



## THERMOREGULATORY AND BEHAVIOUR RESPONSES OF DAIRY HEIFERS RAISED ON A SILVOPASTORAL SYSTEM IN A SUBTROPICAL CLIMATE

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### Abstract

In this study, we investigated the influence of a silvopastoral system on the thermal environment, behaviour and thermoregulation of dairy heifers in a subtropical climate. The experiment was conducted on a dairy farm in Southwest Paraná, Brazil, during the summer of 2014. Crossbred Holstein × Jersey dairy heifers ( $n = 10$ ) were used in a split-plot design. The fixed effects in this study include time of day (9:00–10:00, 13:00–14:00, and 17:00–18:00 h) under silvopastoral or open pastures conditions, which were the main-plot and split-plot factors, respectively. To assess the environmental conditions in both systems, air temperature, relative humidity and wind speed were recorded. In addition, the temperature-humidity index was calculated based on the microclimate measurements. Respiratory rate and hair coat surface temperature of heifers were measured in both groups during time of day (30 days). Diurnal behaviour using focal observations with 0/1 sampling was observed. Data were analysed using Bayesian inference with a mixed effects model. The air temperature was higher ( $P < 0.05$ ) in open pasture conditions than the silvopasture system. Temperature-humidity index values for the silvopasture system were lower than open pasture during the hottest hours of the day. Regarding thermoregulation responses, there was an interaction between time of day and pasture environment ( $P < 0.05$ ). Heifers showed lower respiratory rates and hair coat surface temperature values ( $P < 0.05$ ) when access to shade was provided, mainly during the hottest periods in the afternoon. In addition, there was also an interaction between rumination and water intake ( $P < 0.05$ ), which indicates a higher rumination frequency for animals in the silvopasture system during the hottest period, as well as a higher water intake frequency for heifers in open pasture during midday. These findings imply that the arrangement of trees in a silvopasture system provides better thermal comfort conditions for dairy heifers raised in a subtropical climate.

**Key words:** animal welfare, biometeorology, dairy cow, shading, thermal comfort

Recent studies have focused on identifying best management practices to improve thermal comfort and welfare of thermally stressed livestock (Varella et al., 2016; Angrecka and Herbut, 2016). Low productivity in tropical and subtropical climates is caused by various factors such as inadequate management of reproduction and nutrition under adverse climatic conditions. Therefore, heifers' performance is highly variable, as their production and survival are linked to the level of stress caused by the environment (Collier et al., 2006; Baumgard and Rhoads Jr., 2013).

Silvopastoral (SP) systems have been suggested as a thermal comfort strategy for cattle breeding in relation to open pastures (OP) (Peri et al., 2016). The same authors stated that SP systems involve the insertion of tree species, together with pasture, in the same location, resulting in wood, quality feed for the animal, and improvement in thermal environment. Karki and Goodman (2015) verified a 30–66% reduction in wind speed and 32–63% reduction in total solar radiation in a loblolly-pine silvopasture in Florida (USA). In a research carried out by Karvatte Jr. et al. (2016) in Central West Brazil, higher values for air temperature and black globe temperature were noted in an environment with dispersed trees, which demonstrates the influence of densely shaded environments in relation to pasture with few trees. Concerning relative humidity, the same authors found higher values in an SP system as compared to an environment with dispersed trees. Moreover, these microclimatic modifications promoted by a system of integration between trees and pasture may alter animal behaviour. Mello et al. (2017) observed behavioural alterations in dairy heifers in an SP system in Central West Brazil during the rainy seasons, as grazing level was higher in a system with moderate shade in relation to OP at the hottest times of the day.

Previous studies have discussed the relationship between shade and milk production (Ainsworth et al., 2012), radiation interception in SP systems (Porfirio-da-Silva et al., 2004; Feldhake, 2009), microclimate, and behaviour of dairy cattle in shaded areas (Karki and Goodman, 2010; 2015; Karvatte Jr. et al., 2016). However, there is a lack of knowledge about heifers raised in a SP system in a subtropical climate (Lopes et al., 2016; Mello et al., 2017), thus highlighting the importance of new studies aiming to elucidate microclimatic variations in this environment and its possible effect on thermoregulation of these animals. Therefore, the study aim was to assess the effects of the production system (OP vs. SP) in a subtropical climate on thermal conditions and thermoregulatory responses of dairy heifers. We hypothesized that SP systems can improve thermal conditions, with a consequent reduction of the respiratory rate and hair coat surface temperature of heifers during uncomfortable periods, which allow some desirable behaviours, such as rumination, grazing and water intake.

## Material and methods

All procedures performed in this study were consistent with the Brazilian legislation on animal welfare and met the protocol requirements of animal research of the Ethics Committee for Animal Use of Federal University of Technology – Paraná

(UTFPR). Data collection was performed during usual cattle handling routine procedures.

The study was carried out in summer season (January 2014) on a farm with an SP system in the city of Realeza ( $25^{\circ}46'S$ ,  $53^{\circ}31'W$ , 480 m altitude), Southwest Paraná State, South Brazil. Based on Köppen climate classification, it is characterized as Cfa or humid subtropical, with hot summers and coldest month temperatures between  $-3$  and  $18^{\circ}C$ . The hottest month is equal to or greater than  $22^{\circ}C$ , and average annual rainfall is between 1,900 and 2,200 mm (Alvares et al., 2013).

The SP system was established in 2008 in a pasture area of 12.1 hectares. *Eucalyptus grandis* W. Hill. ex Maiden trees were planted in the area, with a spacing of  $35.0 \times 2.0$  m or 35 m between the lines and 2 m between plants. Between the three lines, a pasture of *Cynodon dactylon* (Tifton 68) was sown. In the experimental area, three plantation lines of *E. grandis* were planted, with 36, 24, and 15 trees planted in the first, second, and third line, respectively.

Table 1. Descriptive statistics (mean  $\pm$  standard deviation) of the dendrometric variables used in the forest inventory

Dendrometric variables	Planting lines		
	1	2	3
Number of selected individuals	6	6	5
Height (m)	$23.9 \pm 3.5$	$26.2 \pm 7.6$	$23.2 \pm 6.1$
Diameter at breast height (cm)	$31.4 \pm 5.9$	$26.4 \pm 6.1$	$25.8 \pm 7.6$
Canopy diameter (m)	$7.5 \pm 1.1$	$8.7 \pm 2.1$	$6.6 \pm 1.9$

A forest inventory of the area was carried out, whereby diameter at breast height (cm), height (m), canopy diameter (m) and spacing between trees (m) were measured. Then the area with trees to be used for data collection was selected, these being sampled following a stratified random sample, based on the height classes. Each of the selected trees was 6 years old. In the first line of the plantation, six trees were selected, with six from the second line and five from the third (Table 1).

Crossbred Holstein  $\times$  Jersey dairy heifers ( $n = 10$ ) were selected from a dairy herd according to their age (between 12 and 15 months) and weight ( $270 \pm 30$  kg). This number of animals can be justified due to the availability of animals for trial purpose in the commercial farm, aiming a minimum interference of the herd handle. Half of the animals were randomly assigned to the control group (i.e., OP treatment), which were maintained in a paddock of *C. dactylon* (Tifton-68) of approximately  $1,360$  m $^2$ . The second treatment group (i.e., SP treatment), with the same size as the open pasture group, remained within a paddock with natural shade, provided by 36 *E. grandis* trees. Both groups had access to water troughs spread around the area.

Data collection included the main environment variables as well as the main thermoregulation variables. All data were collected at three different times of day in each treatment group (OP and SP): 09:00–10:00, 13:00–14:00, and 17:00–18:00 h, during 30 days throughout January 2014.

Table 2. Timely descriptive statistics of different microclimatic variables from open pasture (OP) and silvopasture system (SP) during the experiment, January 2014, city of Realeza, Paraná State, Brazil

Time (h)		Microclimatic variables									
		Air temperature (°C)				Relative humidity (%)				Wind speed (m s <sup>-1</sup> )	
		OP	SP	OP	SP	OP	SP	OP	SP	OP	SP
9:00	Mean ± SEM	24.7±0.78	22.9±0.64	86±2.31	81±8.43	4.7±1.19	3.44±1.06	75±1.2	72±1.4		
	Minimum	21.8	20.3	76	24	0.2	0.0	70	64		
	Median	24.4	22.5	87	88	4.8	3.3	75	73		
	Maximum	28.9	26.6	95	97	9.1	8.6	81	78		
13:00	Mean ± SEM	29.8±1.08	28.0±1.03	73±2.95	75±3.46	5.3±1.07	2.8±0.82	82±1.3	79±1.3		
	Minimum	24.1	22.8	62	66	0.9	0.4	75	73		
	Median	30.2	28.0	72	74	5.7	1.7	83	80		
	Maximum	33.9	31.7	89	94	9.5	6.1	86	84		
17:00	Mean ± SEM	31.6±0.87	30.5±0.86	66±3.48	67±3.0	4.9±1.12	5.1±1.60	83±1.0	82±0.9		
	Minimum	27.4	26.3	54	57	0.6	0.6	79	77		
	Median	32.1	31.1	62	65	5.1	3.8	84	83		
	Maximum	34.1	33.6	80	80	9.1	12.2	86	85		

The evaluated variables include air temperature ( $^{\circ}\text{C}$ ), relative air humidity (%), and wind speed ( $\text{km h}^{-1}$ ). All measurements were collected at 1.50 m above the ground, at intervals of 30 min, near the trees (in the SP treatment) or in the center of the paddock (OP treatment). To measure air temperature and relative humidity, a thermo-hygrometer MTH-I380 was used, with sensor protection against direct solar radiation (resolution from -10 to  $60^{\circ}\text{C}$  and 0 to 100% relative humidity; accuracy of  $0.5^{\circ}\text{C}$  and 5% relative humidity). The wind speed was recorded using a thermo-anemometer HTA-400 (resolution from 0 to 100  $\text{km h}^{-1}$ ; accuracy of 0.001  $\text{km h}^{-1}$ ). The temperature-humidity index (THI) was calculated based on the microclimate measurements, using the equation proposed by Hahn (1999):  $\text{THI} = 0.81 T_{db} + (RH/100) (T_{db} - 14.4) + 46.4$ , where  $T_{db}$  = daily average dry bulb temperature ( $^{\circ}\text{C}$ ) and  $RH$  is the relative humidity. In accordance with the same author, the THI's values intervals were considered for this study: normal,  $\leq 74$ ; alert, 75–78; danger, 79–83; emergency,  $\geq 84$ . The variation throughout the time of day and pasture systems is shown in Table 2.

With regards to thermoregulation of heifers, the collected variables include respiratory rate (RR, breaths  $\text{min}^{-1}$ ) and hair coat surface temperature (MST,  $^{\circ}\text{C}$ ). RR was measured by counting the number of movements of an animal's flank for 15 seconds and then multiplied by four to compute the respirations per minute. MST was measured using an infrared thermometer (Instrutherm model TI-870, resolution from  $-50^{\circ}\text{C}$  to  $550^{\circ}\text{C}$ ; accuracy of  $0.5^{\circ}\text{C}$ ), with a 1-m distance from the five points (front, neck, scapula, thigh, and flank) of an animal's body; the average temperature measurement was then calculated. All thermoregulatory measures in the SP group were collected in the silvopasture system when the animals were in the shade (i.e., below to the canopy shade projection).

Diurnal behaviour using focal observations with 0/1 sampling was observed, accordingly with Broom and Fraser (2007), during the same time intervals of microclimatic measures. A 10 min observation was intercalated with a 30 min interval among observations. An ethogram was used, adapted from Karki and Goodman (2010), as described in Table 3.

For statistical analysis, a split-plot design was used to test the effects of: (1) time of day (9:00–10:00, 13:00–14:00, and 17:00–18:00) and (2) SP and OP pasture environments, which were the main-plot and split-plot factors, respectively. The animals were considered experimental units in this trial. Using Bayesian inference with a mixed effects model, time of day and pasture environments were considered fixed effects, whereas the 30-day evaluation was considered a random effect.

Analyses of thermal environment data, thermoregulation and behaviour were conducted via Bayesian inference. The main reason for choosing this method was based on the lower number of experimental units. According to McNeish (2016), the Bayesian method is a very appropriate and accurate means of estimation for small samples, due to the use of iterative methods, such as the Gibbs sampler. This allows realizing  $N$  simulations with this limited dataset. In addition, the same author discussed other features of this approach, such as the random parameters and fixed data, and the inclusion of prior information, which might increase the accuracy of the prediction.

Table 3. Behavioral ethogram and respective definitions for dairy heifers (adapted from Karki and Goodman, 2010)

Behavior	Definition
Rumination	Regurgitating or rechewing food
Water intake	Ingesting water from drinking troughs
Walking	Displacement, in the pasture area
Interaction within animals	Any movement or contact with other animals
Rest	Lying or standing, without performing any activity
Grazing	Feeding on herbaceous vegetation

For the data analysis of the thermal environment (air temperature, relative air humidity and wind speed), as well as the THI, the variables of interest (Y) showed normal distribution with parameters  $\mu$  and  $\sigma$ . The model considered was:

$$Y_{ki} \sim (\mu, \sigma)$$

$$\mu = \alpha + \beta * X_{ki} + \pi * X^2_{ki} + \rho * X^3_{ki} + u_k + \varepsilon_{ki}$$

$$\alpha \sim \text{Normal}(0, 0.001)$$

$$\beta \sim \text{Normal}(0, 0.001)$$

$$\pi \sim \text{Normal}(0, 0.001)$$

$$\rho \sim \text{Normal}(0, 0.001)$$

$$\sigma \sim \text{Cauchy}(0, 5)$$

where  $X$  refers to the polynomial (first, second and third order) time of day which was verified the value of Y,  $u_k$  the is the random effect of the days, and the indices  $i$  and  $K$  refers to the pasture environment and day, respectively. For the analysis of behavioural data, the variable of interest (Y) follows a Poisson distribution with parameter  $\lambda$ . The model considered for each behaviour within each treatment was:

$$Y_{ki} \sim \text{Poisson}(\lambda_i)$$

$$\text{Log}(\lambda_i) = \alpha + \beta * X_{ki} + \pi * X^2_{ki} + \rho * X^3_{ki} + u_k + \varepsilon_{ki}$$

$$\alpha \sim \text{Normal}(0, 0.001)$$

$$\beta \sim \text{Normal}(0, 0.001)$$

$$\pi \sim \text{Normal}(0, 0.001)$$

$$\rho \sim \text{Normal}(0, 0.001)$$

where  $X$  refers to the polynomial (first, second and third order) time of day when the behaviour was verified, is the random effect of the days, and the indices  $i$  and  $K$  refers to the pasture environment and day, respectively. All data were analysed using the R software package using brms (Bürkner, 2017).

## Results

No interaction was observed between the time of day and pasture environments on microclimatic variables ( $P>0.05$ ). The pasture environment did not have any effect ( $P>0.05$ ) on relative humidity, THI and wind speed. In addition, time of day only influenced the relative humidity ( $P<0.05$ ). However, a difference between the pasture environments was observed for air temperature and THI (Table 4).

Table 4. A posteriori estimate of parameters (mean  $\pm$  SD and credibility intervals) of air temperature and temperature-humidity index (THI) of silvopasture system (SP) and open pasture (OP) throughout the day

Parameter	Percentile (Temperature)		Percentile (THI)	
	2.50%	97.50%	2.50%	97.50%
Intercept	18.85	24.25*	-15.30	31.20 NS
Hour	1.26	2.15*	1.40	2.70*
Treatment	-3.23	-1.04*	-5.68	-1.61*
Hour*Treatment	-0.10	0.41 NS	-0.12	0.81 NS

\*Statistically different based on Bayesian comparisons ( $P < 0.05$ ); NS = not significant.

Table 5. A posteriori estimate of parameters (mean  $\pm$  SD and credibility intervals) of respiratory rate and hair coat surface temperature of dairy heifers in silvopasture system (SP) and open pasture (OP) throughout the day

Parameter	Mean $\pm$ deviation	Percentile		Significance
		2.50%	97.50%	
<b>Respiratory rate:</b>				
intercept	-25.93 $\pm$ 13.62	-51.2	1.57	NS
hour	22.89 $\pm$ 3.73	14.79	29.77	*
treatment	1.94 $\pm$ 5.11	-7.72	12.02	NS
hour*treatment	-6.53 $\pm$ 2.41	-11.15	-1.90	*
<b>Hair coat surface temperature:</b>				
intercept	28.31 $\pm$ 0.81	26.7	29.9	*
hour	2.62 $\pm$ 0.36	1.90	3.36	*
treatment	0.66 $\pm$ 0.74	-0.79	2.11	NS
hour*treatment	-1.03 $\pm$ 0.34	-1.69	-0.35	*

\*Statistically different based on Bayesian comparisons ( $P < 0.05$ ); NS = not significant.

A reduction in air temperature (average of 26.7°C) in the SP system was observed in relation to the OP system (average of 27.1°C) ( $P < 0.05$ ). Otherwise, despite the difference between the environments, the SP system showed a higher amplitude (13.3°C) than the OP system (12.3°C). Concerning the THI, during morning, the average value for OP was 75, classified as alert for dairy heifers and normal in the SP system (THI = 72). During afternoon, in both groups the THI values reached the danger classification (varying from 79 to 83).

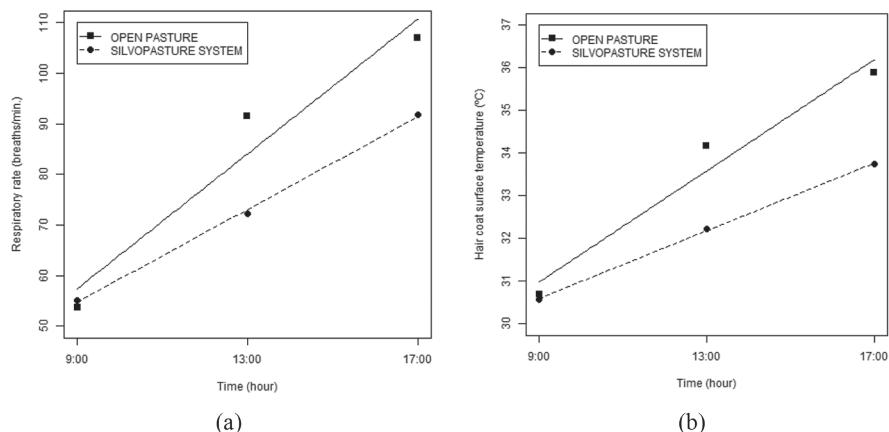


Figure 1. Respiratory rate (a) and hair coat surface temperature (b) of dairy heifers in open pasture and silvopasture system throughout the day

For RR and MST variables, there was an interaction between the time of day and pasture environments ( $P<0.05$ ) (Table 5). During the 13:00 to 14:00 interval, an average difference of 20 breaths per minute was observed in the RR between the animals in each group (72 breaths  $\text{min}^{-1}$  in SP vs. 92 breaths/ $\text{min}$  in OP) (Figure 1a).

Table 6. A posteriori estimate of parameters (mean  $\pm$  SD and credibility intervals) of ingesting water and rumination of dairy heifers in silvopasture system (SP) and open pasture (OP) throughout the day

Parameter	Mean $\pm$ deviation	Percentile		Significance
		2.50%	97.50%	
<b>Ingesting water:</b>				
intercept	-3.63 $\pm$ 0.60	-4.86	-2.50	*
hour	0.32 $\pm$ 1.03	0.07	0.58	*
hour <sup>2</sup>	-0.02 $\pm$ 0.01	-0.03	-0.001	*
treatment 2	1.05 $\pm$ 0.76	-0.45	2.52	NS
hour*treatment 2	-0.52 $\pm$ 0.20	-0.90	-0.14	*
hour <sup>2</sup> *treatment 2	0.03 $\pm$ 0.01	0.01	0.05	*
<b>Rumination:</b>				
intercept	-2.31 $\pm$ 0.39	-3.10	-1.59	*
hour	0.16 $\pm$ 0.10	-0.03	0.36	NS
hour <sup>2</sup>	-0.01 $\pm$ 0.01	-0.02	0.002	NS
treatment 2	-2.19 $\pm$ 0.65	-3.47	-0.91	*
hour*treatment 2	0.61 $\pm$ 0.16	0.31	0.90	*
hour <sup>2</sup> *treatment 2	-0.03 $\pm$ 0.008	-0.04	-0.01	*

Hour<sup>2</sup> = quadratic effect of hour; treatment 2 = silvopasture system; \*statistically different based on Bayesian comparisons ( $P < 0.05$ ); NS = not significant.

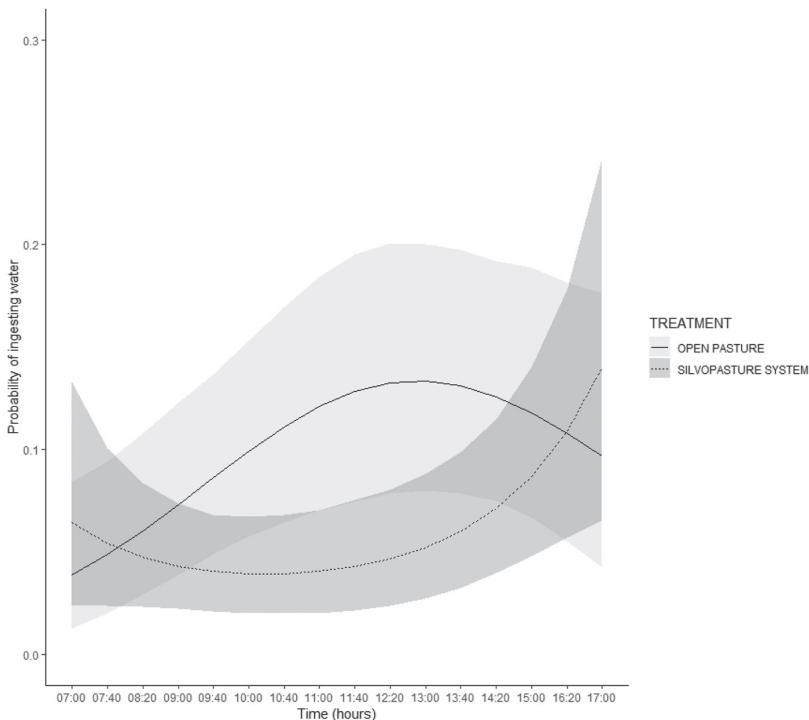


Figure 2. Probability of the occurrence of water intake behavior of dairy heifers in open pasture (—) and silvopasture (....) during the experimental period

As with RR variations, during the hottest hours, a difference between the treatment groups for hair coat surface temperature was noted. From 13:00 to 14:00 and 17:00 to 18:00, the heifers in the SP system presented lower values (72 and 92 breaths  $\text{min}^{-1}$ , respectively) as compared to animals in the OP system (92 and 107 breaths  $\text{min}^{-1}$ , respectively). During the hottest period (13:00–14:00), a  $2.0^\circ\text{C}$  MST difference was noted between the two treatment groups. In addition, no differences were observed between the groups in the morning. However, from 13:00 to 14:00 and 17:00 to 18:00, an increased variation between the groups was observed, wherein animals in the OP system presented higher MSTs ( $34.2$  and  $35.9^\circ\text{C}$ , respectively) as compared to heifers in the SP system ( $32.2$  and  $33.7^\circ\text{C}$ , respectively) (Figure 1b).

Regarding heifers' behaviour, no interaction ( $P>0.05$ ) was found between time intervals and pasture environments on both interaction within animals and rest behaviour. The time of day had effect ( $P<0.05$ ) only for walking and grazing behaviour. For water intake and rumination, an interaction between time of day (quadratic effect) and treatment groups was observed (Table 6).

During the midday period, with increased air temperatures (Table 2), an increased water intake was observed in the OP group (20% probability), when compared with the SP group (5% probability), where heifers reduced the water intake at the same time interval (Figure 2).

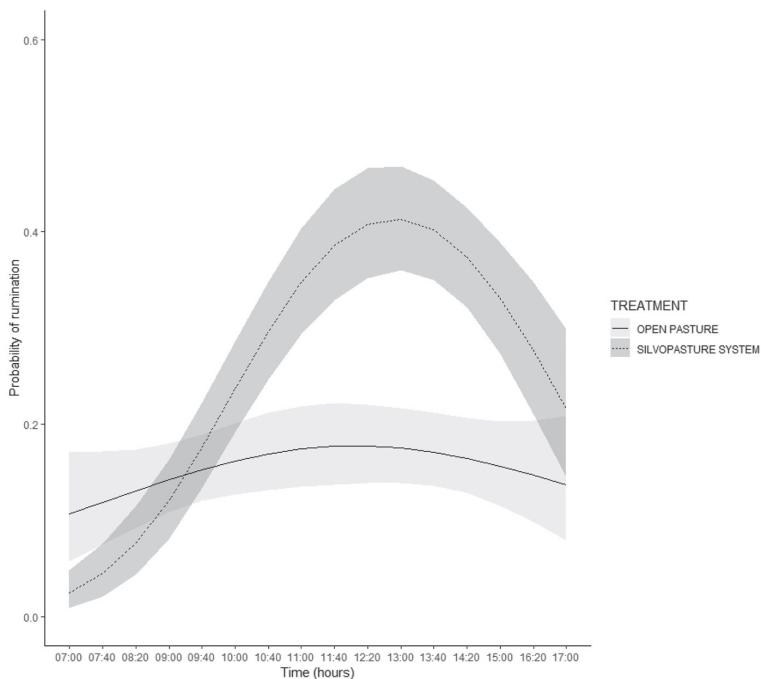


Figure 3. Probability of the occurrence of rumination behavior of dairy heifers in open pasture (—) and silvopasture (....) during the experimental period

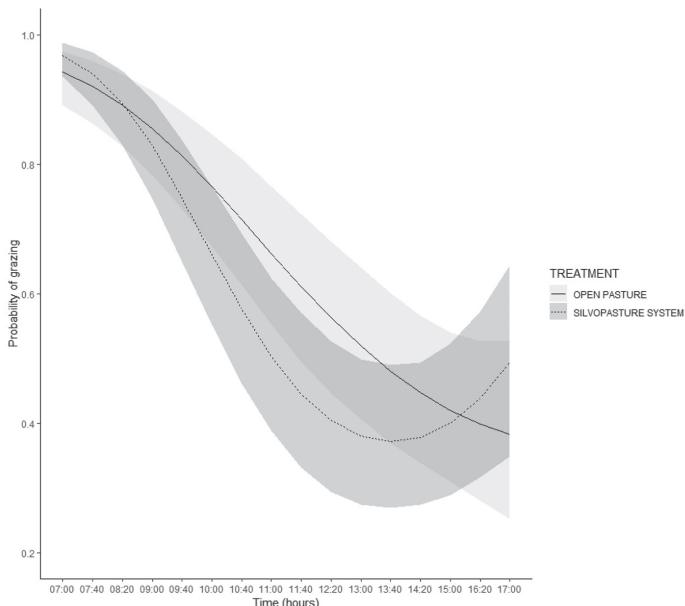


Figure 4. Probability of the occurrence of grazing behavior of dairy heifers in open pasture (—) and silvopasture (....) during the experimental period

Rumination during the hottest periods of the day (11:00 to 13:40) was increased in heifers in the SP system, with 40% of occurrence probability in this period. In OP treatment, the rumination practically remained constant during the diurnal period (Figure 3). A higher probability of grazing behaviour in both groups (Figure 4) was observed during the first hours of the day, which are characterized by the best thermal conditions (Table 2), with strong decrease of probability throughout the day.

## Discussion

The air temperature and relative humidity, jointly with solar radiation and wind speed, are considered the most important factors that determine the exchange of heat between an animal's body and its surroundings (Silva and Maia, 2013). During the morning, the heifers stayed within a thermal comfort zone of 25°C (Berman et al., 1985). However, from 13:00 to 18:00 h, the MSTs of animals were found to be above the upper threshold of the critical zone (above 27°C air temperature) (Collier et al., 2006).

We found in this study that, despite the shade provided by trees, the THI was high in both groups, reaching the danger classification, as demonstrated by Hahn (1999). However, THI in the SP system was lower than the OP system. These results are in agreement with Lopes et al. (2016), who observed that at all points within an open pasture, the animals' thermal comfort was lower than in shaded areas. However, in all systems during afternoon the THI classification was above the normal range. The same was found by Souza et al. (2019), who observed THI values above 76 during day and night times. As discussed by the same authors, the protection provided by the shade was against direct solar radiation, thus the air temperature values were unaffected. In this situation, it is expected that the animals exercise their thermoregulatory mechanisms to deal with the thermal stress caused by the heat (Fuquay, 1981; Armstrong, 1994; Herbut and Angrecka, 2018), thus implying that they use sensible and latent thermal changes, in attempts to reduce thermal load in the system shaded by trees. The highest values of relative humidity were found during the morning and were the lowest in the afternoon. From 13:00, the humidity values, together with high air temperatures, characterize a condition of high heat loss potential for the animals based on respiratory evaporative heat-loss mechanisms such as increased RR (Marcillac-Emberton et al., 2009).

In the present study, we observed the effect of average temperature between groups, with reduced values for SP treatment. The small air temperature difference noted between the studied environments was also reported in previous studies. Karki and Goodman (2015) found small differences of approximately 1–15% between OP and SP systems composed of *Pinus* trees. Otherwise, Baliscei et al. (2013) did not report differences in temperature or relative humidity when comparing OP and SP systems in the northeast of Paraná, Brazil. The same authors justified that the shade provided by trees alters the balance of radiant energy. However, it does not reflect outstanding alterations in temperature or relative humidity. According to Karvatte Jr.

et al. (2016), the most change in microclimate was observed near midday, mainly in shaded areas with *Eucalyptus* sp. Pezzopane et al. (2019) found a remarkable reduction in thermal comfort indexes under SP treatment, when air temperature exceeds 26°C. The same authors reported that the same advance in thermal comfort index values was achieved in an OP system when air temperature was above 24°C.

The lack of differences in RRs between 09:00 and 10:00 may be explained by the behaviour of animals, which probably maintained their grazing activities in the SP system during mild periods (Lopes et al., 2016). The same was observed in Figure 4, with higher frequency of grazing during the morning for both groups. Karki and Goodman (2010) stated that grazing of ruminants in an SP system was increased during the morning. However, in the afternoon, the RR from the physical effort, along with the effort of maintaining the thermal balance, may explain the increase in these values at the referred periods.

According to Silanikove (2000), a bovine RR between 40 and 60 breaths min<sup>-1</sup> represents a low level of stress; RR of 60–80, 80–120, and above 150 breaths min<sup>-1</sup> were categorized as medium, high, and severe stress, respectively. Based on this, the heifers were noted to have low stress levels at 09:00. At 13:00, the animals in the SP system were at a medium level of stress. On the other hand, animals in the OP system presented a high level of stress. The RR increase in an environment without shading reflects the higher necessity to dissipate heat efficiently through respiratory evaporative heat-loss mechanisms. The same behaviour was observed by Domingos et al. (2013) as well, and they reported that after 10:00, animals kept in the shade were able to maintain a more regular RR than animals fully exposed under direct solar radiation, reaching peaks of approximately 75±10 breaths min<sup>-1</sup> at midday.

According to Collier et al. (2006), the fundamental strategy of animals to keep within the zone of thermoneutrality is to maintain body temperature greater than the temperature of the environment. This directs the flow of heat from inside the body to its periphery and thereby constructs a gradient between core body and the environment. The authors also stated that conduction, convection, and radiation depend on this temperature gradient as long as it is below the critical temperature threshold or below 27°C of air temperature. The results of this study are in line with those of previous studies, as it was observed that the variation in MST was accompanied by increasing temperatures of the environment. Furthermore, after 13:00, the air temperature increased above the upper threshold of the critical zone for heifers. Thereafter, an increase in RR was found in an attempt to relieve the physiological effects of the thermal stress. Martello et al. (2010) found similar results, thus confirming that during the summer, high RR and MST values that are comprehensively related to air temperature can be recorded. Moreover, they also found a positive correlation between RR and MSTs, which explains the similar behaviour of these variables in the present study.

According to Karki and Goodman (2010), when inserted in a comfortable condition, dairy cows spent more time grazing, chiefly during morning and post-midday periods. Otherwise, Lopes et al. (2016) indicated more grazing for heifers in an open pasture system, however, silage intake was lower. The results of this present study showed more grazing behaviour during morning, and the rumination was higher in

SP treatment in relation to OP. Grazing and rumination has great correlation, and thus this result regarding rumination is expected in shade systems with less stressful microclimatic conditions, as observed by lower air temperature.

Based on behaviour monitoring data it may be also be stated that during hot periods water intake increases (Kadzere et al., 2002; Cardot et al., 2008). Cows drink water more often, but in smaller quantities, so the total time of water intake increases (Tapki and Sahin, 2006). Regarding water intake, it is expected that in a harsh condition, animals spend more time visiting the water troughs. Our results demonstrate a difference between SP and OP systems during midday, indicating a mechanism to induce evaporative heat lost processes. Mello et al. (2017) found similar results with heifers in a full-sun system with more visits to water points (1.8%), than in shade treatment (0.4%), especially between 10:00 and 15:00 h.

Based on the microclimatic evidence of the two environments, the positive influence of the SP system is evident and it is the favourable thermal environment for the development of dairy heifers. Our results agree with the majority of previous studies, which emphasized the benefits of this system as compared to the OP system or to trees dispersed in pasture (Baliscei et al., 2013; Karki and Goodman, 2015; Karvatte et al., 2016; Mello et al., 2017). However, in some climate types, where summer temperatures exceed 35°C, animals may suffer from severe stress even under an SP system (Lopes et al., 2016).

### Conclusions

SP systems in a subtropical climate provide an adequate microclimate for the thermal comfort of dairy heifers when compared with an OP system. Likewise, heifers raised in a SP system maintain below the level of severe thermal stress throughout the day in the summer season.

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