	.0007
Maximum udder	39.1 ± 0.535	37.9 ± 0.577	.0009
Minimum udder	35.9 ± 0.412	35.3 ± 0.439	.0165
Medium udder	37.3 ± 0.371	36.4 ± 0.406	.0004
Maximum neck	38.1 ± 0.452	37.5 ± 0.495	.0744
Minimum neck	36.4 ± 0.546	36.2 ± 0.609	.518
Middle neck	37.0 ± 0.308	36.3 ± 0.354	.0128

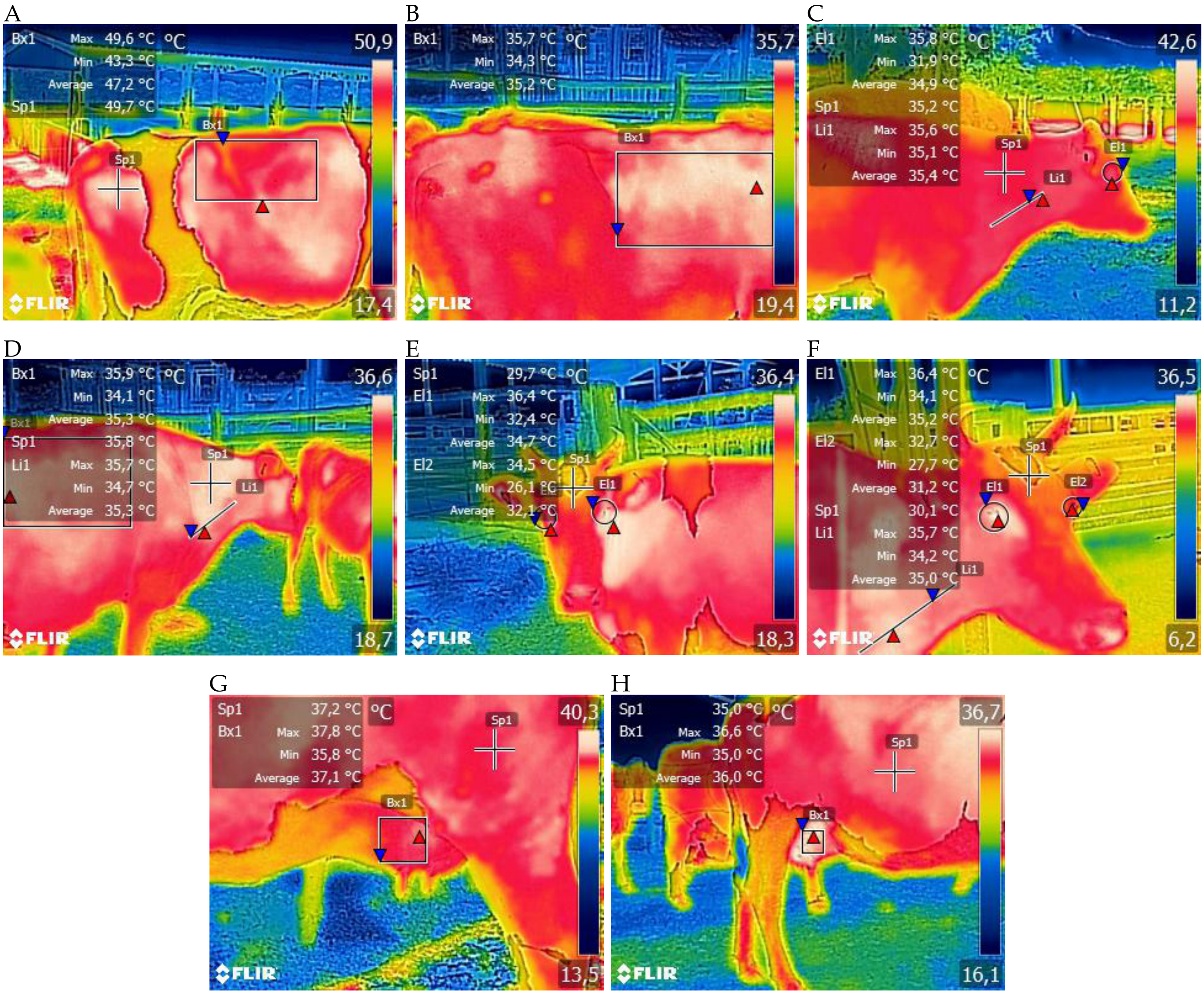

Figure 2. Infrared thermography images of cows raised in the sun and shade: Thermography images of the abdomen, neck, eye, and udder of the animals (A, C, E, G and B, D, F, H, respectively, of the animals raised in shaded and unshaded paddocks).

and afternoon milking with average afternoon humidity. Tao and Dahl (2013) pointed out that high RH can compromise the cow's ability to dissipate heat to the environment, causing heat stress.

With regard to the average and maximum UTIs in the morning and afternoon, the minimum eye temperature had a moderate positive correlation, the maximum udder temperature had a moderate correlation, and the other temperatures, average, maximum, minimum abdomen; minimum and average udder; average and maximum neck; and average and maximum eye, had a low correlation. The measurements vary simultaneously in the same direction, the physiological variables correspond to the increase in the region photographed, since the animals are trying to increase cooling by respiratory evaporation, and are in a panting state at times of greater environmental stress, in order to lose heat to regulate body temperature (Daltro, 2014).

There were a high positive correlation between UTIs and a moderate correlation between average morning and afternoon UTIs and maximum morning and afternoon UTIs with respiratory rate, rectal temperature, and average afternoon temperature. Azevedo et al. (2005) also reported a high positive correlation between respiratory rate and UTI, reporting correlation values of 0.73 between these variables. Respiratory rate showed a moderate positive correlation with rectal temperature (0.51) for Silva (2015). Avila et al. (2013) reported correlation values above 0.80 for the physiological variables of respiratory rate and surface temperature with UTI.

The rectal temperature had a moderate positive correlation with minimum (r = .63) and average (r = .61) udder, with average (r = .59), minimum (r = .63), and maximum (r = .54) eye, neck (r = .53), and maximum udder (r = .54). The moderate positive correlation with environmental variables is due to the fact that this variable has a higher correlation with the time of day than with the temperature variation in the environment (Baccari Júnior, 2001).

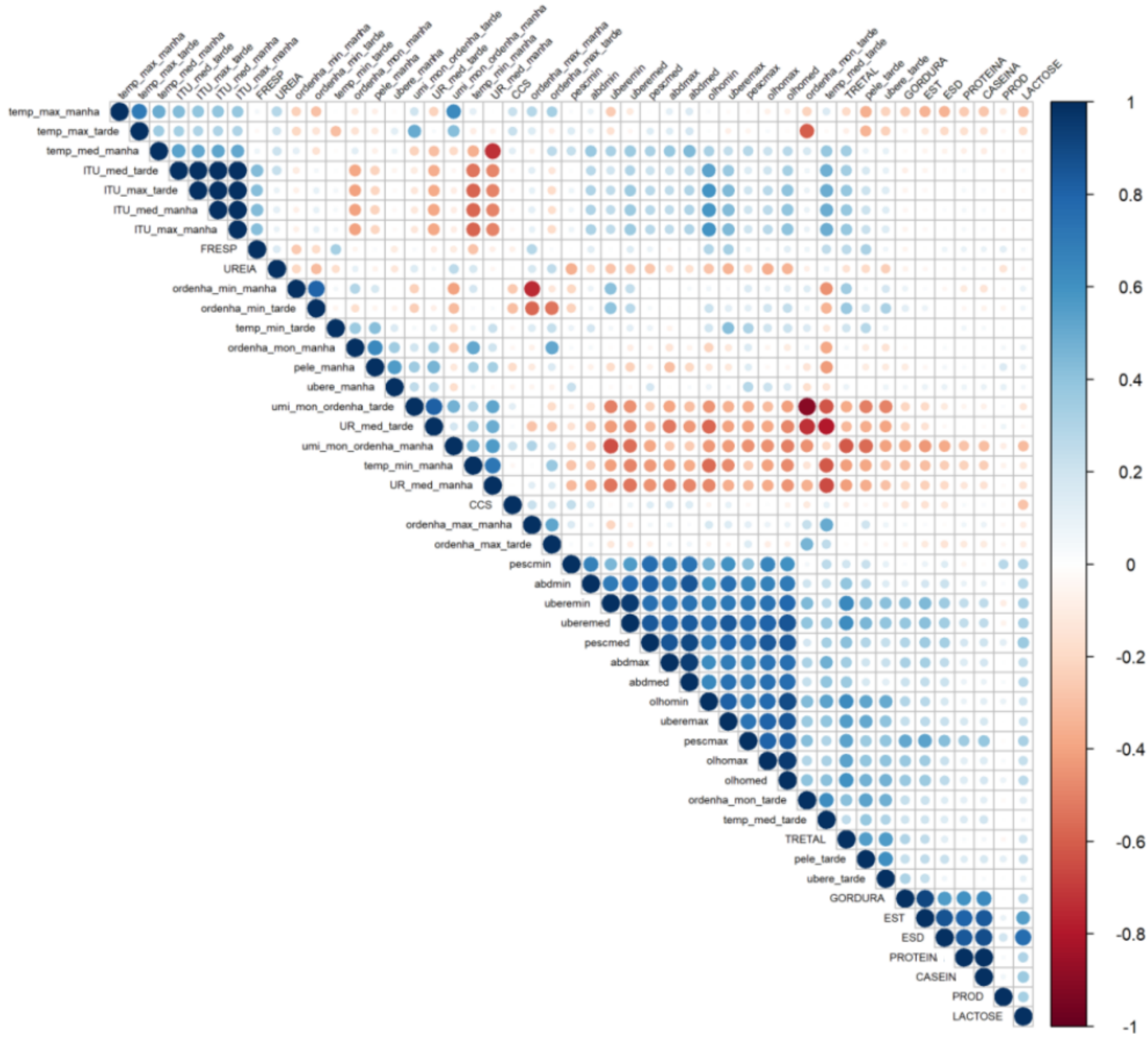

Figure 3. Correlation analysis of the measured variables.

The rectal temperature had a moderate positive correlation with afternoon skin temperature (*r* = .55) and udder temperature (*r* = .55). Amamou et al. (2019) observed variations in skin temperature on different farms during summer and fall, in which skin temperature was highly sensitive to UTI values with a correlation of 0.96. The skin is an important pathway for heat exchange, and skin temperature is the result of the regulation of this exchange between the skin and the core by blood flow. Silva et al. (2007) found positive correlation values ($p < 0.05$) for Holstein and Jersey cows in the states of Ceará and Rio Grande do Norte. Therefore, the animals absorb environmental heat, and the rise in skin temperature activates the homeostatic mechanism, leading to an increase in respiratory rate (Azevedo et al., 2005).

Azevedo et al. (2005), in a study of infrared thermography of the rear and front of the animals, evaluated animals from three genetic groups in the region of Coronel Pacheco – MG and observed a positive correlation between surface temperature and the variables of rectal temperature and respiratory rate.

Among the environmental and physiological variables, an increase in environmental variables tends to an increase in the animals' metabolism in order to maintain homeothermia. For some authors, respiratory rate is positively correlated with UTI and can be a valuable indicator of stress in cattle (Johnson et al., 2011; Kadzere et al., 2002; Zimbelman et al., 2010). Ferreira et al. (2006), when studying cattle in bioclimatological chambers, observed that rectal temperature, respiratory rate, and surface temperature tend to follow the ambient temperature, causing evaporative mechanisms to be activated.

With regard to milk quality, fat had a high positive correlation with TSS (*r* = .90) and a moderate correlation with ESD (*r* = .56), protein (*r* = .60), and casein (*r* = .63). TSS correlated with protein (r = .80) and ESD (r = .85). Peres (2001) stated that, in general, the percentage of protein in milk is positively correlated with the percentage of fat, and that the variation in total solids content is largely dependent on variations in milk fat content.

Protein had a high positive correlation with casein (r = .98). The high correlation between milk protein content and casein is due to the fact that casein accounts for an average of 80% of the total protein in bovine milk, as described by Holt (2016). Protein has a high effect on casein in the same way as ESD, which is the dry matter of milk except for fat, and any variation in ESD will affect the casein in milk (Cowley et al., 2015).

SCC correlated negatively with lactose. Similarly, Dias et al. (2015) also found a negative correlation between SCC and lactose. This correlation could be due to the changes in lactose content that occur due to the passage of lactose from the milk into the blood and the reduction in the glandular epithelium's ability to synthesize lactose as a result of lesions in the epithelium. According to Reis et al. (2013), lactose is the component of milk that decreases as SCC rises.

Contrary to what was observed in this study, some authors have found a positive correlation between fat percentage and SCC. For Pereira et al. (2004), the correlation may be due to increased vascular permeability, which determines the increased passage of substances from the blood to the milk, such as sodium, chlorine, immunoglobulins, and other serum proteins. Sabedot et al. (2011) observed that an increase in SCC values led to an increase in fat percentage.

According to Bueno et al. (2005), the increase in protein associated with an increase in SCC is due to the concentration of plasma proteins in the milk as a result of the inflammatory response, although casein suffers a significant reduction due to the action of leukocyte and blood proteases.

In the present study, protein showed a negative correlation with maximum and minimum temperatures. Nakamura et al. (2012) also found negative correlations, and Depeters and Ferguson (1992) found that high environmental temperatures are factors that reduce the total protein content of milk, also influencing the consistency of the milk clot, which in turn affects quality.

The principal component analysis of the environmental, milk physicochemical, and thermographic temperature variables showed that the first component explained 29.6% and the second 13% of the total variation in the data. Figure 4 shows the principal component analysis using the first two components.

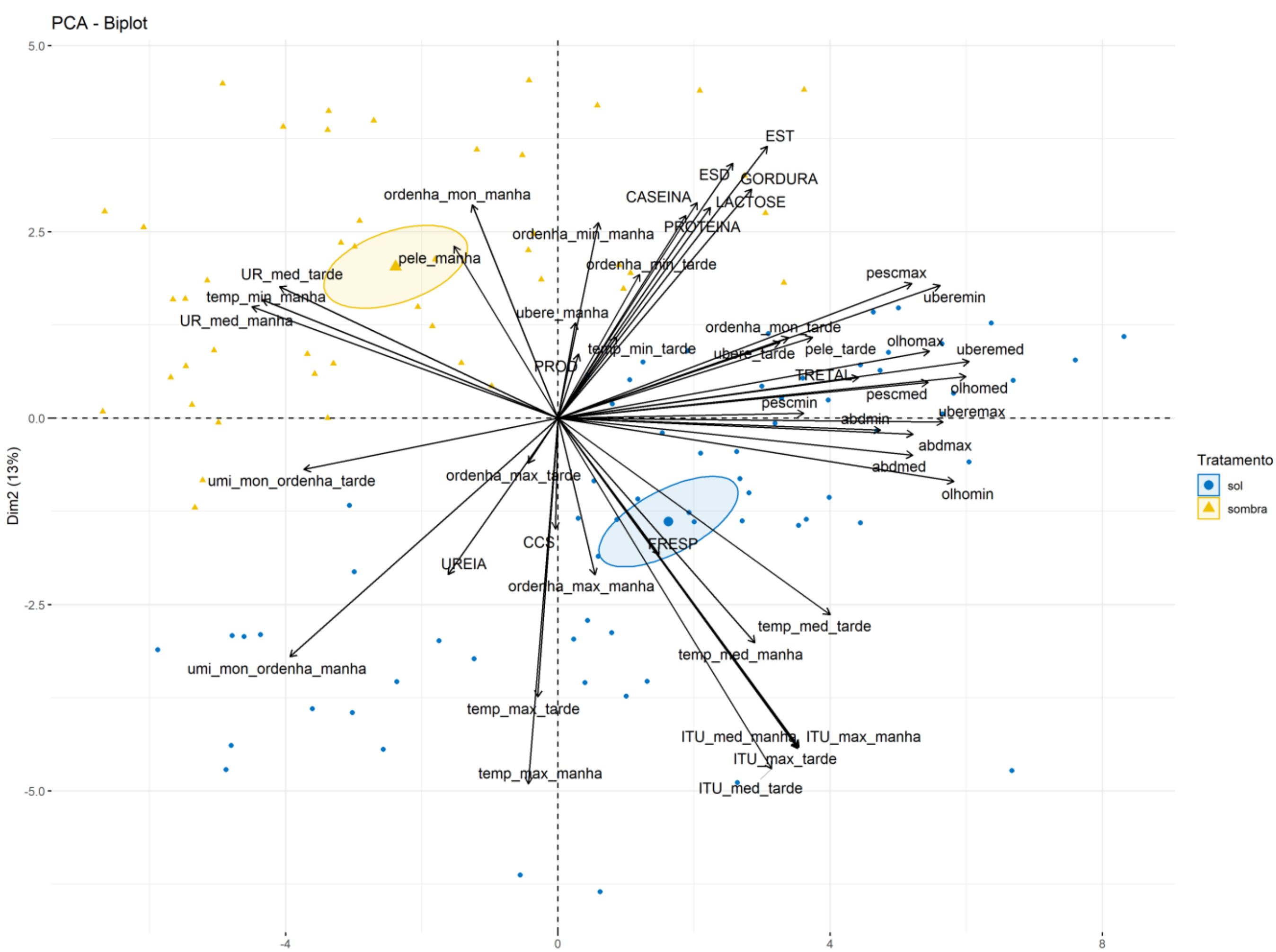

Figure 4. Principal component analysis of the measured variables.

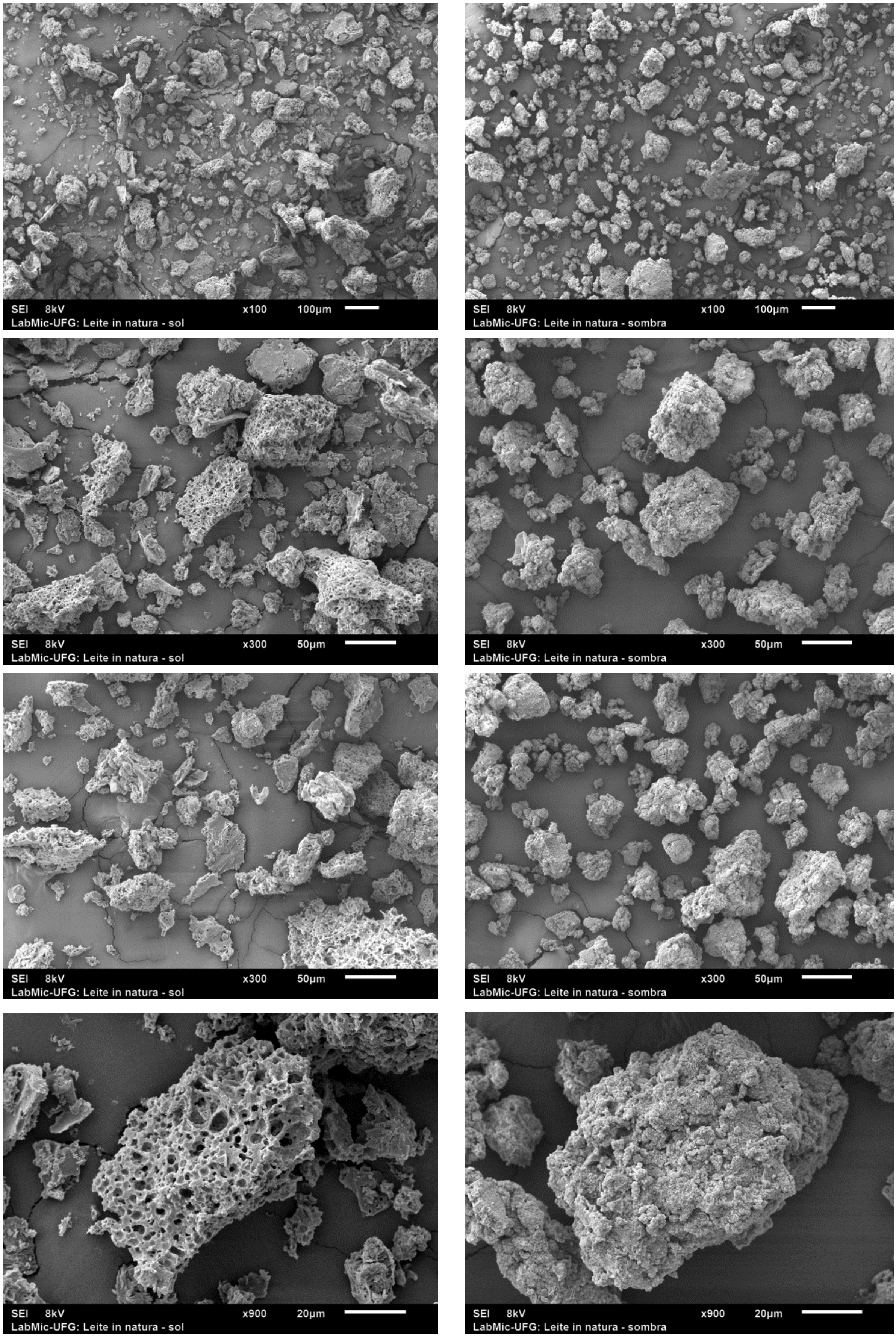

Figure 5. Scanning electron microscopy of milk from Girolando cows kept in the sun and shade.

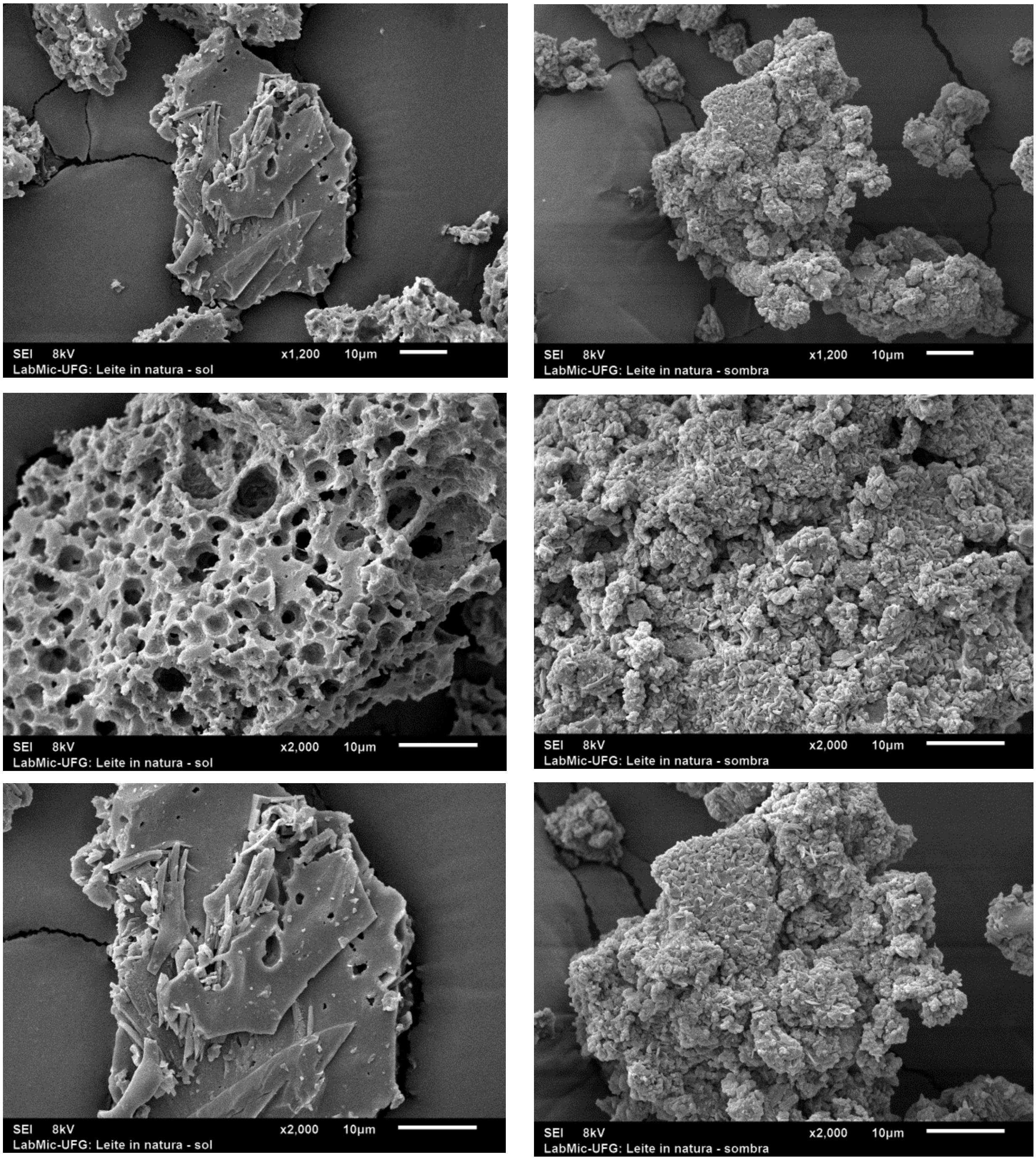

Figure 5. Continuation

The contrast between the treatments can be seen in the variables analyzed. The variables of rectal temperature, maximum and average neck temperature, average, maximum, and minimum abdomen, eye and udder temperature, minimum morning temperature, and average morning and afternoon humidity showed the greatest displacement on the 0 axis of the first principal component, indicating that they showed the greatest variability.

In the second component, the variables of mean and maximum UTI in the morning and afternoon, maximum temperature in the morning and afternoon, and TSS showed the greatest variability. The thermal environment is known to have a strong influence on animal performance since it affects heat transfer mechanisms and thus the regulation of the thermal balance between the animal and the environment.

However, the physiological variables as shown in the graph were higher in the sun animals, but it should be borne in mind that variations in rectal temperature and respiratory rate can be influenced by both intrinsic factors (age, breed, and physiological state) and extrinsic factors (time of day, food and water intake, ambient temperature, wind speed, and season) (Perissinotto et al., 2009).

However, the environmental and physiological variables that showed the greatest variability did not affect the production and physicochemical quality of the milk during the experimental period from May to early September 2020.

Structural analysis of milk from cows kept in the sun and shade showed particles with a relatively homogeneous distribution at 100 × magnification. At 300 × magnification, the sun cows had a more spongy structure compared to the cows kept in the shade (Figure 5).

The structural difference in the micrographs at 300× magnification may be the result of the treatments needed to assess the physical structure of the milk samples. Schmidt and Buchheimi (1992) reported that during technological treatments, the microparticulate constituents of milk, i.e., fat globules, colloidal casein micelles, and the molecular dispersion of whey proteins, undergo significant physical changes and mutual interactions. This gives rise to the characteristic macroscopic structure and physical properties of the products. Therefore, electron microscopy of dairy products is extremely useful for elucidating the relationship between the macroscopic properties of the product and its submicroscopic structure altered by technological treatments.

The spongy structures observed in the milk of the sun animals, at 900 × and 2000 × magnifications, are characteristic of protein agglomerates, with vacuoles that indicate the absence of fat due to sample degreasing for SEM analysis. At 1200 × and 2000 × magnifications, the structures observed correspond to lactose crystals.

4 CONCLUSION

Temperatures above the animal's thermoneutrality zone negatively affect production and well-being, as well as reduce milk constituents. Animals kept in the sun and shade can be affected by average temperatures, as well as maximum and minimum temperatures and the UTI. A higher UTI increases the degree of heat stress to which the animals are subjected and can therefore affect the physiological and productive characteristics of the animals, regardless of breed.

The areas of the body that were thermographed showed different temperatures in the same data collection, showing physiological variations in temperature that help in the clinical assessment of each area, recommending the use of infrared thermography as a complementary test, with the abdomen being the area with the best temperature observation in this study.

There was a correlation between the milk constituents of the animals that were subjected to shaded and unshaded areas, and between the physiological and environmental variables, but this did not affect milk production.

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REFERENCES

Abeni, F., Calamari, L., Maianti, M. G., Cappa, V., & Stefanini, L. (1993). Effetti dello stress termico sulle bovine in lattazione ed accorgimenti alimentari miranti ad attenuarne l'impatto su quantità e qualità del latte prodotto. *Annali UCSC (Piacenza)*, *23*, 151–170.

Aguiar, I. S., & Baccari Junior, F. (2003). Respostas fisiológicas e produção de leite de vacas holandesas mantidas ao sol e com acesso a sombra natural [Physiological responses and milk production of Holstein cows kept in the sun and with access to natural shade]. *Electronic Scientific Journal of Veterinary Medicine*, *1*, 1–4. http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/GkGEPa9bT6f3OpK_2013-5-13-17-0-17.pdf

Almeida, G. L. P., Pandorfi, H., Barbosa, S. B. P., Pereira, D. F., Guiselini, C., & Almeida, G. A. P. (2013). Behavior, production and milk quality of Holstein-Gir cows under acclimatization in the corral. *Brazilian Journal of Agricultural and Environmental Engineering*, *17*(8), 892–899. https://doi.org/10.1590/S1415-43662013000800014

Amamou, H., Beckers, Y., Mahouachi, M., & Hammami, H. (2019). Thermotolerance indicators related to production and physiological responses to heat stress of holstein cows. *Journal of Thermal Biology*, *82*, 90–98. https://doi.org/10.1016/j.jtherbio.2019.03.016

Arcaro Júnior, I., Arcaro, J. R. P., Pozzi, C. R., Del Fava, C., Fagundes, H., Matarazzo, S. V., & Oliveira, J. E. (2005). Physiological responses of dairy cows to fan plus sprinkler in holding pen. *Ciência Rural*, *35*(3), 639–643. https://www.redalyc.org/pdf/331/33135324.pdf

Armstrong, D. (1994). Symposium: Nutrition and heat Stress – Heat stress interaction with shade and cooling. *Journal of Dairy Science*, *77*(7), 2044–2050. https://ia800108.us.archive.org/view_archive.php?archive=/24/items/wikipedia-scholarly-sources-corpus/10.3168.zip&file=10.3168%252Fjds.S0022-0302%252894%252977149-6.pdf

Atrian, P., & Shahryar, H. A. (2012). Heat Stress in Dairy Cows (A Review). *Research in Zoology*, *2*(4), 31–37. https://doi.org/10.5923/j.zoology.20120204.03

Avila, A. S., Jácome, I. M. T. D., Faccenda, A., Panazzolo, D. M., & Müller, E. R. (2013). Evaluation and correlation of physiological parameters and bioclimatic indexes holstein cows in different seasons. *Electronic Journal on Environmental Management, Education and Technology*, *14*(14), 2878–2884. https://doi.org/10.5902/2236117010747

Azevedo, M., Pires, M. F. A., Saturnino, H. M., Lana, Â. M. Q., Sampaio, I. B. M., Monteiro, J. B. N., & Morato, L. E. (2005). Estimation of upper critical levels of the temperature-humidity index for ½, 3/4 e 7/8 lactating Holstein-Zebu dairy cows. *Revista Brasileira de Zootecnia*, *34*(6), 2000–2008. http://doi.org/10.1590/S1516-35982005000600025

Baccari Júnior, F. (2001). *Manejo ambiental da vaca leiteira em climas quentes* [Environmental management of dairy cows in hot climates]. Editora da Universidade Estadual de Londrina.

Barbosa, O. R., Boza, P. R., Santos, G. T., Sakagushi, E. S., & Ribas, N. P. (2004). Efeitos da sombra e da aspersão de água na produção de leite de vacas da raça Holandesa durante o verão [Effects of shade and water sprinkling on milk production of Holstein cows during summer]. *Acta Scientiarum Animal Sciences*, *26*(1), 115–122. https://pdfs.semanticscholar.org/3104/95599be9be98b8ae169383d59d731a2e9baf.pdf

Barnabé, J. M. C., Pandorfi, H.,Almeida, G. L. P., Guiselini, C., & Jacob, A. L. (2015). Thermal comfort and performance Holstein/Gir calves housed in individual shelters with different covers. *Brazilian Journal of Agricultural and Environmental Engineering*, *19*(5), 481–488. https://doi.org/10.1590/1807-1929/agriambi.v19n5p481-488

Bernabucci, U., & Calamari, L. (1998). Effects of heat stress on bovine milk yield and composition. *Zootecnica e Nutrizione Animale*, *24*(6), 247–257. Effects of heat stress on bovine milk yield and composition

Blackshaw, J. K., & Blackshaw, A. W. (1994). Heat stress in cattle and the effect of shade on production and behaviour: a review. *Australian Journal of Experimental Agriculture*, *34*(2), 285–295. https://doi.org/10.1071/EA9940285

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M., & R. Belyea. (2002). The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Animal Research*, *51*(6), 479–491. https://doi.org/10.1051/animres:2002036

Bueno, V. F. F., Mesquita, A. J., Nicolau, E. S., Oliveira, A. N., Oliveira, J. P., Neves, R. B. S., Mansur, J. R. G., & Thomaz, L. W. (2005). Somatic cell count: relationship to milk composition and period of the year in Goiás State, Brazil. *Ciência Rural*, *35*(4), 848–854. https://doi.org/10.1590/S0103-84782005000400016

Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev--Shoshani, G., Schaefer, A. L., & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. *Research in Veterinary Science*, *96*(1), 220–226. https://doi.org/10.1016/j.rvsc.2013.11.006

Collier, R. J. (1985). Nutritional, metabolic, and environmental aspects of lactation. In B. L. Larson (Ed.), *Lactation* (pp. 103–110). Iowa State University Press.

Collier, R. J., Baumgard, L. H., Zimbelman, R. B., & Xiao, Y. (2019). Heat stress: physiology of acclimation and adaptation. *Animal Frontiers*, *9*(1), 12–19. https://doi.org/10.1093/af/vfy031

Collier, R. J., Dahl, G. E., & VanBaale, M. J. (2006). Major advances associated with environmental effects on dairy cattle. *Journal of Dairy Science*, *89*(4), 1244–1253.

Cowley, F. C., Barber, D. G., Houlihan, A. V., & Poppi, D. P. (2015). Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science*, *98*(4), 2356–2368. https://doi.org/10.3168/jds.2014-8442

Cruz, L. V., Angrimani, D. S. R., Rui, B. R., & Silva, M. A. (2011). zEfeitos do estresse térmico na produção leiteira: revisão de literatura. *Revista Científica Eletrônica de Medicina Veterinária*, *9*(16). https://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/3Kbw8tpmIaJpspv_2013-6-26-10-55-41.pdf

Daltro, D. S. (2014). *Uso da termografia infravermelha para avaliar a tolerância ao calor em bovinos de leite submetidos ao estresse térmico* [Master's dissertation, Universidade Federal do Rio Grande do Sul]. LUME Repositório Digital. https://lume.ufrgs.br/handle/10183/95988

Deitenbach, A., Floriani, G. S., Dubois, J. C. L., & Vivan, J. L. (Eds.). (2008). *Manual Agroflorestal para a Mata Atlântica*. Ministério do Desenvolvimento Agrário. https://biowit.wordpress.com/wp-content/uploads/2010/11/manual_agroflorestal_da_mata_atlantica.pdf

DePeters, E. J., & Ferguson, J. D. (1992). Nonprotein nitrogen and protein distribution in the milk of cows. *Journal of Dairy Science*, *75*(11), 3192–3209. https://doi.org/10.3168/jds.S0022-0302(92)78085-0

Dias, M., Assis, A. C. F., Nascimento, V. A., Saenz, E. A. C., & Lima, L. A. (2015). Seasonal variation of milk components and program of payment for quality. *Enciclopédia Biosfera*, *11*(21), 1112–1127. https://www.researchgate.net/publication/365368393_SAZONALIDADE_DOS_COMPONENTES_DO_LEITE_E_O_PROGRAMA_DE_PAGAMENTO_POR_QUALIDADE_SEASONAL_VARIATION_OF_MILK_COMPONENTS_AND_PROGRAM_OF_PAYMENT_FOR_QUALITY

Dikmen, S., & Hansen, P. J. (2009). Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? *Journal of Dairy Science*, *92*(1), 109–116. https://doi.org/10.3168/jds.2008-1370

Ferreira, F., Pires, M. F. A., Martinez, M. L., Coelho, S. G., Carvalho, A. U., Ferreira, P. M., Facury Filho, E. J., & Campos, W. E. (2006). Physiologic parameters of crossbred cattle subjected to heat stress. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *58*(5), 732–738. https://doi.org/10.1590/S0102-09352006000500005

Ferreira, L. C. B. (2010). *Respostas fisiológicas e comportamentais de bovinos submetidos a diferentes ofertas de sombra* [Master's thesis, Universidade Federal de Santa Catarina]. Biblioteca EMATER-DF. http://hdl.handle.net/123456789/221

Fraser, A. F., & Broom, D. M. (1990). *Farm Animal Behavior and Welfare* (3rd ed.). Baillière Tindall.

Giustini, L., Acciaioli, A., Pianaccioli, L., Surace, R., & O. Franci. (2007). Thermic stress effect on the main qualitative parameters of milk produced in the Mugello. *Scienza e Tecnica Lattiero-Casearia*, *58*(6), 383–402. https://www.cabidigitallibrary.org/doi/full/10.5555/20083063393

Holt, C. (2016). Casein and casein micelle structures, functions and diversity in 20 species. *International Dairy Journal*, *60*, 2–13. https://doi.org/10.1016/j.idairyj.2016.01.004

Janeczek, W., Chudoba-Drozdowska, B., Samborski, Z., & Kusz, A. (1995). Skin temperature changes of the cow mammary gland and the heat flux from its surface before and after milking. *Archivum Veterinarium Polonicum*, *35*(1–2), 35–44. https://pubmed.ncbi.nlm.nih.gov/9071450/

Jeelani, R., Konwar, D., Khan, A., Kumar, D., Chakraborty, D., & Brahma, B. (2019). Reassessment of temperature-humidity index for measuring heat stress in crossbred dairy cattle of a sub-tropical region. *Journal of Thermal Biology*, *82*, 99–106. https://doi.org/10.1016/j.jtherbio.2019.03.017

Jodkowska, E., (2000) Analiza zmian temperatury na powierzchni nóg koni podczas treningu wyścigowego. *Folia Universitatis Agriculturae Stetinensis Zootechnica*, *40*, 277–284. https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-article-4f406d5b-515e-4b56-ac23-9be67ac2e9e0

Johnson, H. D., Ragsdale, A. C., Berry, L. L., & Shanklin, M. D. (1962). *Effect of various temperature-humidity combinations on milk production of Holstein cattle* (Research Bulletin No. 846). Missouri Agricultural Experiment Station.

Johnson, S. R., Rao, S., Hussey, S. B., Morley, P. S., & Traub-Dargatz, J. L. (2011). Thermographic eye temperature as an index to body temperature in ponies. *Journal of Equine Veterinary Science*, *31*(2), 63–66. https://doi.org/10.1016/j.jevs.2010.12.004

Kadzere, C. T., Murphy, M. R., Silanikove, N., & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*, *77*(1), 59–91. https://doi.org/10.1016/S0301-6226(01)00330-X

Kamiya, M., Iwama, Y., Tanaka, M., & Shioya, S. (2005). Effects of high ambient temperature and restricted feed intake on nitrogen utilization for milk production in lactating Holstein cows. *Animal Science Journal*, *76*(3), 217–223. https://doi.org/10.1111/j.1740-0929.2005.00259.x

Kendall, P. E., Nielsen, P. P., Webster, J. R., Verkerk, G. A., Littlejohn, R. P., & Matthews, L. R. (2006). The effects of providing shade to lactating dairy cows in a temperate climate. *Livestock Science*, *103*(1–2), 148–157. https://doi.org/10.1016/j.livsci.2006.02.004

Kelly, C. F. (1971). Bioclimatic factors and their measurement. In Nacional Research Council (Ed.), *A Guide to Environmental Research on Animals* (pp. 77–92). National Academy of Sciences.

Knapp, D. M., & Grummer, R. R. (1991). Response of lactating dairy cows to fat supplementation during heat stress. *Journal of Dairy Science*, *74*(8), 2573–2579. https://doi.org/10.3168/jds.s0022-0302(91)78435-x

Lacetera, N., Bernabucci, U., Ronchi, B., & Nardone, A. (2003). Physiological and productive consequences of heat stress. The case of dairy ruminants. In N. Lacetera, U. Bernabucci, H. H. Khalifa, B. Ronchi, & A. Nardone (Eds.), *Interactions between climate and animal production: EAAP Technical Series No.7* (pp. 45–60). Wageningen Academic Publishers.

Leão, J. F. M. (1996). Pastagens dos haras exigem arborização adequada. *A Lavoura*, *99*, 18–19.

Leão, J. M., Lima, J. A. M., Pôssas, F. P., & Pereira, L. G. R. (2015). Uso da termografia infravermelha na pecuária de precisão. *Cadernos Técnicos de Veterinária e Zootecnia*, *79*, 97–109. https://www.alice.cnptia.embrapa.br/alice/handle/doc/1037882

Lemerle, C., & Goddard, M. E. (1986). Assessment of heat stress in dairy cattle in Papua New Guinea. *Tropical Animal Health and Production*, *18*(4), 232–242. https://doi.org/10.1007/bf02359540

Marins, T. N., Almeida, I. G. B., Lôbo, B. V., Pessoa, C. M. B., Teixeira, R. C., Alves, B. G., & Gambarini, M. L. (2020). Stress and thermal comfort indexes associated with physiological parameters and energy status in Girolando cows raised on pasture in the tropical savannah. *Research, Society and Development*, *9*(7), Article e111973672. https://doi.org/10.33448/rsd-v9i7.3672

Matarazzo, S. V., Silva, I. J. O., Perissinoto, M., Fernandes, S. A., Moura, D. J., Arcaro Júnior, I., & Arcaro, J. R. (2007). Electronic monitoring on behavioral patterns of dairy cows in a cooling free stall. Revista Brasileira de Engenharia de Biossistemas, 1(1), 40–49. https://doi.org/10.18011/bioeng2007v1n1p40-49

Mazocco, L. A., Souza, K. A. R., Souza, A. C. B., Silva, P. M. R. S., Ferreira, I. C., & Pimentel, C. M. M. (2017, July 30–August 2). *Efeitos do sombreamento sobre a temperatura de superfície corporal obtida por termografia e sua associação com a produção de leite* [Paper presentation]. VII Congresso Brasileiro de Biometeorologia, Jaboticabal, SP, Brazil. https://doi.org/10.6084/m9.figshare.5178535.v1

Nakamura, A. Y., Alberton, L. R., Otutumi, L. K., Donadel, D., Turci, R. C., Agostinis, R. O., & Caetano, I. C. S. (2012). Correlação entre as variáveis climáticas e a qualidade do leite de amostras obtidas em três regiões do Estado do Paraná. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*, *15*(2), 103–108. https://www.bvs-vet.org.br/vetindex/periodicos/arquivos-de-ciencias-veterinarias-e-zoologia-da-un/15-%282012%29-2/correlacao-entre-as-variaveis-climaticas-e-a-qualidade-do-leite-de-amo/

Nascimento, G. V., Cardoso, E. A., Batista, N. L., Souza, B. B., & Cambuí, G. B. (2013). Indicadores produtivos, fisiológicos e comportamentais de vacas de leite. *Agropecuária Cientifica no Semiárido*, *9*(4), 28–36. https://doi.org/10.30969/acsa.v9i4.349

Navarini, F. C., Klosowski, E. S., Campos, A. T., Teixeira, R. A., & Almeida, C. P. (2009). Conforto térmico de bovinos da raça nelore a pasto sob diferentes condições de sombreamento e a pleno sol. *Engenharia Agrícola*, *29*(4), 508–517.

Nikkhah, A. (2015). Infrared Thermography as a Prognostic Livestock Agrotechnology: A Critique. *Agrotechnology*, *4*(1), Article e112. https://doi.org/10.4172/2168-9881.1000e112

Paulrud, C. O., Clausen, S., Andersen, P. E., & Rasmussen, M. D. (2005). Infrared thermography and ultrasonography to indirectly monitor the influence of liner type and overmilking on teat tissue recovery. *Acta Veterinaria Scandinavica*, *46*(3), 137-147. https://doi.org/10.1186/1751-0147-46-137

Pereira, P. C., Paixão, C., Grossi, B. A., & Telles, M. P. C. (2004, May 28–31). *Avaliação da qualidade do leite amostrado em diferentes épocas do ano* [Conference session]. XIV Congresso Nacional de Zootecnia, Brasília, DF, Brazil.

Peres, J. R. (2001). O leite como ferramenta do monitoramento nutricional. In F. H. D. González, J. W. Dürr, & R. S. Fontaneli (Eds.), *Uso do leite para monitorar a nutrição e o metabolismo de vacas leiteiras* (pp. 30–45). Biblioteca Setorial da Faculdade de Medicina Veterinária da UFRGS.

Perissinotto, M., Moura, D. J., Cruz, V. F., Souza, S. R. L., Lima, K. A. O., & Mendes, A. S. (2009). Thermal comfort on Subtropical and Mediterranean climate analyzing some physiological data through fuzzy theory. *Ciência Rural*, *39*(5), 1492–1498. https://doi.org/10.1590/S0103-84782009005000094

Pires, M. F. A., & Campos, A. T. (2004). *Comunicado Técnico 42: Modificações ambientais para reduzir o estresse calórico em gado de leite*. Embrapa. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/594946

Ponsano, E. H. G., Pinto, M. F., Lara, J. A. F., & Piva, F. C. (1999). Sazonal variations and correlation among properties of the milk quality control. *Higiene Alimentar*, 13(64), 35–39.

Queiros, A. F., Nogueira, F. R. B., Araújo, M. P. R., Nascimento, M. E. L., & Souza, B. B. (2013). Termografia como ferramenta de avaliação do estresse térmico em vacas leiteiras. *Caderno Verde de Agroecologia e Desenvolvimento Sustentável*, *3*(1), 2358–2367. https://www.gvaa.com.br/revista/index.php/CVADS/article/view/1943

Ravagnolo, O., & Misztal, I. (2002). Effect of heat stress on nonreturn rate in Holsteins: fixed-model analyses. *Journal of Dairy Science*, *85*(11), 3101–3106. https://doi.org/10.3168/jds.s0022-0302(02)74397-x

Reis, A. M., Costa, M. R., Costa, R. G., Suguimoto, H. H., Souza, C. H. B., Aragon-Alegro, L. C., Ludovico, A., & Santana, E. H. W. (2013). Effect of the racial group and number of lactation on the productivity and composition of bovine milk. *Semina: Ciências Agrárias*, *33*(6Supl2), 3421–3436. https://doi.org/10.5433/1679-0359.2012v33n6Supl2p3421

Rhoads, M. L., Rhoads, R. P., VanBaale, M. J., Collier, R. J., Sanders, S. R., Weber, W. J., Crooker, B. A., & Baumgard, L. H. (2009). Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *Journal of Dairy Science*, *92*(5), 1986–1997. https://doi.org/10.3168/jds.2008-1641

Rocha, D. R., Salles, M. G. F., Moura, A. A. A. N., & Araújo, A. A. (2012). Índices de tolerância ao calor de vacas leiteiras no período chuvoso e seco no Ceará. *Revista Acadêmica Ciência Animal*, *10*(4), 335–343. https://doi.org/10.7213/academica.7739

Roman-Ponce, H., Thatcher, W. W., Buffington, D. E., Wilcox, C. J., & Van Horn, H. H. (1977). Physiological and production responses of dairy cattle to a shade structure in a subtropical environment. *Journal of Dairy Science*, *60*(3), 424–430. https://doi.org/10.3168/jds.S0022-0302(77)83882-4

Sabedot, M. A., Pozza, M. S. S., Pozza, P. C., Almeida, R. Z., Nunes, R. V., & Eckstein, I. I. (2011). Correlação entre contagem de células somáticas, parâmetros microbiológicos e componentes do leite em amostras de leite in natura. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*, *14*(2), 101–106. https://revistas.unipar.br/index.php/veterinaria/article/view/4142

Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W., & van Keyserlingk, M. A. G. (2012). Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science*, *95*(6), 3212–3217. https://doi.org/10.3168/jds.2011-4741

Schmidt, D. G., & Buchheimi, W. (1992). The application of electron microscopy in dairy research. *Journal of Microscopy*, *167*(1), 105–121. https://doi.org/10.1111/j.1365-2818.1992.tb03223.x

Schütz, K. E. Rogers, A. R., Poulouin, Y. A., Cox, N. R., & Tucker, C. B. (2010). The amount of shade influences the behavior and physiology of dairy cattle. *Journal of Dairy Science*, *93*(1), 125–133. https://doi.org/10.3168/jds.2009-2416

Silva, D. C. (2015). *Índices de conforto térmico, variáveis fisiológicas e desempenho produtivo de vacas leiteiras em sala de espera climatizada* [Master's dissertation, Universidade Estadual de Goiás]. Biblioteca Digital de Teses e Dissertações da UEG. http://www.bdtd.ueg.br/handle/tede/70

Silva, G. A., Souza, B. B., Peña Alfaro, C. E., Azevedo Neto, J., Azevedo, S. A., Silva, E. M. N., & Silva, R. M. N. (2006). Influência da dieta com diferentes níveis de lipídeo e proteína na resposta fisiológica e hematológica de reprodutores caprinos sob estresse térmico. *Ciência e Agrotecnologia*, *30*(1), 154–161. https://doi.org/10.1590/S1413-70542006000100022

Silva, R. G. (2000). *Introdução à bioclimatologia animal* (1st ed.) [Introduction to animal bioclimatology]. Nobel.

Silva, R. G., Morais, D. A. E. F., & Guilhermino, M. M. (2007). Evaluation of thermal stress indexes for dairy cows in tropical regions. *Revista Brasileira de Zootecnia*, *36*(4), 1192–1198. https://doi.org/10.1590/S1516-35982007000500028

Staples, C. R, & Thatcher, W. W. (2011). Heat Stress: Effects on Milk Production and Composition. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences* (2nd ed., Vol. 4, pp. 328–334). Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.21237-7

Stelletta, C., Gianesella, M., Vencato, J., Fiore, E., & Morgante, M. (2012). Thermographic Applications in Veterinary Medicine. In R. V. Prakash (Ed.), *Infrared Thermography* (pp. 117–140). IntechOpen. http://doi.org/10.5772/29135

Stewart, M., Stafford, K. J., Dowling, S. K., Schaefer, A. L., & Webster, J. R. (2008). Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. *Physiology and Behavior*, *93*(4–5), 789–797. https://doi.org/10.1016/j.physbeh.2007.11.044

Tao S., & Dahl, G. E. (2013). Invited review: Heat stress effects during late gestation on dry cows and their calves. *Journal of Dairy Science*, *96*(7), 4079–4093. https://doi.org/10.3168/jds.2012-6278

Tavares, F. A. S., & Salman, A. K. D. (2020). Aptidão leiteira dos bovinos na Amazônia. In A. K. D. Salman & L. F. M. Pfeifer (Eds.), *Pecuária Leiteira na Amazônia* (pp. 69–87). Embrapa.

Wheelock, J. B., Rhoads, R. P., VanBaale, M. J., Sanders, S. R., & Baumgard, L. H. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science*, *93*(2), 644–655. https://doi.org/10.3168/jds.2009-2295

Zimbelman, R. B., Baumgard, L. H., & Collier, R. J. (2010). Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. *Journal of Dairy Science*, *93*(6), 2387–2394. https://doi.org/10.3168/jds.2009-2557