



Thermal comfort and grazing behavior of Girolando heifers in integrated crop-livestock (ICL) and crop-livestock-forest (ICLF) systems

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ABSTRACT. To compare the integrated crop-livestock-forest (ICLF) and crop-livestock (ICL) systems in relation to thermal comfort and grazing behavior of Girolando heifers, a 2 × 2 crossover trial (two system and two periods) was carried out with eight ¾ Holstein × ¼ Gyr heifers in Xaraés–palisade grass pasture under intermittent stocking with 10 days of occupation period and 20 days of resting. In ICLF, crown cover of eucalyptus planted in rows was 65% at the beginning of the experiment. Animal behavior was assessed through bioacoustics by recording heifers' sounds for 48 hours. Concomitantly, heifers' internal temperature (IT, °C) was recorded every 10 min using data logger thermometers adapted to hormone-free intravaginal devices. Air temperature and humidity data were collected by thermohygrometers located in the center of ICLF and ICL for calculating Temperature–Humidity Index (THI). According to THI values, ICLF and ICL did not vary in terms of thermal comfort. However, THI indicated moderate stress (82.26±4.40) during day (06:00 to 17:59h) and mild stress (75.76±4.38) at night (18:00 to 05:59h) in both systems. During day, heifers from ICL had significantly ($p < 0.05$) higher IT (39.51±0.56°C) than those from ICLF (39.41±0.56°C); the former spent more time grazing and less time drinking water in ICLF (581.35±30.1 and 4.87±1.09 min, respectively) when compared with those from ICL (436.88±28.1 and 10.25±1.09 min, respectively). Therefore, although thermal comfort index did not vary between the systems, under moderate stress, crossbred heifers have lower internal temperatures, resulting in longer diurnal grazing and shorter water drinking time in Eucalyptus-shaded pastures.

Keywords: agrosilvopastoral; wellbeing; heat stress.

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Introduction

Cattle ranching in the pasture-based systems in tropical regions are a very challenging activity owing to the negative effects of the associated heat stress on herd reproduction and production performance (Atrian & Shahryar, 2012). Bovines are homeothermic, with behavioral, physiological, and metabolic circadian patterns for maintaining constant body temperature, where heat production (thermogenesis) and heat loss (thermolysis) are in equilibrium (Reyes et al., 2010). Heat stress occurs when the mechanisms for heat exchange are not enough to dissipate the excess heat produced by animal metabolism to the environment, thereby leading to a thermal disequilibrium (Barnabé, Pandorfi, Almeida, Guiselini, & Jacob, 2015). In dairy cattle, thermal disequilibrium is more pronounced owing to their specialization for milk production. This increases bovine feed consumption, which subsequently results in high metabolic heat production owing to rumen fermentative processes (Vasconcelos & Demétrio, 2011).

Under heat stress, animals change their behavior to reduce the amount of endogenous heat produced and promote heat loss (Schütz, Rogers, Cox, Webster, & Tucker, 2011). Thus, the daily grazing time is reduced, while the time spent for resting and drinking water increases (Perissinotto, Moura, Silva, & Matarazzo, 2005; Silva et al., 2009). Reduction in grazing activity decreases dry matter intake, with negative effects on body weight gain, milk yield, and reproductive efficiency, leading to economic losses resulting from such less than optimal herd performance (Almeida, Pandorfi, Guiselini, Henrique, & Almeida, 2011; Soren, 2012; Mellado et al., 2016).

In this context, it is important to adopt herd management and livestock systems with focus on maximum performance of the whole farm system by optimizing the interaction among pasture utilization, supplementary feeding strategy, and animal performance. The integrated crop-livestock-forest (ICLF) is an alternative with benefits for soil and pasture (Freitas et al., 2013) as well as animals owing to the thermal comfort offered by shade of the forest component within the system (Schütz, Cox, & Tucker, 2014). This is related to increase in live weight gain and, consequently, improvement of animal performance (Paciullo et al., 2011).

The objective of this study was to compare the integrated crop-livestock-forest (ICLF) and crop-livestock (ICL) systems with respect to thermal comfort and grazing behavior of Girolando heifers.

Material and methods

The Ethics Committee for the Use of Animals (CEUA) of Brazilian Agricultural Research Corporation (Embrapa Rondônia) has approved all the management practices applied to experimental animals (process number 06-2015).

Trials were carried out in the experimental field of Embrapa, Porto Velho, Rondônia, Brazil (8°48'03.89" S and 63°50'53.08" W) from 10th September to 11th November of 2015. The predominant climate in this region is Am according to Köppen classification actualized by Alvares, Stape, Sentelhas, Gonçalves, and Sparovek, (2014). This is characterized by a dry season (from May to September) and a rainy season (from October to April). The means of annual air temperature and annual rainfall are 26°C and 2095 mm, respectively.

Eight 25±6.8 month-old Girolando ($\frac{3}{4}$ Holstein × $\frac{1}{4}$ Gyr) heifers with initial live weight (LW) of 268±83 kg were used. They were distributed in a 2 × 2 crossover design between two integrated (crop-livestock-forest: ICLF and crop-livestock: ICL) systems. There were two 30-day experimental periods (period 1 and period 2), 10 days for adaptation and 20 days for data collection, with a total of 60-days of experimental period (Figure 1).

An area of 10 ha was divided in two five ha areas for each of the ICL and ICLF systems. In each area, the *Urochloa brizantha* 'Xaraés' pasture was divided into four paddocks of 1.25 ha and managed by intermittent grazing (10 days of occupation and 30 days of resting periods) with stocking rate of 2.5 animal unit (AU) per ha. In the center of each pasture system, there was a place for offering water and mineral salt mix *ad libitum*. The ICLF system had seven tiers of four rows each of eucalyptus trees planted in March 2013 with 3 × 3 m of distance between the plants. At the beginning of the trial, the trees had average diameter at breast height 11.9 ± 2.7 cm, total height 13.8 ± 2.5 cm, and crown cover 65%.

For estimating the available forage and the post-grazing residue, the forage was sampled in 1 m² plots randomly placed in different areas of the paddock before and after the occupation period. With a stainless steel 100 cm long graduated ruler, the grass height was measured just before cutting and weighing with a portable digital dynamometer (DD2000 *Instrutherm*®, São Paulo, Brazil). Grass samples were oven-dried at 65°C until constant weight and grounded (1 mm mesh) for determination of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin content (LIG) (Silva & Queiroz, 2006) (Table 1).

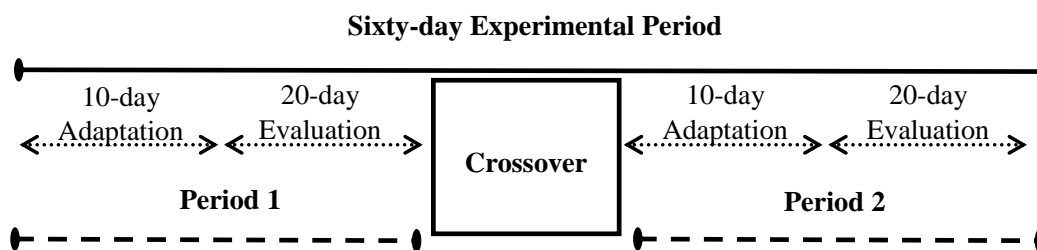


Figure 1- Scheme of the experimental period for the crossover design.

Table 1. Forage available and chemical composition of palisade grass (*Urochloa brizantha* 'Xaraés') within integrated crop-livestock-forest (ICLF) and crop-livestock (ICL) systems.

System	Forage Available (kg DM.100 kg of Live Weight ⁻¹)	DM (%)	CP	NDF	ADF	LIG
			(% DM)			
ICL	41.9	30.6	9.5	59.9	27.5	2.2
ICLF	32.3	25.9	12.5	60.2	28.8	2.5

DM – Dry Matter, CP – Crude Protein, NDF – Neutral Detergent Fiber, ADF – Acid Detergent Fiber, LIG – Lignin.

Grazing behavior was recorded from 14th to 16th day of the experimental period by bioacoustics (Veit, Salman, Cruz, Souza, & Schmitt, 2018). Audio data were taken by MP3 recorders tied to the heifer's halters for registering the sounds during the activities of grazing, ruminating, resting, and drinking. The audios were continually recorded for 48 h and reproduced by Audacity® software for identification, and quantification of the amount of time (in minutes) spent in each activity according to the period, which was divided into daytime (06:00 to 17:59h) and nighttime (18:00 to 05:59h). The time spent in each activity in each hour fraction (60 min) during a 24-h day was calculated as the average of the two days of audio collection (48 h).

Alongside recording of the behavior data, the internal temperature (IT) of heifers were taken during the 48 h using data logger thermometers (DS1921H-F5# *Thermochron, Innovation Drive Whitewater, WI 53190 United States*) adapted to hormone-free intravaginal devices and programmed for registering data every 10 min. Additionally, air temperature and humidity data were recorded from thermal hygrometers (ITLOG-80 *Instrutherm©, São Paulo, Brazil*) within PVC shelters placed in the center of each system. They were programmed for registering data every 10 min during 48 h.

The Temperature–Humidity Index (THI) was calculated using the equation proposed by Kibler (1964):

$$THI=1.8T-(1-H*100^{-1})(T-14.3)+32$$

where: T = Temperature (°C) and H = Humidity (%).

Statistical analysis of the behavioral and internal temperature data was performed using the mixed procedure of SAS (Statistical Analysis System), according to the following model:

$$Y_{ijklm} = \mu + S_{ij} + P_k + T_l + H_m + TH_{lm} + e_{ijklm}$$

where: Y_{ijklm} = variable answer; μ = general mean; S = effect of the i^{th} animal (1 to 8) within the j^{th} sequence (1 or 2); P = fixed effect of the k period (1 or 2); T = fixed effect of the l system (ICL or ICLF); H = fixed effect of the m period of the day (daytime or nighttime); e_{ijklm} = unexplained random error

These means were compared by Tukey-Kramer and F tests at 5% significance.

Regression analysis was applied to verify the influence of THI variation during the day on the time spent drinking water or on the internal temperatures of heifers. It uses the REG procedure of SAS and the equation parameters were compared by t test.

Results and discussion

Numerically, the air temperature was higher during the day and the relative humidity was higher at night. The values of these parameters and the THI were similar between both the systems (Table 2).

The variations in air temperature that is considered to be comfortable for bovines of European breed is from -1 to 16°C and for Indian breeds it is from 20 to 27°C (Santos et al., 2005). In both the systems, the mean of daytime air temperature was above the values of thermal neutral zone of bovines from both the genetic origins. There was no effect of shading on air temperature because the tree works as a protection against radiant heat only and not for the own heat load, leading to unaffected air temperatures (Souza et al., 2010a). Besides, considering the forest component within ICLF, the tree arrangement and tree species have influence on radiation interception by the tree crown and on the air circulation within the systems, resulting in microclimate changes (Caron et al., 2012; Karvatt Junior et al., 2016), which were not measured in our study.

Table 2. Means of air temperature (T), relative humidity (H), and Temperature-Humidity Index (THI) (\pm pattern deviation) registered within ICL and ICLF systems at daytime and nighttime.

	ICL		ICLF	
	Daytime	Nighttime	Daytime	Nighttime
T (°C)	32.11±4.1	24.65±1.4	31.93±3.6	24.91±1.4
H (%)	58.85±16.5	92.24±6.1	58.04±14.6	91.06±6.7
THI	82.47±4.1	75.62±1.8	82.06±3.7	75.89±1.7

Dairy cattle can show heat stress signals when high air temperature is combined with high relative humidity, because the evaporation is the best mechanism used by animals for losing heat when the air temperature is higher than the body temperature. This process is more effective when the air humidity is low (Atrian & Shahryar, 2012; Renaudeau et al., 2012). It is desirable for the air relative humidity to be around 60-70% (Souza et al., 2010a). The daytime relative humidity of both the systems had values similar to those considered optimal for thermal comfort of bovines. However, the nighttime relative humidity was above the values considered optimal for thermal comfort of bovines, while the nighttime air temperature was mild.

The THI was similar between the two systems, but the daytime THI was higher than the nighttime THI (Table 2). For dairy cattle, heat stress is classified according to the THI variation as mild (72-78), moderate (79-88), and severe (89-98); the THI below 72 characterizes an environment without heat stress (Armstrong, 1994). Following this classification, heifers were under varying heat stress along the evaluation period. It was moderate during the daytime and mild during the nighttime (Table 2). According to Azevedo et al. (2005), crossbred dairy cattle are more adapted to tropical climate and can tolerate THI as high as 77. In this case, heifers experienced thermal comfort during the nighttime and were uncomfortable during the daytime, regardless of the system.

The heifers' internal temperatures (IT) followed the THI variation through the day (Figure 2), with lower ($p < 0.05$) values during the night ($39.37 \pm 0.56^\circ\text{C}$) than those during the day ($39.46 \pm 0.56^\circ\text{C}$), being this last a slightly higher than the variation (38.0 - 39.3°C) considered normal for dairy cattle (Costa, Feitosa, Montezuma, Souza, & Araújo, 2015). During the day, heifers in the ICL system had a significantly higher IT ($39.51 \pm 0.56^\circ\text{C}$) than those in the ICLF system ($39.41 \pm 0.52^\circ\text{C}$), where the tree shades reduced the direct exposure of heifers to the solar radiation, consequently, reducing the heat absorbed by them (Souza et al., 2010b). At night, no statistical differences were found between the systems with the IT of heifers in ICL ($39.39 \pm 0.56^\circ\text{C}$) and in ICLF ($39.35 \pm 0.51^\circ\text{C}$) being within the normal range for this animal category (Costa, Feitosa, Montezuma, Souza, & Araújo, 2015). In both the systems, grazing activity was performed with preference timings as: early in the morning (06:00 to 08:00h), late in the day (16:00 to 17:00h), and middle of the night (22:00 to 01:00h) with averages of 74.62, 102.28, and 47.30 min, respectively (Figure 3). There was a significant interaction between systems and period of the day (Table 3). The grazing time was higher during the day than during night, regardless of the system, highlighting the bovine diurnal habits for grazing, especially at the beginning of the morning and end of the afternoon (Kilgour, Uetake, Ishiwata, & Melville, 2012).

Regardless of the system, daily grazing time was around the mean 480 min (8 h) observed for bovines in pastures with adequate forage allowance that does not limit dry matter intake (Hodgson, Clark, & Mitchell, 1994). During the daytime, heifers of ICLF spent more time grazing than those of ICL (Table 3), with significantly higher grazing times in ICLF at 07:00 and 22:00 (Figure 3). This observation is probably a consequence of the positive effect of the tree shading on grazing activity. At 7:00h, shading caused better thermal comfort by limiting direct exposure to solar radiation thereby enabling the heifers to spend more time grazing (Araújo et al., 2017).

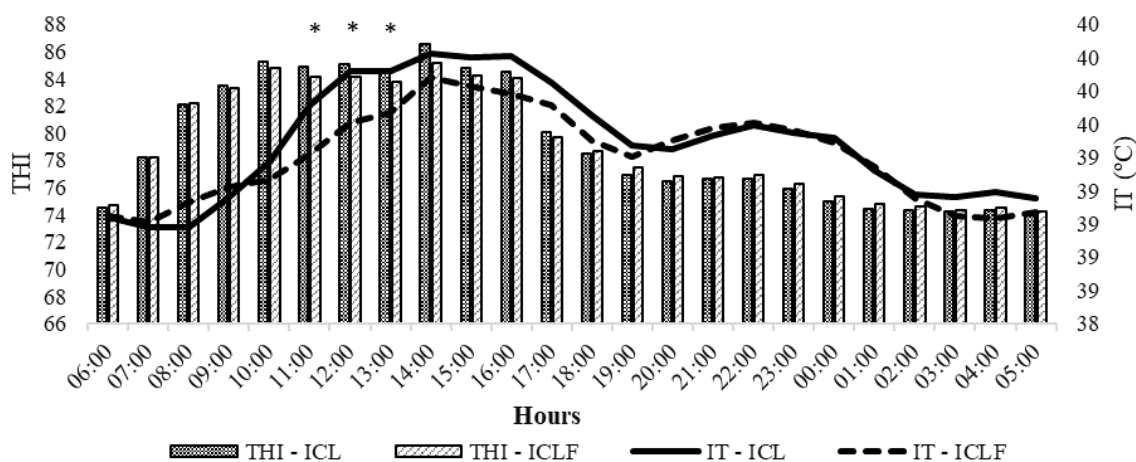


Figure 2. Temperature–Humidity Index (THI) of Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems and internal temperature (IT) of Girolando heifers (¾ Holstein × ¼ Gyr) along the day. * Different by F test at 5% of significance.

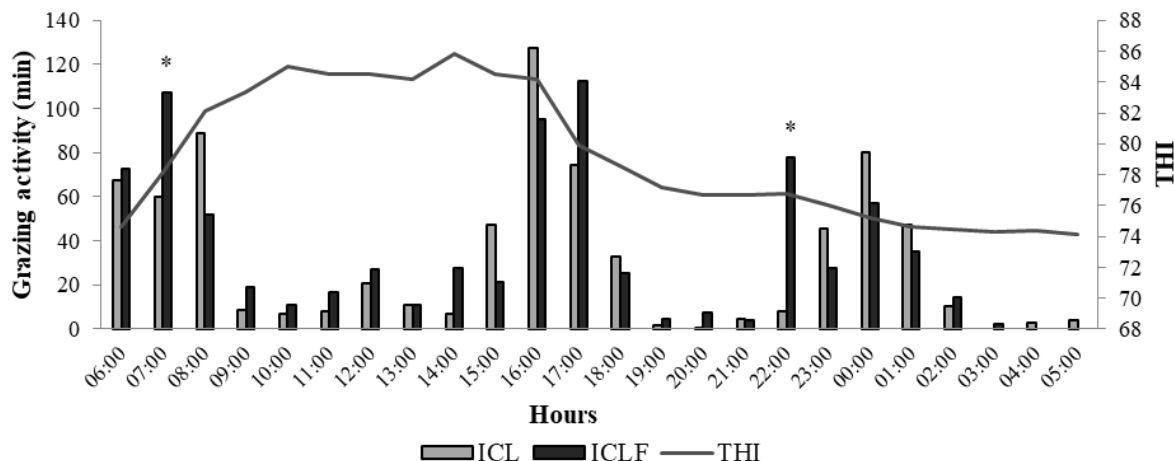


Figure 3. Variations in grazing activity (minutes) and Temperature–Humidity Index (THI) throughout the day in the Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems. * Different by F test at 5% significance.

The greater grazing time spent at 22:00h in the ICLF system could be as a result of the greater time spent in rumination at 18:00h (Figure 4), which permitted the heifers to empty their rumen and return to grazing activity at 22:00h. This time was one hour earlier than that of the heifers of the ICL system, the latter having a significantly higher resting time at this point (Figure 5). In fact, shading helps to anticipate the rumination activity in pasture-based systems (Titto, Titto, Titto, & Mourão, 2011), which should be a consequence of lower IT observed in animals in shaded pastures. Later in the day (17:00 - 18:00h) heifers of the ICLF system had IT numerically lower than that of the heifers of the ICL system (Figure 2), which justifies the anticipation of the time spent in rumination.

Comparing the two systems, there were no differences observed in terms of the rumination time (Table 3), which was usually during the night when THI value was lower. Heifers spend significantly more time in this activity at night (Figure 4), in agreement with many previous findings (Soriani, Panella, & Calamari, 2013; Mendes et al., 2013; Desnoyers, Giger-Reverdin, Sauvant, & Duvaux-Ponter, 2011). This is owing to the high amount of endogenous heat produced during rumination, which forces the animals to avoid this activity during the warmer periods of the day and choose the night when air temperature is milder (Vilela et al., 2013). Beside climatic conditions, diet traits also have effects on the rumination time, with elevated diet NDF level increasing the rumination time (Missio et al., 2010). The similar NDF level of grass from both the systems (Table 1) explains the lack of difference between them with respect to this parameter (Missio et al., 2010).

During the day, ruminants distribute their time among grazing, rumination, and resting (Mercês et al., 2012). Under heat stress, bovines tend to make changes in their behavior to reduce heat production and/or to promote heat loss by avoiding additional stock of body heat, decreasing grazing time, and increasing resting time (Borges, Azevedo, Lima, Brasil, & Ferreira, 2012).

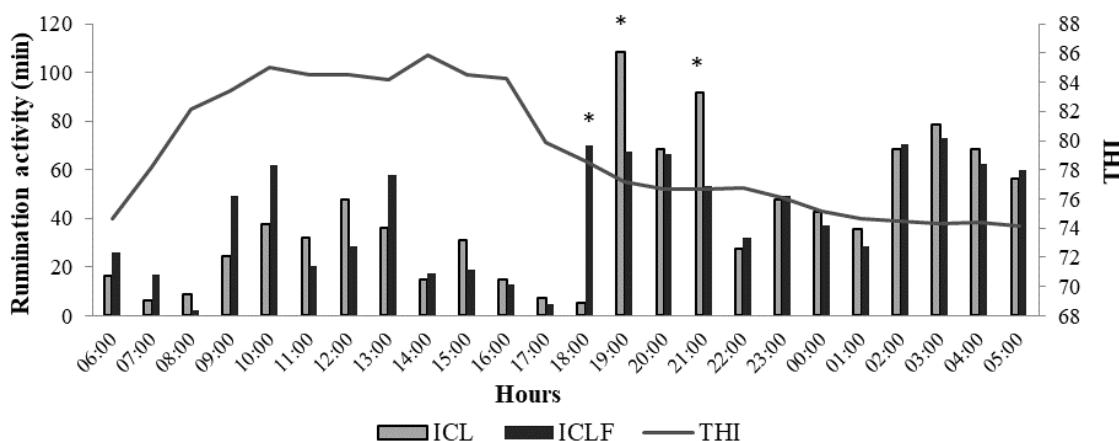


Figure 4. Variations in rumination activity (minutes) and Temperature–Humidity Index (THI) throughout the day in the Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems. * Different by F test at 5% significance.

Although grazing time was higher ($p < 0.05$) in the ICLF system (Table 3), leisure activity was not different between the systems. Although the resting time spent is distributed throughout the day, heifers prefer doing it during the dawn and early morning (Zanine, Santos, Parente, Ferreira, & Cecon, 2007), which explains the peaks of resting times observed in the ICLF system at 04:00 and 06:00 (Figure 5). Tree shading in ICLF allowed the heifers to rest according to the period of their preference, while in the ICL system, the animals concentrated this activity preferably at 09:00 when the THI reached the value of 83 (Figure 5) and at 22:00, just after a rumination peak (Figure 4).

In the ICL system, heifers spent more time in drinking water (Table 3), especially at 7:00, 9:00, and 15:00 (Figure 6) when THI values were above 77, which is considered as the limiting value of thermal stress tolerance for crossbred dairy cattle in tropical climate (Azevedo et al., 2005). The animals spent more time with this activity in the daytime period than in the nighttime (Table 3). Usually, water intake by animals raised in tropical regions is intense during the daytime due to the high temperatures, which causes thermal discomfort for animals resulting in increased sweating rate and the body water losses (Atrian & Shahryar, 2012). Thus, in environments with adequate shading, where animals are not directly exposed to solar radiation, the water intake tends to be lower (Schütz, Rogers, Poulouin, Cox, & Tucker, 2010).

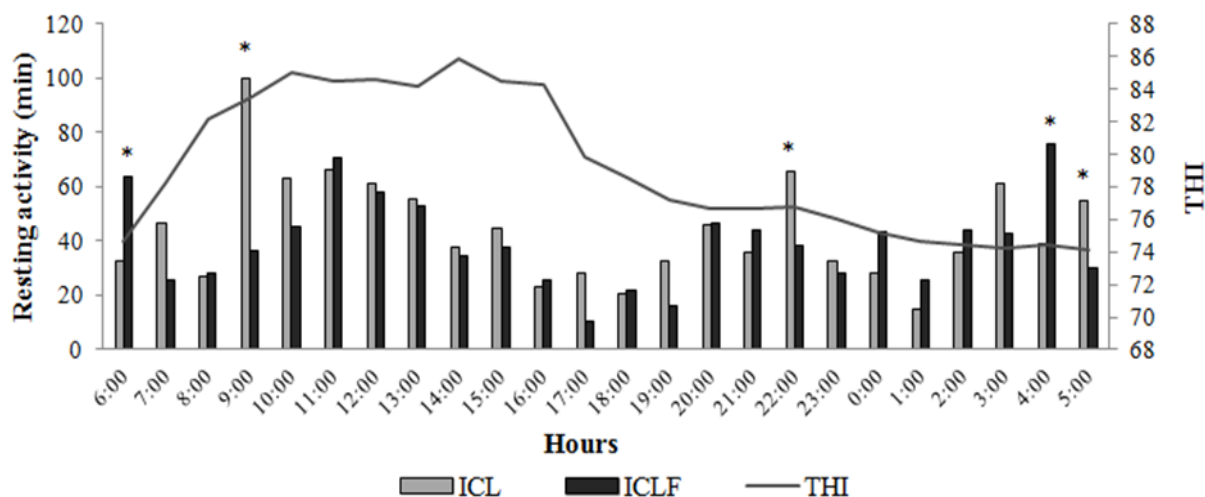


Figure 5. Variations in resting activity (minutes) and Temperature–Humidity Index (THI) throughout the day in the Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems. * Different by F test at 5% significance.

Table 3. Average (\pm standard error) of the time spent on grazing, rumination, resting and water intake by Girolando heifers (3/4 Holstein \times 1/4 Gyr) in the Crop-Livestock Integration (ICL) and Forest (ICLF) systems during daytime periods (06:00 to 5:59h) and nighttime (6:00 to 5:59h).

Grazing (minutes)			
Day period	ICL	ICLF	Average
Daytime	436.88 \pm 28.1 Ba	581.35 \pm 30.1 Aa	509.11 \pm 22.7
Nighttime	228.00 \pm 28.1 Ab	238.06 \pm 30.1 Ab	233.03 \pm 22.7
Average	332.44 \pm 22.1	409.71 \pm 23.6	
Rumination (minutes)			
Daytime	237.00 \pm 21.4 Ab	301.16 \pm 22.5 Ab	269.08 \pm 13.1
Nighttime	648.50 \pm 21.4 Aa	670.16 \pm 22.5 Aa	659.33 \pm 13.1
Average	442.75 \pm 12.6	485.66 \pm 12.9	
Resting (minutes)			
Daytime	490.62 \pm 45.4	497.50 \pm 4.2	494.06 \pm 38.4 a
Nighttime	446.87 \pm 45.4	485.64 \pm 48.2	466.26 \pm 38.4 a
Average	468.75 \pm 37.6 A	491.57 \pm 39.7 A	
Water drinking (minutes)			
Daytime	10.25 \pm 1.09 Aa	4.87 \pm 1.09 Ba	7.56 \pm 0.77
Nighttime	1.50 \pm 1.09 Ab	1.37 \pm 1.09 Ab	1.43 \pm 0.77
Average	5.87 \pm 0.77	3.12 \pm 0.77	

Average followed by different letters (uppercase in the row and lowercase in the column) differ from each other by the Tukey-Kramer test at 5% significance.

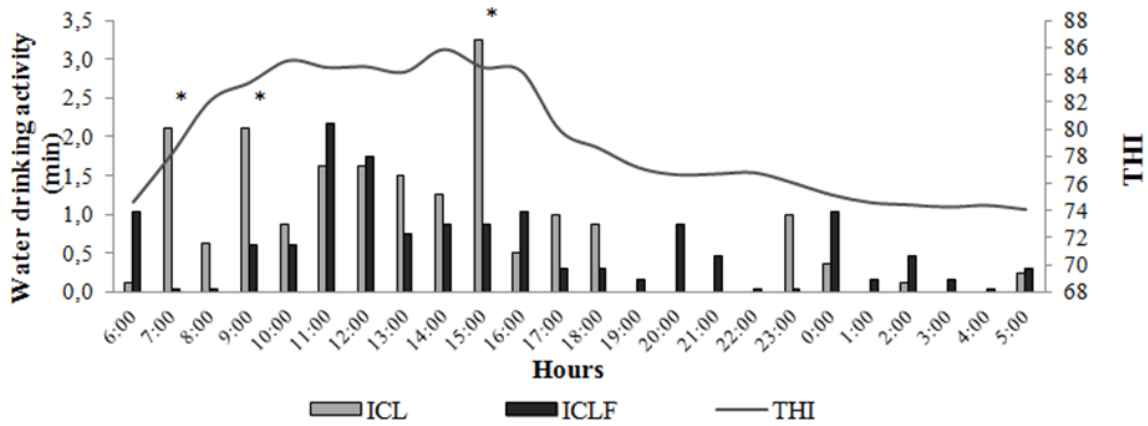


Figure 6. Variations in water drinking activity (minutes) and Temperature–Humidity Index (THI) throughout the day in the Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems. * Different by F test at 5% significance.

Regressing the time spent on water drinking and the internal temperature values on the variation of THI during the day (Figure 7) has shown that at each THI unit there was an increase in the time spent on drinking water by heifers of ICL and ICLF systems by 0.14 and 0.067 min, respectively (Figure 7A). The difference in the time is around 50% between the two systems. Regarding IT, each THI unit represented an increase of 0.045 and 0.031°C in IT of heifers in the ICL and ICLF systems, respectively (Figure 7B), which corresponds to a difference of 31% between the systems. In addition, the coefficient of determination (r^2) and the correlation coefficient (r) had higher values in the ICL system than in the ICLF system (Figure 7), demonstrating that climatic condition had higher effect on the water drinking activity and internal temperature of heifers in the unshaded pasture. Our results are in agreement with the reports of Schütz, Rogers, Poulouin, Cox, & Tucker (2010), who reported that increasing heat load results in greater water drinking and higher time spent around the drinkers in unshaded pasture, demonstrating the importance of shading for dairy cattle.

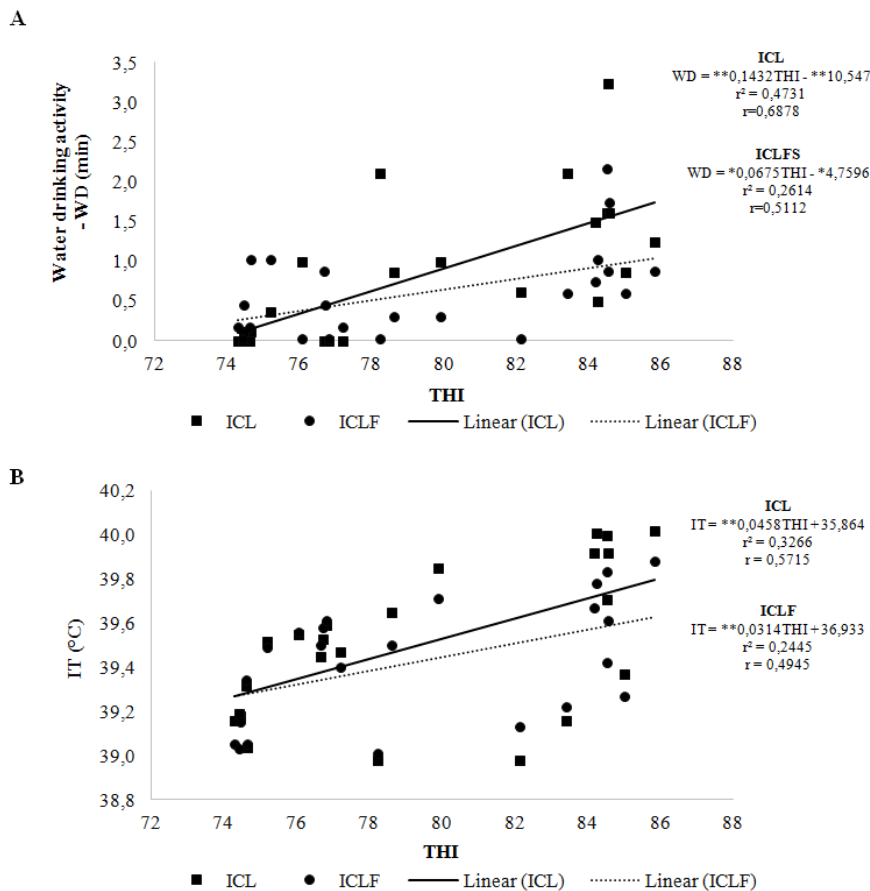


Figure 7. Effect of Temperature–Humidity Index (THI) variation along the day on the time spent in water drinking (A) and on internal temperature (B) of Girolando heifers in the Integrated Crop-Livestock (ICL) and Crop-Livestock-Forest (ICLF) systems. **Significant at 1% and *Significant at 5% by the *t*-test.

Conclusion

Although THI values did not indicate differences in thermal comfort between the ICL and ICLF systems; during the daytime, Girolando heifers have a lower internal temperature, graze for longer, and spend less time drinking water in shaded pastures. Climatic conditions have a more prominent effect on the water intake activity and internal temperature of heifers in a completely unshaded pasture. This indicates that the presence of trees in pastures can positively influence the grazing behavior of the animals.

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