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Physiological and behavioral responses of dairy heifers in an integrated-crop-livestock-forestry system

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The integrated crop-livestock-forestry system has been an option in tropics to mitigate the effects of heat stress on pasture-based system for dairy cows. We evaluate the effects of shade arrangements in integrated crop-livestock-forestry systems on physiological and behavioral responses of crossbred dairy heifers. Twenty-four crossbred European-Zebu heifers with 330.0±36.7 kg of body weight were assigned in three treatments: no shade, partial shade or total shade. Air temperature (34.4°C), black globe temperature (41.6°C), temperature humidity index (84) and heat load index (98) were higher ($P<0.05$) for the No shade treatment. Higher values of respiratory rate (99 $mov.min^{-1}$) and skin temperature (38.1°C) were also found for the no shade treatment. Shade availability affected the grazing time ($U = 246.5$, $P<0.05$). Heifers spent 10% more time in pasture on shade treatments. No difference was found on time spent in rumination, drinking or walking on shade treatments. Shade provision was an efficient strategy to reduce respiratory rate and skin temperature as well as to increase grazing time in integrated crop-livestock-forestry system.

Key words: Cattle, *Eucalyptus*, heat stress, integrated farming, pasture, shade.

INTRODUCTION

The integration of pastures with tree species in livestock-forestry systems (ICLF) has been an option to overcome the effects of thermal stress in tropics (Salton et al., 2014; Ainsworth et al., 2012). Heat stress affects the animal physiology and behavior and compromises their welfare (West, 2003; Schütz et al., 2010). Physiological responses include sweating, increase of body temperature, respiratory frequency and reduced rate of metabolism and feed intake (Collier et al., 2006). Also, cows spend less time lying in order to expose a greater surface area to lose heat by convection (Mader et al., 1997).

Shade provision by tree species protects animals against solar radiation and produces an environment with

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mild temperatures and consequently, better thermal comfort (Valtorta et al., 1997; Tucker et al., 2008; Rovira and Velazco, 2010). Ferreira et al. (2014) evaluated the shade availability in the paddock on physiological and behavioral response of crossbreed dairy cattle. Dairy cows spent around 57% of their time under the shade and the absence of shade on pasture caused heat stress. Treatment with insufficient shade helps to reduce stress at an intermediate level. These results show that shade should be available for all cows at the same time, as previously reported by Schütz et al. (2010).

Forage production may be reduced when shade levels exceed 50% of incident radiation. It occurs due to the decrease in a photosynthetic rate of C_4 grasses (Devkota et al., 2009; Paciullo et al., 2010). However, in moderate shade conditions, there is evidence of the maintenance or an increase in forage production at sunny conditions (Baruch and Guenni, 2007). Mello et al. (2017) assessed the influence of shade level (full sun, moderate shade, and intensive shade) on dairy heifer behavior during three seasons in Midwest of Brazil. The system with intensive shade shown negative effects on sward structure. On the other hand, the grazing activity was positively affected.

Additionally, an increase of forage nutritive value was found under shade, which could improve the performance of animals on a pasture-based system (Sousa et al., 2010; Yamamoto et al., 2007). Our objectives were to evaluate the effects of shade arrangements in integrated crop-livestock-forestry system on the respiratory rate, skin temperature and behavioral responses of crossbred dairy heifers.

MATERIALS AND METHODS

Location of the study, animals and treatments

The experiment was conducted in the experimental area in the ICLF of Embrapa Agrossilvopastoral, located in Sinop, Mato Grosso, Brazil (Latitude 11°51´43´´S, Longitude 55°35´27´´O) and all procedures involving animals were approved by the Animal Ethics Committee. The climate of the region corresponds to the Aw type (tropical climate with dry season), according to the Köppen classification, with dry winters and rainy summers. The data were collected during February and March 2013, and the evaluations were carried out on sunny and overcast days. Because of the safety standards of the Embrapa experimental field, collections were not held in rainy days.

Twenty-four Holstein x Zebu crossbred heifers (24.5±2.9 months; 330.0±36.7kg) were divided into group of eight in three treatments balanced by genetics groups. The treatments involved shade arrangements of *Eucalyptus camaldulensis* in the crop-livestock-forestry system: no trees (no shade); presence of trees (385 trees ha^{-1}) in two rows with 2.0 m distance between plants and 3.0 m between lines and with a distance of 49 m between the rows (partial shade); and trees arranged in three-line rows with 2.0 m distance between plants and 3.0 m among lines and with a distance of 49 m between the rows, resulting in a density of 720 trees ha^{-1} (total shade) (Figure 1).

A crop-livestock-forestry system was installed in January, 2011. It presented a 10 ha area formed by *E. camaldulensis* clone, pasture of Piatã grass (*Urochloa brizantha* cv. Piatã) in integration with corn for silage. Planting lines were oriented East to West and the *Eucalyptus* high was 12 m. All treatments approximately had the same pasture area formed by Piatã grass. Ten paddocks (0.25 ha) enclosed by electrified fences were used. Rotational grazing was carried out and the entry of animals in each paddock occurred when grazing reached 95% of light interception.

Environmental variables

Dry bulb temperature (DBT), relative humidity (RH) and black globe temperature (BGT) was monitored in 20-min intervals from 8:00 h to 16:00 h using an electronic system of data acquisition (HOBO®H8), installed at 1.60 m above the ground. For the no shade treatment, a sensor was placed in the center of the paddock, and for partial and total shade treatments, sensors were located in the middle of the rows of eucalyptus and set 0.5 m apart from the trees. The wind speed (WS) was measured with a digital anemometer (accuracy of $\pm$ 0.03 m s^{-1}) at the height of other sensors, at intervals of 2 h, between 8:00 h and 16:00 h.

Temperature humidity index (THI) was calculated hourly using the equation developed by Berry et al. (1964) and the heat load index (HLI) according to Gaughan et al. (2008) as follows:

$$THI = (1.8 \times DBT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times DBT - 26)];$$

$$HLI_{TGN>25} = 8.62 + (0.38 \times RH) + (1.55 \times BGT) - (0.5 \times WS) + [e^{2.4 - WS}];$$

$$HLI_{TGN<25} = 10.66 + (0.28 \times RH) + (1.3 \times BGT) - WS.$$

Where:
e = base of natural logarithm (approximate value e = 2.71828).

Physiological variables

Respiratory rate (RR) and skin temperature (ST, °C) were measured twice a day at 8:00 h and at 14:00 h. The RR expressed in movements per minute was obtained by counting number of rises of the flank. The ST was measured with a portable digital infrared thermometer (Instrutemp, ITTI 1000 model), equipped with laser sights, set with emissivity (ε)=0.95, 1.5% accuracy and optical resolution of 30:1. The measures were taken at approximately 1.5 m from the animal in the dorsolumbar region.

Animal behavior

Heifers were individually identified with colorful necklaces. Behaviors were recorded with focal sampling (Martin and Bateson, 1993) every 15 min from 8:00 to 16:00 h. The behavioral variables observed were the following: location of animals (under the shade or sun), posture (standing or lying down), and activities (grazing, resting, rumination, walking). Under the shade was considered when the animal was with 50% or more of the body under the eucalyptus' shade. Standing was considered to be an inactive posture (no locomotion). Lying behavior was considered to be when the flank was in contact with the ground. Heifers were considered to be grazing if grass was being consumed or could be seen in the mouth. Resting was defined when heifers have not shown apparent activity. Data were expressed as the percentage of the time in each behavior regarding the total time of observation.

Statistical analyses

The effects of the treatments on environmental variables (DBT, RH,

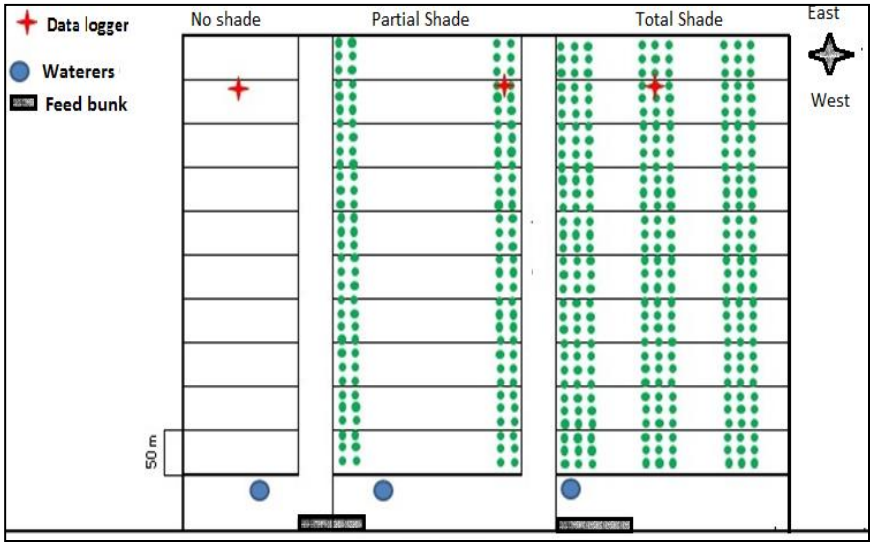

Figure 1. Schematic view of experimental area.

BGT and WS), thermal comfort indexes (THI and HLI) and physiological variables (RR and ST) were performed using the procedure for mixed models in SPSS® program version 16 (IBM Software). Treatments, day, time and interaction between treatments were considered as fixed effects. The normality of the variance was evaluated for all variables using the Kolmogorov-Smirnov test. The averages were adjusted for multiple comparisons by the method of least significant difference (LSD) being adopted with a significance level <0.05.

Pearson correlation coefficients ($r_{Pearson}$) were determined among physiological variables and HLI. The proportion of the shade usage, grazing activities, water intake, rumination, idleness, as well as the time spent standing, lying or walking were analyzed by Kruskal-Wallis non-parametric test (KS). Medians were compared by the Mann-Whitney test (U) when a significant difference among treatments was observed by KS.

RESULTS

Environmental variables

The shade availability affected the microclimate of the paddocks (Table 1). The DBT, BGT, WS, THI and HLI were higher ($P<0.05$) at no shade in comparison with partial shade or total shade. On the other hand, the RH was higher ($P<0.05$) in the total shade treatment in comparison with no shade and partial shade treatments.

No shade treatment presented higher DBT values ($P<0.05$) than those treatments with shade during the day-time. The maximum DBT (37.1°C) was observed at 15:00 h. At this time, shade treatments reduced the DBT by approximately 4°C (33.0°C).

Smaller values of RH (47.3% vs. 58.5%) were verified at 15:00 h respectively, for no shade and partial shade treatments. The values found for the BGT in the no shade treatment were higher ($P<0.05$) than those treatments with shade all day long. Both thermal comfort indexes THI and HLI showing highest values ($P<0.05$) for no shade treatment.

Physiological variables

Animals with shade availability showed smaller values ($P<0.05$) for RR and ST. The RR of the heifers was higher ($P<0.05$) in the no shade treatment (77 mov min^{-1}) followed by total shade (69 mov min^{-1}) and partial shade (64 mov min^{-1}). The RR in the afternoon was higher than in the morning. The treatments with partial shade (76 mov min^{-1}) and total shade (83 mov min^{-1}) showed lower values of RR compared with the no shade treatment (99 mov min^{-1}).

The ST was higher ($P<0.05$) in the no shade treatment in comparison with partial shade (31.8°C) and total shade (32.7°C) treatments, which did not differ from each other. ST in the afternoon was higher than in the morning. Partial shade (33.7°C) and total shade (34.1°C) treatments showed lower values of ST compared with No shade treatment (42.1°C).

The HLI showed a positive relationship with RR ($r_{Pearson}=0.67$) and ST ($r_{Pearson}=0.51$). As expected, RR and ST were increased when HLI was increased. No shade treatment animals were more vulnerable to this effect when compared with shade treatments (Figure 2).

Animal behavior

The shade availability affected the grazing time of the heifers ($U=246.5$, $P<0.05$, Figure 3). The animals grazed 10% more in shade treatments compared with no shade treatment. The animals in no shade treatment decreased the grazing activity from 12:00 h to 13:00 h time that

Table 1. Average values (minimum and maximum) of environmental variables and thermal comfort indexes during the trial period.

Environmental variable	No Shade		Partial Shade		Total Shade	
	Mean	Range	Mean	Range	Mean	Range
Dry bulb temperature (°C)	34.4[a]*	28.3-37.1	30.7[b]	27.0-32.9	30.6[b]	26.7-33.3
Relative Humidity (%)	55.7[c]	47.3-73.1	66.5[b]	58.6-82.0	71.9[a]	62.1-85.7
Black globe temperature (°C)	41.6[a]	33.0-45.9	32.6[b]	27.4-35.3	32.8[b]	27.4-36.3
Wind speed ($m.\ s^{-1}$)	0.9[a]	0.7-1.2	0.6[b]	0.5-0.8	0.3[c]	0.1-0.4
THI[1]	84[a]	79-87	81[c]	78-84	82[b]	78-85
HLI[2]	98[a]	90-104	88[c]	82-93	93[b]	84-98

*Row with differing superscripts are significantly different (P<0.05); [1]THI= Temperature–humidity index; [2]HLI= Heat load index.

coincides with the highest values of HLI (HLI=100) and in the late afternoon (16:00 h) grazing activity was increased.

No differences were found among treatments for rumination time (KS= 0.60, P=0.74), displacement (KS=0.22, P=0.89) and water intake (KS=5.37, P= 0.07). The average time spent with the activities of rumination and displacement were 30 and 8% of the total time, respectively.

Heifers in no shade treatment remained longer (43.7%) on resting behavior (U=284.0, P=0.001, Figure 4) when compared with the heifers in partial shade (37.7%). Resting time differed (U=287.5, P=0.001) among treatments with 37.7 and 38.2% for partial and total shade, respectively.

Heifers of the total shade treatment were recorded more often (U=315, P=0.003) standing (68.6%) than those at no shade (58.2%). Time spent lying was higher (41.8%) for animals in the no shade treatment (U=315.0, P=0.01) when compared with heifers with access to total shade (31.4%).

No difference was found in the use of shade between partial and total shade treatments (U=513.5, P=0.69, Figure 5). This feature was effectively used by heifers and corresponding on average 60% of the time in the shade.

DISCUSSION

Shade provides by trees modified the microclimate of the pasture resulting in a reduction of approximately 4°C air temperature and increase of relative humidity. Trees introduction reduced the wind speed and the solar radiation, creating a mild temperature regime with higher humidity levels. This occurred because the aerial part of the trees (canopy) can become a physical protection for the pasture, reducing the wind speed, decreasing direct losses from the soil and its humidity evaporation (Franke and Furtado, 2001).

The microclimatic conditions in a silvopastoral systems and its effects on heifers grazing behavior were evaluated by Lopes et al. (2016). The average temperature were higher than 30°C during the experimental period, and the two strategies with *Eucalyptus* rows adopted were not able to mitigate the heat stress conditions. Although better conditions were found under the tree canopy in both silvopastoral arrangements.

Thermal comfort indexes THI and HLI were high in all treatments ranging from 78 to 87 for THI, and 82 to 104 for HLI. The values were higher than the recommended limits in all treatments showing stressful thermal environment conditions to dairy heifers. Maximum productive potential of heifers could be expressed under THI and HLI around 72 and 77 units, respectively. However, these limits are only a guide and may be higher or lower depending on other factors, such as diet, feed intake and breed. Also, we can recognize that shade environments reduced the heat load index.

Shade availability decreased the RR between 17 and 10%, respectively, for partial and total shade treatments. The RR can quantify the severity of heat stress: 40 to 60 frequencies mov min^{-1} animals are in mild stress, 60 to 80 mov min^{-1} in medium stress, 80 to 120 mov min^{-1} in high stress and over 120 mov min^{-1} in severe stress (Silanikove, 2000). All heifers experienced stress in our experiment; in partial shade (76 mov min^{-1}) they were under medium stress, while in total shade (83 mov min^{-1}) and no shade treatments (99 mov min^{-1}) they were under high stress. Even though crossbred heifers are considered to be higher heat tolerant than pure dairy-bred, we found that heat stress has a significant effect on physiological responses. Consequently, shade provided by trees could mitigate the heat stress on pasture-based systems in tropical areas.

Rovira and Velazco (2010) evaluated the effects of artificial and natural shade on the respiratory rate, behavior and development of steers during the summer, observed that animals under shade showed a lower respiratory rate average than animals without shade, 64 and 74 mov min^{-1}, respectively. Steers with access to natural shade showed 6 mov min^{-1} less than those with access to artificial shade.

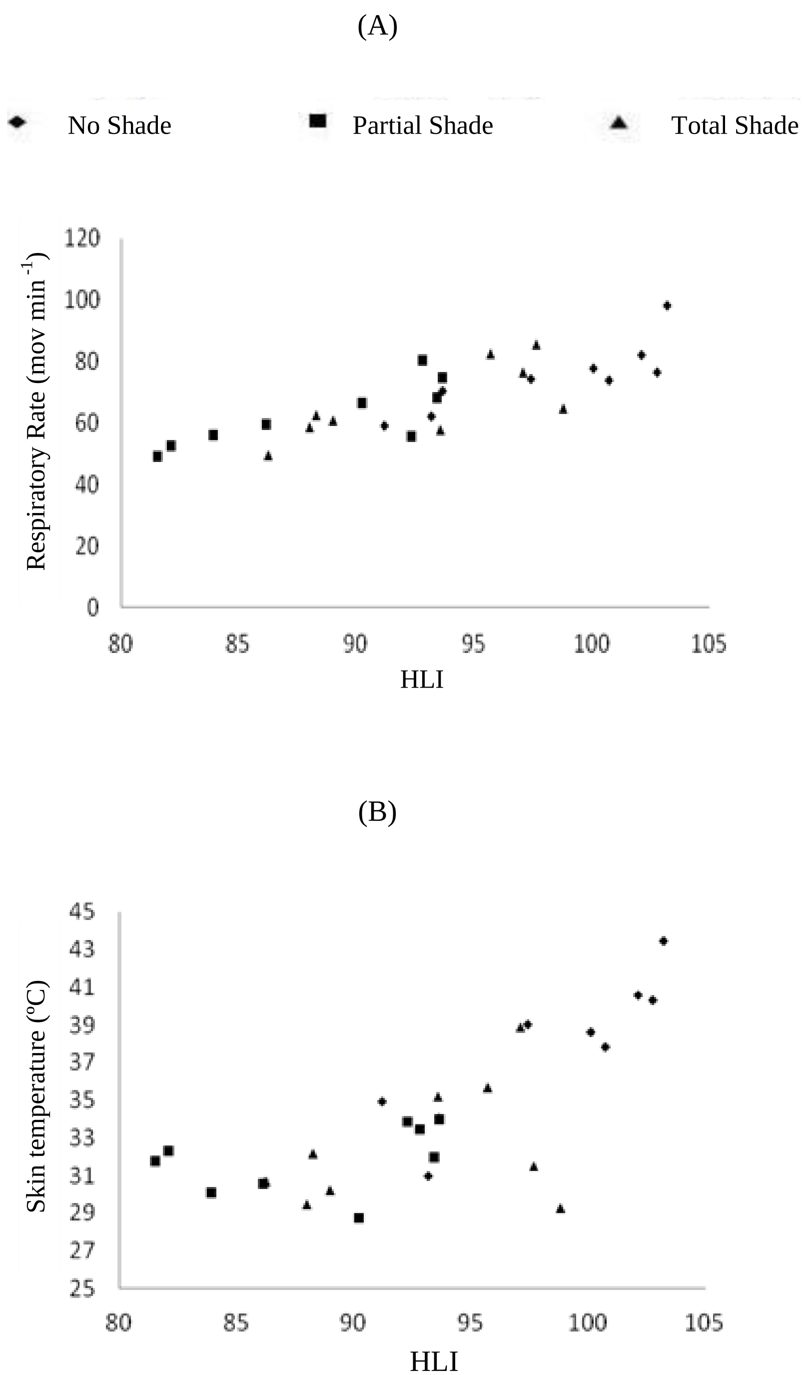

Figure 2. Relationship between (A) heat load index (HLI) and respiratory rate and (B) skin temperature of heifers in no shade, partial shade or total shade treatments.

High environment temperature could decrease the ability of cattle to dissipate body heat and it results in increased body temperature with negative influences on the productive performance. Thus, environmental modifications such as shade or evaporative cooling should be adopted to facilitate heat exchanges (West, 2003).

Heifers in shade treatments had lower ST with values of 31.8 and 32.8°C for partial and total shade, respectively. Shade reduced ST 5 to 6°C compared with no shade treatment. According to Collier et al. (2006) when skin surface temperature is lower than 35°C, the

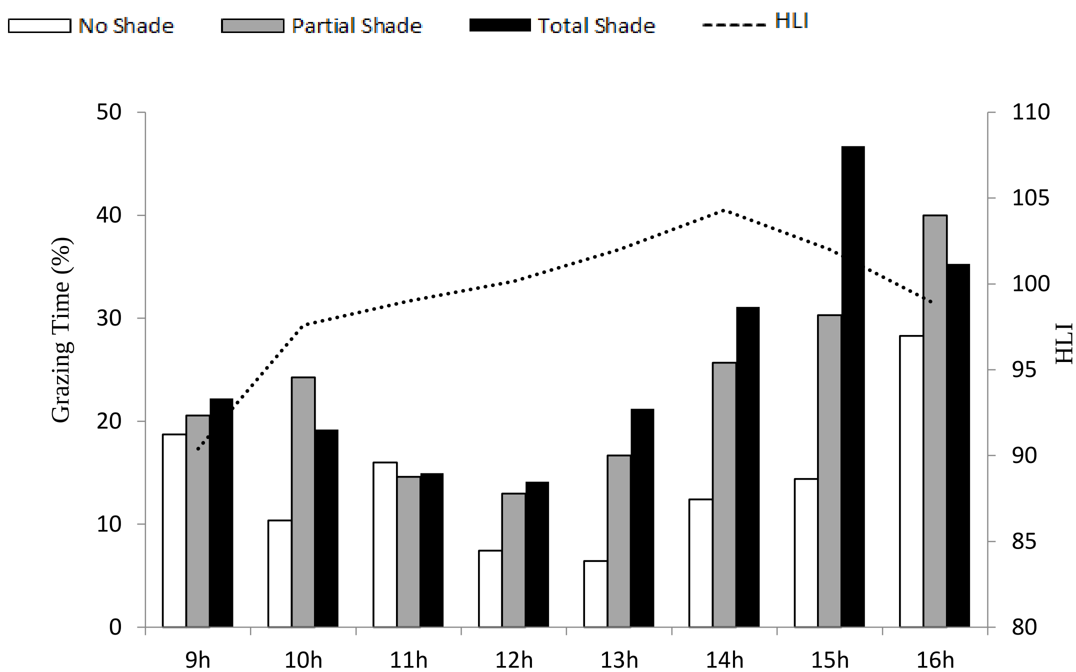

Figure 3. Percentage of grazing time of the dairy heifers from 9:00 h to 16:00 h in no shade, partial shade or total shade treatments.

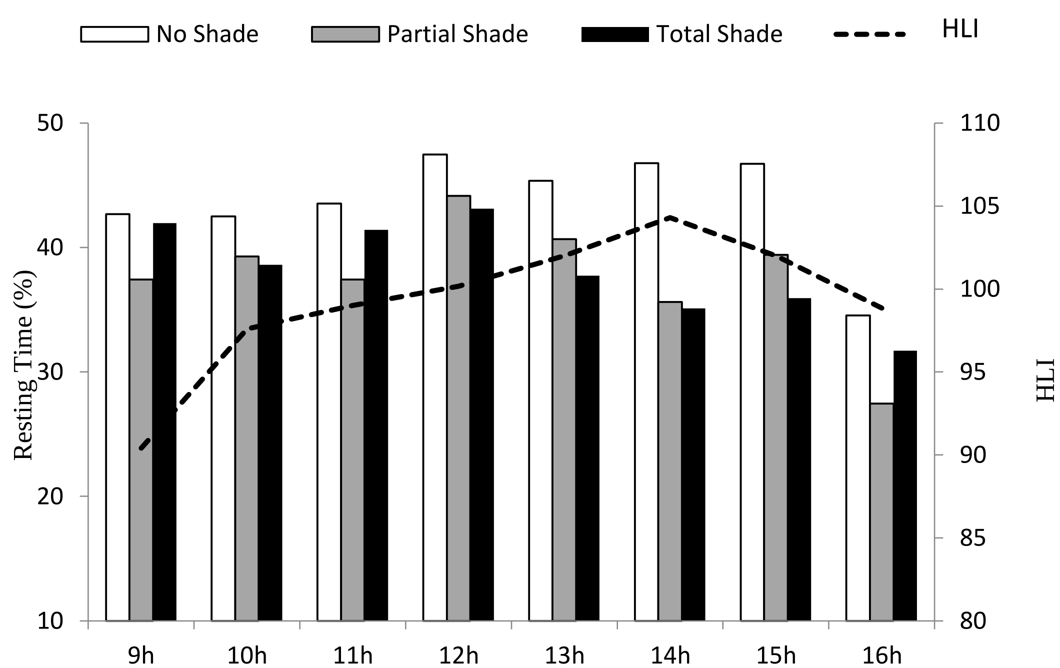

Figure 4. Percentage of the time spent in rest from 9:00 h to 16:00 h in no shade, partial shade or total shade treatments.

temperature gradient between body core and skin is enough for the animals to effectively use the four basic methods (conduction, convection, radiation and evaporation) of heat exchange. As a result, the shade provision reduced skin temperature showing that animals exposed to a partial shade had better thermal comfort.

Our findings shown heifers with shade access spent an average of 60% of the time using this resource. It was

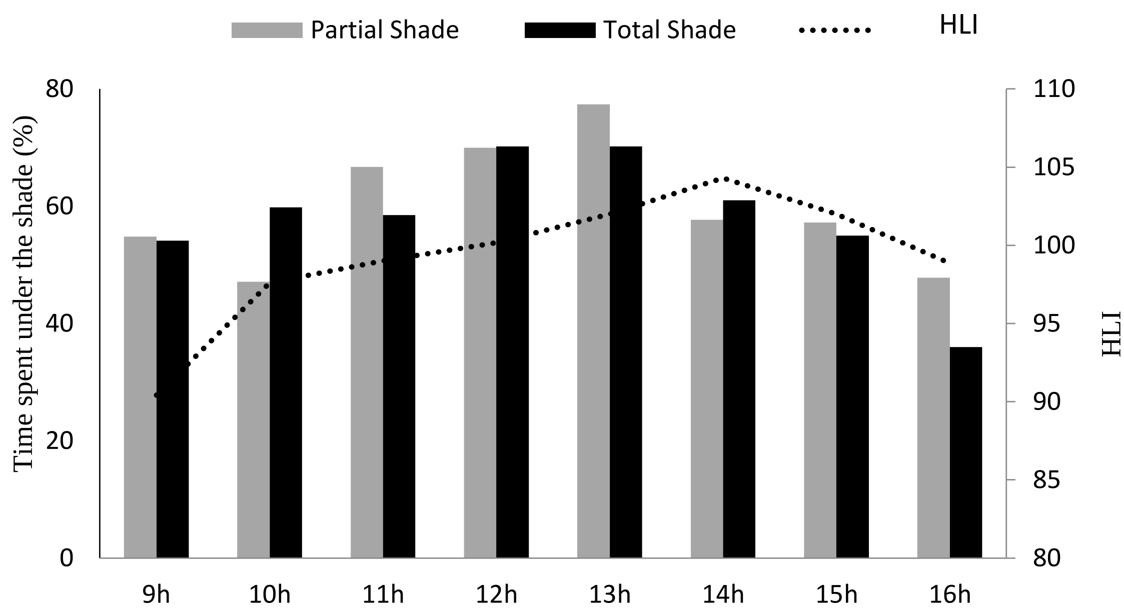

Figure 5. Percentage of time spent under the shade in partial and total shade treatments from 9:00 h to 16:00 h.

expected, since these cows tend to remain under the shade due to the coolest microclimate. Both treatments with trees had enough shade area where heifers were able to share it and not compete for it as recommended by Schütz et al. (2010).

Grazing behavior could be affected by daytime heat accumulation. Also, time spent under the shade is positively related to ambient temperature, solar radiation and rectal temperature (Sprinkle et al., 2000; Bennett et al., 1985). Shade availability increased the heifers´ grazing time even during the hottest hours. It was a beneficial response considering that dairy herds could reduce the feed intake to minimize the thermal imbalance and maintain the homeothermy (Yousef, 1985).

Previous research with livestock-forestry system found similar results with crossbred Holstein x Zebu and concluded this system could provide a thermal comfort for animals resulting in longer time on grazing (Paes Leme et al., 2005).

Heifers in shade treatments remained standing for a longer period (66.4%). However, as reported previously, this pattern was associated with the increase on grazing time. Specific environmental condition can stimulate more than one behavioral response and animal learns to employ the most efficient one (Curtis, 1981).

The longest resting time (43.7%) found in the no shade treatment might be associated as an endogenous strategy to reduce heat production. Although no difference was observed in the time spent on water intake, this was a frequent animal behavior in no shade treatment. These results supporting previous findings, where the time around the water trough was increased for animals with shade availability reduced or no shade treatment (Schütz et al., 2010; Mader et al., 1997). According to these authors, the water evaporation produces a microclimate that cools the animals.

Conclusion

Shade availability in a livestock-forestry increased the heifers´ grazing time and decreased respiratory rate and skin temperature.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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