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Variation in available shaded area changes behaviour parameters in grazing dairy cows during the warm season

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ABSTRACT - The objective was to investigate the effects of available shaded area on behavioural characteristics of lactating cows during the warm season in the subtropics. Twelve Holstein cows were divided into groups, with 2 (SH2) or 10 m² (SH10) of available shade per cow (six cows per treatment). The behaviour was recorded when animals were grazing from 09.00 to 16.00 h (GMT −02.00 h) throughout the trial. Data considered in the statistical analysis were restricted to the five days when the maximum temperature was ≥ 25 °C. Cows in the SH10 group had lower panting score, but higher proportions of time in shade and lying down and stayed more time per visit in the shaded area than cows in the SH2 group. Cows in the SH10 group had less events of competition near the water trough and in the shaded area than cows in the SH2 group. The solar radiation threshold that triggered the use of the shade was lower in SH10 than in SH2 group. Hourly proportion of time spent grazing or ruminating differed between shaded areas at specific times. The 2 m² of available shaded area per cow is sufficient for all cows to enter the shaded area at the same time. In spite of that, 2 m² of available shaded area per cow negatively affects the proportion of time spent lying and in the shaded area; in addition, it enhances agonistic behaviours between cows when compared with 10 m² of shade per cow.

Keywords: animal welfare, behaviour, dairy cows, heat stress, lactation, shade

Introduction

Dairy production systems based on grazing can help the health and welfare of animals, as well as improve the public perception of the dairy sector (Ventura et al., 2016). However, during the warm season and especially when shade provision is not adequate, animals may be exposed to heat stress (Kendall et al., 2006), triggering changes in social and feeding behaviours (Fisher et al., 2008; Schütz et al., 2010; Vizzotto et al., 2015) as well as in physiological attributes (Van Laer et al., 2015a), milk yield (Kendall et al., 2006; Van Laer et al., 2015b), and milk composition (Van Laer et al., 2015a).

Providing shade is an efficient strategy to reduce heat load and solar radiation, preventing increase in body temperature, maintaining physiological and productive parameters (Schütz et al., 2010), thus reducing heat stress in dairy cows (Van Laer et al., 2014). The available shaded area may influence behaviour and physiological and productive traits. Schütz et al. (2010) compared the behaviour of

grazing cows without shade or with 2.4 or 9.6 m^2 of shade per cow for five days and observed that the larger the amount of shade, the longer the permanence of cows in shade and the lower the number of aggressive interactions. In addition, these authors observed that cows that had only 2.4 m^2 of available shade per cow were unable to use shade at the same time and had higher respiration rates. Diverging from these results, Schütz et al. (2014) observed that 2 m^2 of shaded area per cow was sufficient for all cows to be able to use the resource concomitantly. These previous studies associating shaded area with behaviour were conducted in the temperate zone under moderate air temperature, relative humidity, and temperature-humidity index (THI) (22 and 23 °C, 59 and 59%, and 69 and 70%, respectively).

The hypothesis of this study is that 2 m^2 of shaded area per cow does not allow cows to use the shade at the same time in the subtropics during the warm season, and it will negatively affect feeding and social behaviours. The objective was to verify the changes on feeding and social behaviours when grazing cows have access to 2 and 10 m^2 of available shaded area per cow during the warm season in the subtropics.

Material and Methods

This study was approved by the local Ethics Committee on Animal (case number 21901). The experiment was conducted during the warm season in the southern hemisphere, during a period of 27 days (from January 30 to February 25, 2015), with the first six days as the adaptation period, followed by 21 days of measurements (experimental period – days 7 to 27). The experiment was conducted in Lages, SC, Brazil (latitude: −27°48'58"; longitude: 50°19'34"; altitude of 950 m asl). The average relative humidity is approximately 79.3%, and the climate is humid subtropical temperate climate.

Twelve Holstein cows were selected from the herd of the experimental station according to their body weight (BW), body condition score (BCS), days in milk (DIM), milk production, parity, and coat colour. Before the study, all cows grazed quicuio grass (*Pennisetum clandestinum*), with unrestricted access to natural shade provided by *Eucalyptus coolabahs* trees.

Cows were divided into two groups balanced for BW, DIM, parity, and coat colour, and further they were allocated to one of the following two treatments: access to 10 m^2 (SH10) and to 2 m^2 of shade per cow (SH2). At the beginning of the study, SH2 cows, on average, weighed (mean±SEM) 571.5±29.0 kg of BW, presented 2.9±0.2 of BCS, produced 22.9±2.4 kg milk per day, and were at 140±37 DIM. The SH10 cows, on average, weighed 575.2±29.0 kg of BW, presented 3.0±0.2 of BCS, produced 26.1±2.6 kg milk per day, and were at 144±37 DIM.

The shadow was provided by Eucalyptus (*Eucalyptus coolabah*) trees with more than 5 m in height. Every week, the area beneath the projection of shade of the tree was delimited with a string, and its largest width and length were used to calculate the shaded area. This procedure was performed at 09.00, 12.00, and 16.00 h (GMT −02.00 h), and an average arithmetic value was calculated. We used electrical fences to control the available shaded area per group of cows. During the adaptation phase (six days), all cows had access to 10 m^2 of available shade per cow.

Cows remained in paddocks of approximately 1.7 ha with quicuio grass (*Pennisetum clandestinum*). In SH2 and SH10 paddocks, respectively, average forage mass was 5,186.3±409.4 and 4,459.5±409.4 kg DM ha^{-1}, canopy height was 21.1±2.5 and 21.1±2.5 cm, leaf to blade ratio was 0.7:1±0.2 and 0.8:1±0.2, and leaf blades mass offered was approximately 2.4 and 2.1 kg DM 100 kg BW^{-1} per day.

Cows were milked twice daily at 07.00 and 17.00 h in a herringbone parlour. Behaviour was registered between milkings, from 09.00 to 16.00 h, when cows were in the paddocks. Cows were fed 3 kg of concentrate offered before milkings. Concentrate was composed by 200 g kg^{-1} of soybean meal, 750 g kg^{-1} of ground corn, 30 g kg^{-1} of mineral mix, and 20 g kg^{-1} of sodium bicarbonate, and contained 880 g DM, 160 g crude protein, 80 g neutral detergent fiber, and 750 g total digestible nutrients per kg of DM.

Panting scores (PS) were assigned on days 11, 13, 21, 25, and 27 once per hour from 09.00 to 16.00 h in a 0 to 4 scale, in which 0 = no panting and 4 = severe panting (Mader et al., 2006).

Milk production (kg) was recorded on days 11, 13, 21, 25, and 27, at the morning and evening milkings using DeLaval milk meter MM25.

Behaviour was recorded daily throughout the trial when animals were grazing from 09.00 to 16.00 h, but because a number of days were cool and wet, data analysis was restricted to five days (11, 13, 21, 25, and 27) when the maximum temperature was ≥ 25 °C. The time budgets for grazing, rumination, standing without grazing, lying, staying in the shade (use of shade), and walking were recorded with instantaneous scan sampling performed at 10-min intervals (Martin and Bateson, 1993). Results were expressed as the proportion of the observation time per hour (09.00 to 16.00 h) for each behavioural activity, except for the mean duration of visit to the water trough, expressed as minutes per visit (Table 1). The number of visits to the water trough, to the shaded area, and the number of competition events for shade were observed continuously and recorded whenever they occurred during the 7-h period. Competition for shade and number of visits to shade were expressed as the number of the events per hour, while the number of visits to the water trough and competition events near the drinkers were expressed as the total number during the 7-h period due to uneven distribution. The animals within and across treatments had visual contact with each other; water was provided *ad libitum* with two drinkers (1.5×0.8 m), one in the sun and one in the shade installed in each paddock.

All cows in both treatments were identified with coloured collars and had numbers painted with water-based ink on both flanks. Behavioural evaluations were performed by four previously trained experimenters, who have previously worked together in this kind of behavioural evaluations. Two observers watched one group of cows at any given time, but we switched observers between treatments on each measurement day to avoid bias favoring one of the treatments. Interobserver reliability for the scan observations was not accessed.

Air temperature (dry bulb temperature) and relative humidity were registered at hourly intervals using meteorological stations protected from direct solar radiation (Incoterm, USB – TFA 35.175) placed 1.5 m above the ground in the shaded and unshaded areas of the paddocks, irrespective of the treatments. Data loggers were surrounded by portable fences to prevent animals to approach and damage it. Maximal distance between data loggers and cows was approximately 200-250 m. Sensors to measure ambient air temperature and relative humidity have the following technical specifications: accuracy of ±1 °C and ±5%, respectively, and resolution of 0.1 °C and 1%, respectively (Incoterm

Table 1 - Ethogram describing the evaluated behavioural activities

Behaviour characteristics	Description of activity
Grazing	Time spent seizing and chewing the pasture (expressed as % per hour).
Rumination	Time spent in regurgitating, remasticating, and reswallowing the ruminal bolus (expressed as % per hour).
Standing	Time spent standing, excluding grazing activity (expressed as % per hour).
Lying	Time spent with the flank in contact with the floor (expressed as % per hour).
Walking	Time spent walking with head in the upright position, excluding grazing activity (expressed as % per hour).
Duration of visits to the water trough	Time spent per visit (expressed as minutes per visit).
Number of visits to the water trough	Number of events when cows stood and faced the drinker and involved the action of swallowing water (expressed as total number of events during the observation period, from 9.00 to 16.00 h).
Use of shade (visits)	Cow having 50% or more of its body in the shaded area (expressed as number of events per hour).
Position exchange events	Number of times cows exchanged position from standing to lying and vice-versa (expressed as number of events per hour).
Water trough competition events	Number of physical and non-physical attempts to move another animal in the area near the water trough (less than 2 m) (expressed as the total number of events during the observation period, from 9.00 to 16.00 h).
Shade competition events	Number of physical and non-physical attempts to move another animal that is occupying the shade (expressed as number of events per hour).

Manual User guide). Solar radiation data were obtained from an automated meteorological station (Campbell Scientific, Inc. Brand), equipped with a CR200 data logger and Pyranometer CS300, placed 500 m from the paddocks.

Data were analysed considering the randomised design. Animal served as the experimental unit. Data were tested for normality using the PROC UNIVARIATE (option normal, Shapiro-Wilk test). Proportions of time spent per hour in each behavioural activities (expressed as % of the time, per hour) as well as the number of visits to shade, number of position exchanges, competitions for shade, and panting score (measured per hour) were subjected to a repeated measurements ANOVA, using PROC MIXED procedures of SAS (Statistical Analysis System, version 9.4) , testing the fixed effects of treatment, hour, and interaction between treatment and hour, and solar radiation measured on each hour nested within days was used as a covariate. Measurement days and cows were considered as random effects.

The number of visits to the water troughs and the number of events of competition close to the water troughs were summed and expressed as total number from 09.00 to 16.00 h. Data were tested for normality using the UNIVARIATE (option normal, Shapiro-Wilk test) and further subjected to a repeated measurements ANOVA, using MIXED procedures of SAS, testing the fixed effects of treatment (shaded area) and solar radiation as a covariate. Measurement days and cows were considered as random effects. In both analyses, a structural selection test was performed using the Bayesian information criterion (BIC). Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. When differences were observed, pair-wise means were compared using the Lsmeans option pdiff (t test). The significance criterion was taken as $P<0.05$. The effect of shaded area on the relationship between behavioural data and solar radiation was tested with linear regression analysis using REG procedure of SAS. The existence of differences in these relationships between treatments was tested using MIXED procedure of SAS evaluating the interaction between treatment and solar radiation.

Results

During the trial, the overall means of air temperature, relative humidity, and solar radiation were (mean SEM): 25.9±1.4 °C, 62.9±4.7%, and 531.1±108.3 W m^{-2}, respectively. The accumulated precipitation throughout the experimental period was 55.4 mm. There was no rain on the days when behaviour was registered. Highest values of temperature were observed on days 13, 21, and 27 (Figure 1C), while the highest solar radiation values were observed on days 21, 25, and 27 (Figure 1D).

Cows in the SH10 group spent higher proportion of time per hour lying than cows in SH2 (Table 2). Significant interactions ($P<0.05$) between treatment and hour were detected for the hourly proportion of time spent standing, in shade, ruminating, and grazing (Table 2). Cows in SH2 spent a higher proportion of time per hour grazing ($P<0.05$) from 10.00 to 11.00 h and ruminating from 15.00 to 16.00 h than cows in SH10. Cows in SH2 stood up during a higher proportion of time ($P<0.05$) from 11.00 to 12.00 h and from 14.00 to 15.00 h compared with cows in SH10. Cows in SH10 used the shade during a higher proportion of time per hour ($P<0.05$) than cows in SH2 during the whole period, except from 14.00 to 15.00 h, when SH10 cows tended ($P<0.10$) to stay more in the shade than SH2 cows. The available shaded area did not affect the hourly proportion of time spent walking ($P>0.05$). Cows in SH10 had more visits per hour ($P<0.05$) to shade from 9.00 to 11.00 h, but had fewer visits to the shade from 13.00 to 15.00 h than cows in SH2. Cows in SH2 were assigned with higher panting scores ($P<0.05$) than cows in SH10 from 12.00 to 16.00 h. The average number of cows using the shade per hour was higher in the SH10 group than in SH2 group. The number of competitions events for shade per hour was higher ($P<0.05$) in SH2 than in SH10 group. The number of position exchanges was higher ($P<0.05$) in the SH10 group than in the SH2 group.

The average duration of visits to shade was greater for the SH10 group than in the SH2 group (130.0 vs 77.1 min, respectively; $P<0.05$; Table 3). The number of competition events near the water troughs was higher in SH2 than in SH10 (0.8 vs 0.2, respectively; $P<0.05$), but the available shaded area did not affect the number of visits to the water trough ($P>0.05$).

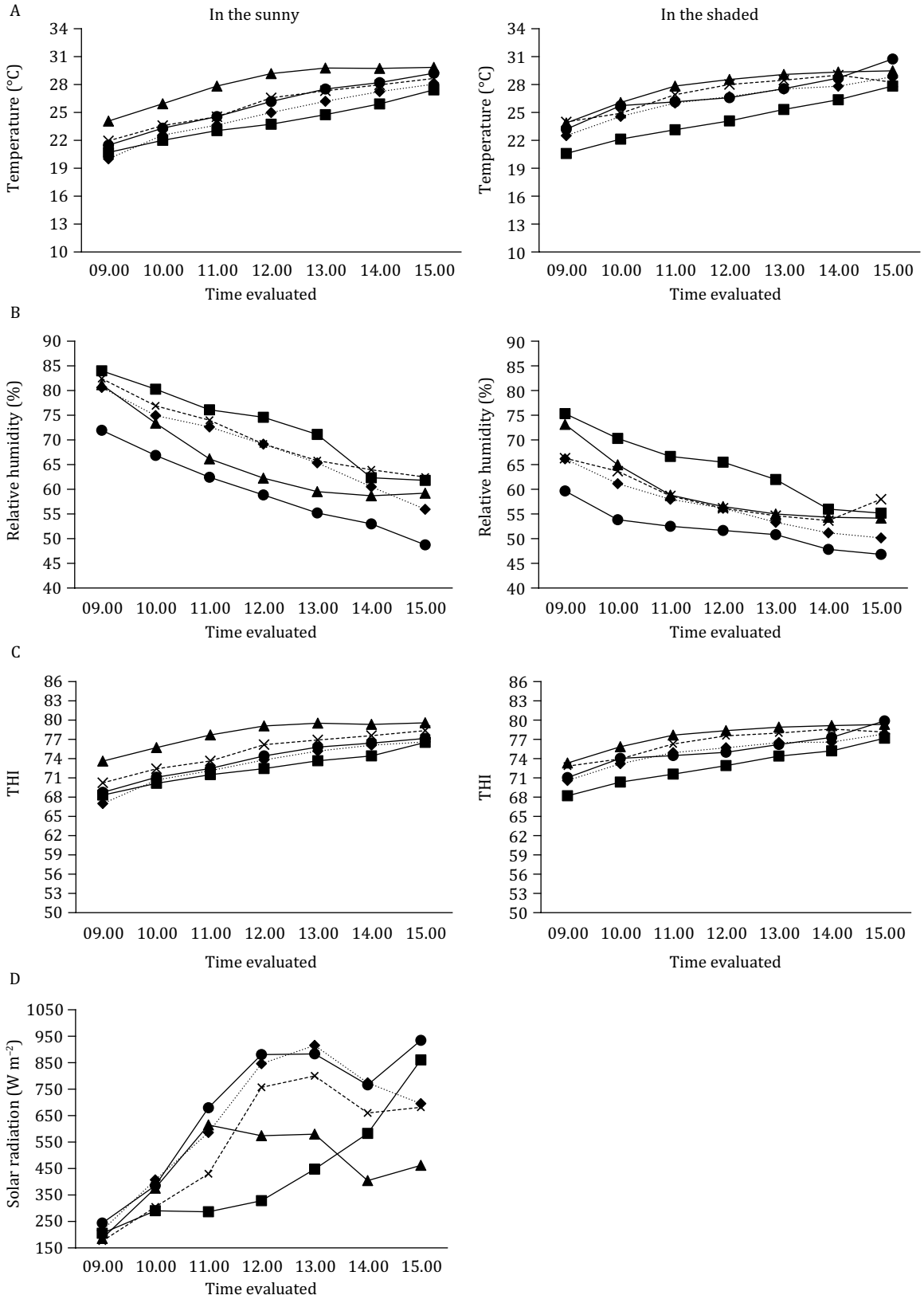

Figure 1 - Values of air temperature (A), relative humidity (B), temperature-humidity index (THI) (C), and solar radiation (D) measured in the sun and in the shaded area of paddocks on days 11 (■), 13 (▲), 21 (×), 25 (♦), and 27 (●).

Cows with access to 2 m^2 of shade used the shaded area almost exclusively during the hottest hours of the day, unlike cows with access to 10 m^2 of shade, as they distributed their visits more uniformly during the observation period (Figure 2). The solar radiation when cows entered the shaded area was lower for SH10 cows compared with SH2 cows (P<0.05; Table 2).

Proportion of time spent grazing and the number of competition events near the water trough decreased as solar radiation increased in both available shaded areas (Table 4). The proportions of time spent lying, walking, as well as the number of exchange positions and number of visits to the water troughs did not present a linear relation with solar radiation. On the other hand, the relationships between proportions of time spent ruminating, in shade, and standing as well as the number of visits to the shade, number of animals in shade, and panting score with solar radiation differed between the available shaded areas. The proportions of time spent ruminating, standing, and in shade as well

Table 2 - Hourly values of the behavioural parameters and physiological traits of lactating grazing cows with access to 10 or 2 m^2 of shade

Trait	Treatment[1]		SEM	P>F Treatment	P>F Treatment × Hour
	SH10	SH2			
Behaviour expressed as proportion of time (%) per hour, from 9.00 to 16.00 h					
Standing	48.6	52.0	3.5	ns	**
In shade	73.1	42.2	10.9	***	***
Lying	15.7	6.8	2.2	**	ns
Ruminating	25.2	27.2	5.5	ns	***
Grazing	32.9	38.0	3.7	ns	***
Walking	1.3	1.7	0.6	ns	ns
Number of events or animals (n/hour) from 9.00 to 16.00 h					
Visits to shade	0.5	0.4	0.2	ns	***
Animals in shade	5.2	3.5	0.9	***	ns
Competition for shade	0.2	0.5	0.1	*	ns
Position exchange events	0.4	0.2	0.1	**	ns
Physiological traits (per hour) from 9.00 to 16.00 h					
Panting score	0.3	0.7	0.1	***	***

[1] SH10: cows with access to 10 m^2 of shade; SH2: cows with access to 2 m^2 of shade.
SEM - standard error of the mean; ns - not significant.
* P<0.05; ** P<0.01; *** P<0.001.

Table 3 - Overall mean values of the behavioural parameters and productive traits of lactating grazing cows with access to 10 or 2 m^2 of shade

Trait	Treatment[1]		SEM	P>F Treatment
	SH10	SH2		
Number of events (n/period from 9.00 to 16.00 h)				
Visits to the water trough	2.8	2.9	0.3	ns
Competitions near the water trough	0.2	0.8	0.2	*
Overall means per visits to shade				
Duration of visits to the shade (min. per visit)	130.0	77.1	15.0	**
Solar radiation at visit to shade (W m^{-2})	453.3	572.3	21.9	**
Productive traits				
Milk yield (kg)	21.3	23.3	0.8	ns

[1] SH10: cows with access to 10 m^2 of shade; SH2: cows with access to 2 m^2 of shade.
SEM - standard error of the mean; ns - not significant.
* P<0.05; ** P<0.01; *** P<0.001.

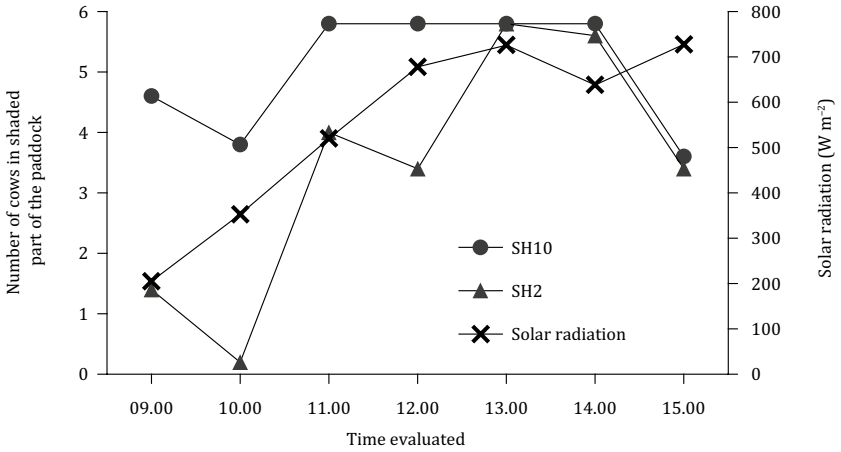

Figure 2 - Distribution of the number of cows using the shaded area when available shade was 2 and 10 m² per cow during the evaluation period.

Table 4 - Relationships between behavioural and physiological attributes with solar irradiation during the five days of observation

Variable	Solar irradiation (W m⁻²)			
	Slope	SE	P-value Solar radiation	P-value Trt × Solar radiation
In shade (% h⁻¹)	0.09	0.007	<0.001	<0.001
Standing (% h⁻¹)	0.1	0.006	<0.001	0.001
Lying (% h⁻¹)	−0.001	0.005	0.721	0.196
Ruminating (% h⁻¹)	0.04	0.006	<0.001	0.010
Grazing (% h⁻¹)	−0.10	0.006	<0.001	0.306
Walking (% h⁻¹)	0.001	0.001	0.219	0.392
Visits to shade (n h⁻¹)	0.000	0.000	0.176	<0.001
Animals in shade (n h⁻¹)	0.004	0.001	0.002	<0.001
Position exchange (n h⁻¹)	0.000	0.000	0.94	0.77
Competitions for shade (n h⁻¹)	−0.002	0.0005	0.625	0.016
Animals in shade (n h⁻¹)	0.004	0.001	0.002	<0.001
Visits to the water trough (n 7 h⁻¹)	−0.003	0.002	0.184	0.344
Competitions for water (n 7 h⁻¹)	−0.004	0.001	0.005	0.056
Panting score (n h⁻¹)	0.001	0.0001	<0.0001	<0.0001

SE - standard error; Trt - treatments (cows with access to 2 or 10 m² of shade).

as panting score increased linearly with solar radiation, but this effect was more pronounced in SH2 (higher slope) than in SH10. The number of visits to shade decreased with solar radiation in SH10 but increased in SH2. The number of animals in shade increased linearly with solar radiation in SH2, while the number of animals in shade varied much less during the observation period in SH10.

Discussion

This study prioritised the behavioural observation during the hottest hours of the day, coincident with highest solar radiation and air temperature, and when maintaining homeostasis, it may become a challenge for lactating dairy cows (Kadzere et al., 2002). The region where the study was held shows large variations in the temperature and solar radiation range. The air temperature and solar radiation measured on the evaluation days during the diurnal part of the day ranged from 20.0 to 30.4 °C (average of 25.9 °C) and from 175.3 to 935.6 W m⁻² (average 549.7 W m⁻²), respectively (Figure 1).

Usually, shade relieves heat load due to the direct reduction of solar radiation, which is the main factor contributing to increase body temperature in free range conditions (Fisher et al., 2008). This beneficial effect of shade can help lowering respiration rates (Schütz et al., 2010; Van Laer et al., 2015a), preventing hyperventilation and its negative side effects, such as respiratory alkalosis (Calamari et al., 2007). In the present study, the available shaded area influenced panting scores and its relation with solar radiation, as cows in the SH2 group augmented panting scores with increased solar radiation and presented higher panting scores from 11.00 to 15.00 h compared with cows in the SH10 group, which was coincident with the highest values for solar radiation and THI (Figure 2 and Table 4). These differences in panting scores are compatible with differences observed in the use of shade by the two groups of animals, evidenced by the higher proportion of time spent in shade, longer average duration of visits to shade, and higher number of visits to shade from 9.00 to 11.00 h, as well as by the higher overall average number of cows using the shade concomitantly observed in the SH10 group than in SH2 group (Tables 2 and 3 and Figure 2). Our results agree with those reported by Schütz et al. (2010), in which cows with access to 9.6 m^2 of shade per cow spent more than twice as much time in the shade than cows with 2.4 m^2 of shade per cow. Previous studies evidenced that during the warm season, lactating dairy cows seek shade (Schütz et al., 2008, 2010, 2014; Vizzotto et al., 2015).

Usually the limitation to use a resource increases the number of competitions events, which was confirmed by the increased number of aggressive interactions at the shaded area in SH2 compared with SH10 group (Table 2). Schütz et al. (2010) reported that cows with access to 9.6 m^2 of shade had less aggressive interactions per m^2 of shade and shared the area rather than competing for it, unlikely when only 2.4 m^2 of shaded area per cow was available.

Cows in both groups sought the water trough on average 2.8 times from 09.00 to 16.00 h, but apparently the drive to drink was higher for SH2 cows, as they tripled the number of competition events near the drinkers compared with SH10 cows (Table 3). This behaviour may be linked to their shorter permanence time in shade that increased their need to drink water. Schütz et al. (2010) reported that cows with 2.4 m^2 of available shade per cow spent more time near the water trough, indicating their higher motivation to drink than those cows with 9.6 m^2 of shade.

The availability of shade also affected other behaviours such as time spent lying, standing, grazing, and ruminating. The rank of the most prevalent behaviours changed between shaded areas, as in SH2 group, cows spent the highest proportions of time standing, in shade, and grazing, while in SH10 group, cows spent the highest proportions of time in shade, standing, and grazing, wich is in a close agreement with the results presented by Schütz et al. (2010) comparing shaded areas of 2.4 and 9.6 m^2 per cow. Cows in SH10 doubled the proportion of time spent lying compared with SH2 cows, which together with extended time in shade, the higher number of animals using concomitantly the shade and less competition events indicates that cows felt more comfortable when they had larger available shaded area to rest (Kovács et al., 2018).

In the present study, the increased proportion of time spent standing up of the SH2 group compared with SH10 is probably linked to the attempt to increase body surface exposed to air and facilitate heat dissipation (Palacio et al., 2015), as SH2 cows spent less time in shade and were exposed to solar radiation during an extended time compared with SH10 cows, confirming previous results of Schütz et al. (2010) and of Vizzotto et al. (2015), who reported that cows without access to shade increased time spent standing compared with those with access the shaded area. Kendall et al. (2006) evaluated the behaviour of dairy cows with 1.8 m^2 of shade or without shade and also noted the preference of cows to remain standing in the shade. The reduced number of exchanged positions between lying and standing observed in SH2 cows may be explained by the higher proportion of time spent standing up and that cows were less prone to lay down even in the shade compared with SH10 animals.

The limited effects of the available shaded area on the proportion of time spent ruminating and grazing verified in the present study are partially in agreement with Schütz et al. (2010, 2014), who did not report changes in total time spent grazing and ruminating between cows with access to different shaded areas. Before 10.00 h, grazing was the prevailing behavior irrespective of the shaded area. Further, from

10.00 to 11.00 h, cows in SH2 continued to graze, while cows in SH10 sought shade and decreased grazing. When shaded area was limited to 2 m^2 per cow, cows did not seek shade until solar radiation was higher than 400 W m^{-2}, in agreement with results reported by Oliveira et al. (2014). The linear negative effect of solar radiation on grazing activity was evidenced in the present study irrespective of the shaded area. Kendall et al. (2006), evaluating the effect of providing or not shade to grazing lactating cows, reported that cows with shade grazed less during the hottest hours of the day compared with cows without shade access.

Both groups increased their time spent ruminating during the hottest hours, especially the SH2 cows from 15.00 to 16.00 h, when they decreased physical activity (e.g. grazing) and remained in shade in an adaptive way to cope with highest solar radiation, in agreement with Oliveira et al. (2014).

Under the conditions of the present study, in the high-altitude subtropical region, provision of 2 m^2 of shade per cow changed the way of using the shade when compared with provision of 10 m^2 of shade per cow, compromising some behavioural parameters such as shortening the proportion of time spent lying and in shade and the duration of visits to the shade, while increasing the aggressive interactions in the shade and near the drinkers.

Conclusions

During the warm season in the high-altitude subtropical region, lactating grazing cows seek shade. Postural and social behaviours as well as the use of shade are modified by the amount of available shaded area. The supply of 2 m^2 of shade increase behaviours related to poor welfare such as augmented aggressive interactions and shorter lying time.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: S.C.B. Stivanin, D. Werncke and V. Fischer. Data curation: S.C.B. Stivanin and D. Werncke. Formal analysis: D. Werncke, E.F. Vizzotto and V. Fischer. Funding acquisition: A. Thaler Neto. Investigation: S.C.B. Stivanin, D. Werncke, E.F. Vizzotto and V. Fischer. Methodology: S.C.B. Stivanin, D. Werncke, E.F. Vizzotto, A. Thaler Neto and V. Fischer. Project administration: A. Thaler Neto and V. Fischer. Resources: A. Thaler Neto and V. Fischer. Supervision: A. Thaler Neto and V. Fischer. Validation: M.T. Stumpf. Visualization: M.T. Stumpf. Writing-original draft: S.C.B. Stivanin, M.T. Stumpf and V. Fischer. Writing-review & editing: V. Fischer.

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