R. Bras. Zootec., 51:e20210047, 2022
https://doi.org/10.37496/rbz5120210047

Ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Productive performance and ingestive behavior of crossbred heifers in integrated livestock-forest systems

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Received: March 10, 2021
Accepted: December 1, 2021

How to cite: Santos, J. M. F.; Andrighetto, C.; Mateus, G. P.; Lupatini, G. C.; Daldon, M. S.; Lima, L. P.; Luz, P. A. and Bello, H. J. S. 2022. Productive performance and ingestive behavior of crossbred heifers in integrated livestock-forest systems. Revista Brasileira de Zootecnia 51:e20210047.
https://doi.org/10.37496/rbz5120210047

ABSTRACT - The present study aimed to assess the productive performance, ingestive behavior, and thermal condition of crossbred beef heifers in an integrated livestock-forest (ILF) system with different tree-planting designs. Seventy-two ½ Angus ½ Nellore heifers were divided in three treatments—conventional system (CS) without eucalyptus, integrated livestock system with 187 eucalyptus ha^{-1} (ILF-1L), and integrated livestock system with 446 eucalyptus ha^{-1} (ILF-3L)—, in the winter and summer. Total dry mass of forage (TDM), chemical analysis of Marandu palisadegrass, average daily gain (ADG), stocking rate (SR), weight gain per area (WGA), behavior, microclimatic variables, and thermal comfort indexes were evaluated. A higher percentage of crude protein and lower TDM and SR were observed in ILF-1L and ILF-3L, with no differences for ADG and WGA. Ambient temperature, temperature-humidity index, black globe-humidity index, and radiant heat load were better in ILF-1L and ILF-3L. Heifers in ILF-1L and ILF-3L carried out grazing, resting, rumination, and other activities in the shade. In the summer, heifers in ILF-1L and ILF-3L spent less time resting and more time in rumination and performing other activities than those in the CS. In conclusion, the better forage quality in ILF-1L and ILF-3L does not alter the performance of heifers or WGA, despite the decreased TDM and SR. Furthermore, ILF-1L and ILF-3L provide better thermal comfort, and heifers that do not have access to shade decrease their activities as a strategy to reduce the use of energy for thermal regulation process.

Keywords: animal welfare, silvopastoral system, thermal comfort

1. Introduction

The demand for products generated via efficient use of natural resources and animal welfare has increased in recent years (Broom, 2017). Thermal stress in tropical regions due to high temperatures can cause deleterious effects on behavior, animal welfare (Vizzotto et al., 2015; Broom, 2017; Giro et al., 2019), and production (Vizzotto et al., 2015; Pantoja et al., 2017; Kamal et al., 2018).

An integrated livestock-forest (ILF) system or a silvopastoral system includes three components: the animal, the forage, and the trees. These systems are an alternative that can sustainably diversify production and increase productivity and income of rural producers. In addition, they improve animal welfare through better thermal comfort provided by the tree shade (FAO, 2010). The amount of shade afforded in the ILF system modifies the environment, and thus, protects the animal from excessive heat emitting due to direct solar radiation (Emerenciano Neto et al., 2013) and decreases the air temperature and animal surface body temperature, which allows heat transfer between animal and environment (Giro et al., 2019) aiming at reducing heat stress.

The shade provided by trees is an important ally in minimizing the effects of high temperatures in the tropics. European breeds and their crossbreeds are more sensitive to high temperatures (Nardone et al., 2010); thus, introducing shade can be a management strategy that would help to breed these animals in tropical environments. However, there is still little information on the impact of ILF system on the productive and behavioral responses in beef cattle that are crosses between European and Zebu breeds.

Based on the hypothesis that crossbreding between Zebu and European breeds are sensitive to high temperatures and solar radiation, in tropical regions, and that the use of trees can improve animal thermal comfort and to change the behavior, the objective of the present study was to assess the productive performance, ingestive behavior, and thermal condition of crossbred beef heifers in an ILF system with different tree-planting design.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (case no. 03/2017.R1).

The experiment was conducted in Andradina, São Paulo, Brazil (20°53'38" S, 51°23'1" W at an altitude of 400 m). The predominant Köppen climate classification in the region is Aw (Alvares et al., 2013). The soil in the experimental area is classified as Red Yellow Dystrophic Argisol (Santos et al., 2013), with an average ground slope of 6%.

From November 2012 to March 2013, the tree seedlings were manually planted, following the contour lines present in the area. The eucalyptus used was *Eucalyptus urograndis* (I-224 clone); the average height of the eucalyptus during the experimental period was 18.0±4.7 and 18.2±4.6 m, while the diameter at breast height was 17.8±4.3 and 17.6±4.0 cm for ILF-1L and ILF-3L, respectively.

The forage used was palisadegrass, *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandu. In March 2017, the area was prepared for the experiment: 100 kg urea ha^{-1} was applied, corresponding to 45 kg N ha^{-1}, and the grazing was deferred until June 2017, when ½ Angus ½ Nellore heifers were introduced to the experiment. The second fertilization was carried out in January 2018, using 180 kg ha^{-1} of a 20:5:20 fertilizer mix (36 kg N ha^{-1}, 86.8 kg ha^{-1} of 0.5 kg phosphorus pentoxide [P_2O_5], and 36 kg ha^{-1} of potassium oxide [K_2O]).

The experimental design was arranged in randomized blocks, with three treatments and four repetitions per treatment and two seasons (winter and summer), in a split plot system. The blocks were separated by weight ranges (301.1±5.8, 283.9±6.9, 269.6±8.3, and 252.2 kg ± 9.1) and divided into the following treatments: CS, without an arboreal component; ILF-1L, with eucalyptus planted in a simple line, the distance between the lines was 17–21 m, the distance between plants was 2 m, and the density was 187 trees ha^{-1}; and ILF-3L, with eucalyptus planted in triple lines, the distance between eucalyptus lines was 3 m, the distance between the eucalyptus rows was 17–21 m, the distance between plants was 2 m, and the density was 446 trees ha^{-1}.

The experimental period was from June to September 2017, corresponding to the winter, and from December 2017 to March 2018, corresponding to the summer. Seventy-two heifers originating from the ½ Angus ½ Nellore crossbreed, all with an average age of nine months, were divided into three treatments (CS, ILF-1L, and ILF-3L), totaling 24 animals per treatment and four replicates per treatment (six animals in each replicate). The paddock was repeated, with 12 paddocks used in total. The initial average weights of heifers were 276.0±21.6, 276.9±19.9, and 277.2±19.6 kg, respectively, for CS, ILF-1L, and ILF-3L.

The experimental area was 25.7 ha and the average paddock area was 2.14±0.3 ha, a total of twelve paddocks. All paddocks were provided with a drinking trough and a trough for the supplement. In the winter, the supplement had 40% crude protein (CP) and 32% total digestible nutrients (TDN), while in the summer it was 20% CP and 50% TDN. The average supplement intake was 0.1% of body weight during both seasons.

The grazing method adopted was continuous stocking with a variable stocking rate (SR), using the "put and take" technique (Mott and Lucas 1952). In each paddock, six tester animals and a variable number of regulators were used, according to the need to adjust the SR to maintain the management target, with an average pasture height of 30 cm (Euclides et al., 2014). The pasture management target in the experimental plots was monitored through measurements using a graduated ruler at 90 random points in each plot, at average intervals of 14 days during each season. The average height of the pasture was 35, 35, and 33 cm in the winter and 30, 34, and 32 cm in the summer for CS, ILF-1L, and ILF-3L, respectively.

The forage collection was carried out every 28 days. The evaluation of the total dry mass of forage (TDM) was done by cutting all material present inside a 1 × 0.5 m (0.5 m^2) metal frame, in nine representative points of each experimental plot, close to the ground and with the aid of a STIHL® brand electric brush cutter, with a model HL-KM 145° pruning bar.

The cut forage was packed in plastic bags and weighed. The sample was homogenized, and a subsample was removed from it, which was then also weighed, packed in a paper bag, and placed in an oven with forced air circulation at 65 °C, until reaching a constant weight. After drying, the subsamples were weighed again to determine the partially dry matter (DM). Based on the 0.5 m^2 sampling area, the weight of the first sample collected in the field and its DM content, the data were transformed and expressed in kilograms of the DM of forage per hectare.

For the chemical analysis of the forage, grazing simulation was performed every 28 days, with identification of the locations and parts of the plant selected by the animals throughout the experimental plot and then simulating manually the process of picking and harvesting forage, according to the methodology described by Johnson (1978).

With these samples, analyses of DM and CP contents were carried out according to the methodology described by the AOAC International (1995). The levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) were determined according to the methodology described by Van Soest (1991), as adapted by Mertens (2002). Hemicellulose was determined through the difference between the levels of NDF and ADF, while cellulose was determined according to the AOAC (1995). Mineral material (MM) was determined by burning the samples at 550 °C in a muffle furnace. *In vitro* dry matter digestibility (IVDMD) was assessed using the ANKOM® DAISYII incubator according to the technique mentioned by Tilley and Terry (1963) and the methodology of Holden (1999).

Animals were subjected to solid fasting for 16 h and weighed every 28 days on a model VF-B digital electronic scale by Valfran® with an accuracy of up to 1 kg. To assess the average daily gain (ADG), the difference between the final and initial weights was used, divided by the number of days in the period.

Weight gain per area (WGA) was calculated by multiplying ADG by the average number of animals per hectare and number of grazing days. The SR was obtained by the sum of the weight of the tester and regulator animals during grazing, divided by the area of the experimental plot, with the animal unit corresponding to 450 kg live weight.

The behavioral assessment was carried out in the winter (June 2017) and the summer (March 2018). For behavioral assessment, the methodology recommended by Martin and Bateson (1993) was used, with instantaneous and continuous harvesting and focal sampling and a 10-min interval between the samplings. The sampling was carried out directly, in continuous 12-h periods.

The behavioral variables studied were: grazing, rumination, resting, and other activities, including water and supplement intake, displacement, interaction with other animals, urination, and defecation. All activities were evaluated in two positions (in the sun and in the shade). The behavior was considered exposed to the sun when ≥ 50% of the heifer's body was under the sun and in the shade when ≥ 51%

of the heifer's body was exposed to the shade. The behavioral variables were evaluated in minutes, totaling 720 min during the whole evaluation period for each season.

Grazing time was considered the time spent on foraging and harvesting activities in the pasture, with the animal ingesting actively. Rumination time was considered the period when the animal was not grazing but chewing, characterized by repetitive and cyclic mandibular movements. The periods when the animal was not performing other activities were considered as rest.

At the time of behavioral assessment, the black globe temperature, air temperature, and relative humidity were measured and recorded every hour using HOBO® model U12-012 dataloggers (Onset Computer Corporation/EUA) with the following conditions: temperature precision, ±0.35 °C; relative humidity precision, ±2.5%; threshold value of temperature, −20 °C to +70 °C and threshold value of relative humidity 5 to 95%). The equipment was placed 1.4 m from the ground, simulating the height of the center of mass of large ruminants. The black globe was built using plastic floats painted with black mat paint, and a thermometer was inserted inside the float. The thermometer was connected in the datalogger to measure the black globe temperature. In CS, one datalogger was utilized per paddock and in ILF-1L and ILF-3L, two dataloggers were utilized for the paddock, one was positioned in the sun and other in the shade and was kept in the area until the end of behavioral assessment (6:00 until 18:00 h). Wind speed (WS) was measured every hour by a previously trained evaluator using Instrutemp ITAN-700 digital anemometer under the following condition precision, ± 0.2 m s^{-1}, threshold value 0.4 to 20 m s^{-1}.

In the CS, the equipment was placed in the sun, while in the ILF-1L and ILF-3L systems, it was placed in the sun and in the shade. After the evaluation, the following thermal comfort indexes were determined. The temperature-humidity index (THI) was calculated using the equation proposed by Thom (1959): THI = AT + 0.36 DPT + 41.2, in which AT is the ambient temperature and DPT is the dew point temperature. The black globe-humidity index (BGHI) was calculated using the equation proposed by Buffington et al. (1981): BGHI = BGT + 0.36 DPT + 41.2, in which BGT is the black globe temperature. Finally, the radiant heat load (RHL) was calculated using the equation proposed by Bond et al. (1954) apud Esmay (1978): RTL = $\sigma\,(MRT)^4$, in which σ is the Stefan–Boltzmann constant (5.67×10^{-8} W m^{-2} K^{-4}) and MRT is the mean radiant temperature, calculated using the following equation: $MRT = 100(\sqrt[4]{2.51}\,\sqrt{WS}\,(BGT - AT) + \left(\frac{BGT}{100}\right)^4)$, in which WS is the wind speed and BGT is the black globe temperature.

The experiment was designed in randomized blocks. The treatments were organized in a 3–2 split plot, with three systems (CS, ILF-1L, and ILF-3L) and two seasons (winter and summer), for ADG, WGA, final weight, SR, dry mass of forage per hectare, chemical composition of forage, and behavior. For microclimatic variables, dataloggers were placed in the shade and in the sun. The position of dataloggers determined the five treatments: CS, with dataloggers placed in the sun; ILF-1L sun, with dataloggers placed in the sun; ILF-1L shade, with dataloggers placed in the shade; ILF-3L sun, with dataloggers placed in the sun; and ILF-3L shade, with dataloggers placed in the shade.

The following mathematical model was used:

$$Y_{ijk} = \mu + \beta_j + \alpha_i + e_{ji} + €_k + (\alpha \times €)_{ik} + \varepsilon_{ijk},$$

in which in μ represents a constant common to all observations, β_j is the effect of the j-th level of the block factor, α_i is the effect of the i-th level of the system factor (plot), e_{ji} is the random error attributed to plot, $€_k$ is the effect of the k-th level of the season factor (subplot), $(\alpha \times €)_{ik}$ is the interaction between system and season, and ε_{ijk} is the random error attributed to each subplot.

Data were subjected to statistical analysis using the R program (R Development Core Team, 2009). The residue normality was tested with the Shapiro–Wilk test, and the function psub2.dbc of the ExpDes.pt package was used for analyses. Tukey's test was used to compare the adjusted means of the system and season main effects, and to study the simple effects of interactions, P<0.05 was considered significant.

3. Results

There was no treatment × season interaction for TDM (P = 0.2647), DM (P = 0.5926), CP (P = 0.5013), NDF (P = 0.0545), ADF (P = 0.7953), LIG (P = 0.6847), MM (P = 0.1487), and IVDMD (P = 0.0512). The TDM was higher in the CS and lower in the systems with the tree component (P = 0.0018), with no differences for TDM (P = 0.0991) between the seasons (Table 1). The ILF-1L and ILF-3L systems showed 1294.96 and 1372.98 kg ha^{-1}, respectively, less TDM than the CS. The DM was higher in SC (P = 0.0421) and lower in ILF-3L. There were no differences between ILF-1L system and the treatments SC and ILF-3L for DM. There were no differences for NDF (P = 0.1576), ADF (P = 0.0710), LIG (P = 0.1674), MM (P = 0.4627), and IVDMD (P = 0.3598) between the evaluated systems (Table 1). The percentage of CP was higher in the ILF-1L and ILF-3L systems and lower in the CS (P = 0.0022). The CP content of palisadegrass was 25.65% higher in the ILF-1L system and 32.22% higher in the ILF-3L system when compared with the CS.

For SR, there was a system × season interaction (P = 0.0243; Table 2). In the winter (Table 3), SR was higher in CS and lower in ILF-1L (239.31 kg) and in ILF-3L (240.61 kg) systems, while in the summer there was no difference between treatments. In the ILF-1L and ILF-3L systems, the SR in the winter was lower than in the summer, and in the CS, there were no differences in the SR between the seasons.

There were no differences between treatments for the final live weight (FLW, P = 0.7650), ADG (P = 0.7555), and WGA (P = 0.7706). With regards to FLW, ADG, and WGA (Table 2), a difference was observed between the two seasons (P<0.0001). Average daily gain, WGA, and SR were 0.390, 147.51, and 138.71 kg, respectively, which were superior in the summer than in the winter.

There was no treatment × season interaction for the microclimatic variables and thermal comfort indexes (Table 4). The ambient temperature (P = 0.0017), BGT (P = 0.0035), THI (P = 0.0113), and BGHI (P = 0.0018) were lower in the shade of the ILF-1L and ILF-3L systems in relation to the CS. The ambient temperature was 1.53 and 1.73 °C, and the BGT was 4.20 and 4.29 °C in the ILF-1L and ILF-3L systems, respectively. The BGHI of ILF-1L shade was 3.27 and ILF-3L shade was 4.33, which was lower than in CS. The relative humidity was 3.12% higher in ILF-1L shade and 3.63% in ILF-3L shade than that in

Table 1 - Total dry mass of forage (TDM) and chemical composition of *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandu in the conventional system (CS) and the integrated livestock-forest systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	TDM (kg ha^{-1})	DM (%)	CP (%)	NDF (%)	ADF (%)	LIG (%)	MM (%)	IVDMD (%)
System								
CS	4250.00a	33.42a	10.80b	60.04	30.49	2.74	8.57	55.12
ILF-1L	2955.04b	31.60ab	13.57a	61.12	32.39	2.78	8.89	54.75
ILF-3L	2877.02b	30.85b	14.28a	60.85	31.56	3.23	8.93	53.38
Season								
Winter	3247.01	34.15a	11.67b	60.28	31.02	3.03	8.82	56.00a
Summer	3474.30	29.75b	14.09a	61.07	31.94	2.80	8.77	54.91b
P-value								
System	0.0018	0.0421	0.0022	0.1576	0.0710	0.1674	0.4627	0.3598
Season	0.0991	0.0014	<0.0001	0.1103	0.1690	0.4381	0.0585	0.0271
Interaction	0.2647	0.5926	0.5013	0.0545	0.7953	0.6847	0.1487	0.0512
CV system	14.02	4.92	9.04	1.64	2.42	16.73	6.71	3.96
CV season	6.89	7.44	4.98	2.49	5.71	20.60	2.04	1.69

DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; LIG - lignin; MM - mineral matter; IVDMD - *in vitro* dry matter digestibility; CV - coefficient of variation (%).
a,b - Means followed by different letters differ significantly by Tukey's test (P<0.05).

Table 2 - Average daily gain (ADG) of ½ Angus ½ Nelore heifers, stocking rate (SR), and weight gain by area (WGA) in the conventional system (CS) and in integrated livestock-forest (ILF) systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	FLW (kg)	ADG (kg day^{-1})	WGA (kg)	SR (kg ha^{-1})
System				
CS	339.57	0.492	299.28	1053.61a
ILF-1L	346.59	0.534	287.59	914.02b
ILF-3L	344.68	0.522	282.47	859.10b
Season				
Winter	305.99b	0.315b	71.13b	872.90b
Summer	382.59a	0.705a	218.64a	1011.61a
P-value				
System	0.7650	0.7555	0.7706	0.0141
Season	<0.0001	<0.0001	<0.0001	0.0003
Interaction	0.3053	0.3036	0.3568	0.0243
CV system	3.64	12.81	16.15	9.73
CV season	2.35	13.49	19.32	6.51

FLW - final live weight; CV - coefficient of variation (%).
a,b - Means followed by different letters differ significantly by Tukey's test (P<0.05).

Table 3 - Stocking rate (kg ha^{-1}) in the conventional system (CS) and in the integrated livestock-forest systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	CS	ILF-1L	ILF-3L
Winter	1032.91Aa	793.60Bb	792.30Bb
Summer	1074.33Aa	1034.51Aa	925.92Aa

Lowercase letters compare means in the rows, while uppercase letters compare means in the columns, in which the same lowercase letters and the same uppercase letters do not differ significantly by Tukey's test (P>0.05).

Table 4 - Microclimatic variables and thermal comfort indices in the conventional system (SC) and in integrated livestock-forest systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	AT (°C)	RH (%)	BGT (°C)	WS (m s^{-1})	THI	BGHI	RHL
System							
CS	26.58a	54.94b	31.49a	1.42a	74.00a	78.34a	556.20a
ILF-1L sun	26.02a	55.04b	30.47a	1.07b	73.41a	76.84a	522.34ab
ILF-1L shade	25.00b	58.06a	27.29b	1.05b	72.26b	75.07b	491.23b
ILF-3L sun	26.11a	54.88b	31.19a	0.86b	73.48a	78.24a	529.49ab
ILF-3L shade	24.85b	58.57a	27.20b	1.04b	72.24b	74.01b	488.45b
Season							
Winter	18.10b	55.87	21.31b	1.94a	62.84b	65.19b	477.92b
Summer	33.32a	57.13	37.75a	0.24b	83.30a	87.84a	557.16a
P-value							
System	0.0017	0.0014	0.0035	0.0313	0.0113	0.0018	0.0030
Season	<0.0001	0.0502	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction	0.2098	0.01826	0.4739	0.9991	0.2576	0.2022	0.5442
CV system	2.15	2.12	5.59	21.26	0.67	1.82	4.18
CV season	2.82	3.32	5.78	81.80	1.21	1.40	3.33

AT - ambient temperature; RH - relative humidity; BGT - black globe temperature; WS - wind speed; THI - temperature-humidity index; BGHI - black globe-humidity index; RHL - radiant heat load; CV - coefficient of variation (%).
a,b - Means followed by different letters differ significantly by Tukey's test (P<0.05).

CS, but it was not significantly different in ILF-1L and ILF-3L sun (P = 0.0014). The WS was higher in CS (P = 0.0313).

The RHL was 11.7% lower in the ILF-1L shade and 12.2% lower in the ILF-3L shade compared with the CS. The ILF-1L sun and ILF-3L sun did not differ from the other treatments (P = 0.0030).

In the evaluated seasons, the summer had higher AT, BGT, THI, BGHI, and RHL, as well as lower WS (P<0.0001). Relative humidity did not differ between seasons (P = 0.0502).

The behavior of ½ Angus ½ Nellore heifers was altered by the presence of the tree component in the pasture (Table 5). Heifers spent less time grazing (279.61 and 285.73 min), ruminating (68.35 and 67.80 min), resting (140.43 and 149.60 min), and performing other activities (45.81 and 41.32 min) in the ILF-1L and ILF-3L in the sun, respectively, compared with CS. The animals that remained in the ILF-1L and ILF-3L systems spent the same time grazing, ruminating, resting, and performing other activities in the sun and shade (Table 5). In the evaluated systems, when the pasture had an arboreal component, the animals preferred to carry out their activities in the shade and not in the sun and increased their total rumination (Table 5 and Figure 1).

There was a treatment × season interaction (Table 5) regarding the time heifers spent ruminating (P = 0.0446), resting (P = 0.0043), performing other activities (P = 0.0009) in the sun as well as in total rumination (P = 0.0297) and total resting time (P = 0.0120).

In the summer, total rumination time of heifers in ILF-1L and ILF-3L remained longer (37.59 and 93.6 min, respectively) than in CS (Table 6). In contrast, in the same season, the heifers that were kept in the CS spent more time ruminating in the sun, in particular, 110.28 and 111.5 min more than in ILF-1L and ILF-3 L, respectively. The rumination behavior in the sun was 48.25 and 51.79 min higher in the winter for heifers that were kept in ILF-1L and ILF-3L systems, respectively.

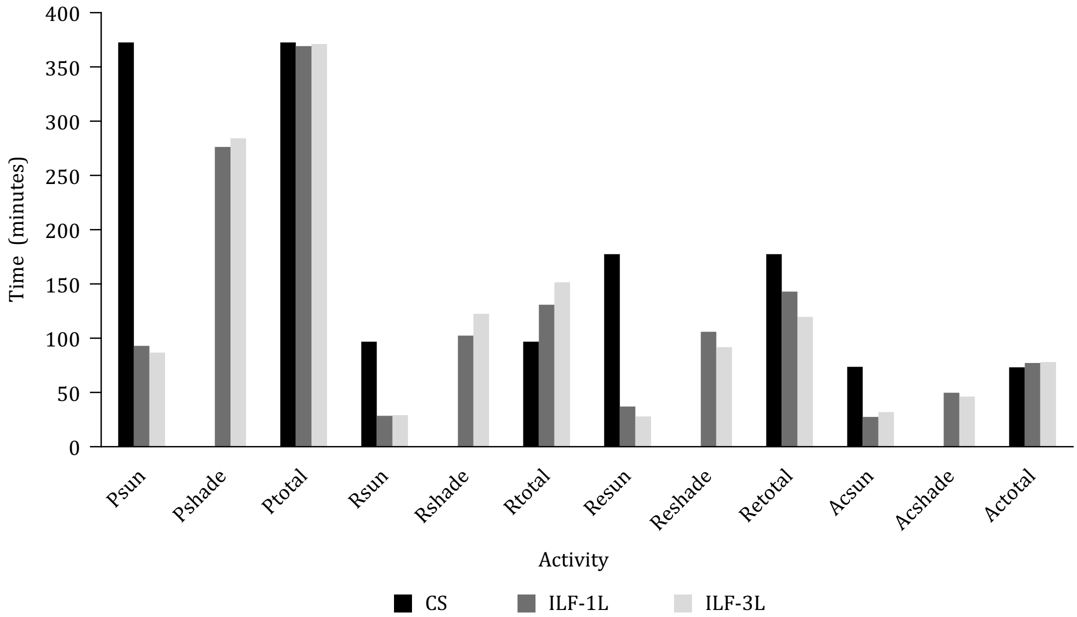
In the summer, animals that remained in CS exhibited a longer time resting, spending 135 min more than heifers in ILF-1L and ILF-3L in this activity. The same pattern was observed for resting in the sun, and heifers in CS spent 244.22 and 252.90 min more than heifers kept in ILF-1L and ILF-3L systems, respectively.

Table 5 - Behavior of ½ Angus ½ Nellore heifers, in minutes, in conventional system (CS) and in integrated livestock-forest systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	Psun	Pshade	Ptotal	Rsun	Rshade	Rtotal	Resun	Reshade	Retotal	Acsun	Acshade	Actotal
System												
CS	372.53a	-	372.53	96.81a	-	96.81b	177.49a	-	177.49	73.16a	-	73.16
ILF-1L	92.92b	276.30	369.22	28.46b	102.29	130.75a	37.06b	105.87	142.93	27.35b	49.75	77.10
ILF-3L	86.80b	284.24	371.04	29.01b	122.38	151.39a	27.89b	91.66	119.55	31.84b	46.19	78.03
Season												
Winter	228.55a	178.34	406.87a	62.16	32.12b	94.29b	70.70	56.61	127.30	58.46a	33.07	91.53a
Summer	139.62b	195.37	334.98b	40.68	117.65a	158.34a	90.92	75.08	166.01	29.77b	30.89	60.66b
P-value												
Systems	0.0028	0.8325	0.9863	0.0047	0.3938	0.0164	0.0045	0.6014	0.1448	0.0261	0.7841	0.9626
Season	0.0175	0.4598	0.0278	0.1293	0.0010	0.0005	0.2934	0.1370	0.0912	0.0001	0.7703	0.0171
Interaction	0.5544	0.3692	0.1626	0.0446	0.0694	0.0297	0.0043	0.2104	0.0120	0.0009	0.5038	0.0936
CV system	36.34	20.41	9.28	36.07	28.74	14.50	48.28	40.53	26.98	43.55	41.08	42.43
CV season	31.58	19.33	14.24	50.42	23.25	16.16	46.11	26.18	27.56	16.06	37.62	26.34

Psun - pasture in the sun; Pshade - pasture in the shade; Ptotal - total pasture; Rsun - rumination in the sun; Rshade - rumination in the shade; Rtotal - total rumination; Resun - resting in the sun; Reshade - resting in the shade; Retotal - total resting; Acsun - other activities in the sun; Acshade - other activities in the shade; Actotal - total other activities; other activities included: interaction with other animals, drinking water, salt licking, urination, defecation, and displacement; CV - coefficient of variation (%).
a,b - Means followed by different letters differ significantly by Tukey's test (P<0.05).

The other activities in the sun (interaction with other animals, water drinking, salt licking, urination, defecation, and displacement) were performed by animals with less intensity in CS, 63.89 min less in the summer than that in the winter. In systems with an arboreal component (ILF-1L and ILF-3L), there were no differences in these behaviors between seasons.



Psun - pasture in the sun; Pshade - pasture in the shade; Ptotal - total pasture; Rsun - rumination in the sun; Rshade - rumination in the shade; Rtotal - total rumination; Resun - resting in the sun; Reshade - resting in the shade; Retotal - total resting; Acsun - other activities in the sun; Acshade - other activities in the shade; Actotal - total other activities; other activities included: interaction with other animals, drinking water, salt licking, urination, defecation, and displacement.

Figure 1 - Activities of ½ Angus ½ Nellore heifers in the conventional system (CS) and in integrated livestock-forest (ILF) systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L).

Table 6 - Time (min) spent on total rumination, rumination in the sun, total resting, resting in the sun, and activities in the sun of ½ Angus ½ Nellore heifers, in the conventional system (CS) and in integrated livestock-forest systems with a density of 187 eucalyptus ha^{-1} (ILF-1L) and 446 eucalyptus ha^{-1} (ILF-3L) in the winter and summer

	CS	ILF-1L	ILF-3L
Total rumination			
Winter	79.01Aa	109.29Ba	94.57Ba
Summer	114.61Ab	152.20Aa	208.21Aa
Rumination in the sun			
Winter	79.02Aa	52.58Aa	54.90Aa
Summer	114.61Aa	4.33Bb	3.11Bb
Total resting			
Winter	98.35Aa	164.85Aa	118.72Aa
Summer	256.63Ba	121.01Ab	121.00Ab
Resting in the sun			
Winter	98.34Aa	61.70Aa	52.04Aa
Summer	256.63Ba	12.41Ab	3.73Ab
Activities in the sun			
Winter	105.11Aa	30.64Ab	39.64Ab
Summer	41.22Ba	24.05Aa	24.04Aa

In the columns, means followed by the same lowercase letters and the same uppercase letters do not differ significantly according to Tukey's test (P>0.05).

4. Discussion

In this study, we observed that livestock-forest systems influence forage characteristics, WGA, SR, thermal comfort, and behavior of heifers (½ Angus ½ Nellore), in winter and summer, by expanding the knowledge of ILF systems in different tree densities. Previous studies showed a reduction in TDM and increase in CP in integrated crop livestock systems with trees (Paciullo et al., 2011a; Paciullo et al., 2011b; Oliveira et al., 2014; Lopes et al., 2017), which was also verified in the results of this study (Table 1).

The reduced TDM in ILF-1L and ILF-3L systems is attributed to the reduced light in the system as well as competition between trees and forage for nutrients and water (Pontes et al., 2018; Oliveira et al., 2014). Furthermore, the shade reduced tiller density, changed forage structure to promote the elongation of stem (Paciullo et al., 2008), and reduced the accumulation rates (Paciullo et al., 2008; Andrade et al., 2004), which are factors that also contribute to the reduction of TDM in experimental period.

It was expected that in the winter the TDM would be lower than in the summer due to the seasonality of forage production, resulting from the low temperature and reduced water availability (Paciullo et al., 2011a). However, there was no such difference between the seasons (Table 1); this fact can be explained by fertilization and deferred grazing on the pasture before starting the experiment. This approach provided greater forage offer in all systems during the winter, and thus there was no difference from the summer.

The increase in the CP content in systems with the tree component occurs due to shading, which provides less plant growth, causing an imbalance in the assimilation of carbon and nitrogen exceeding the metabolic capacity (Dale and Causton, 1992). Lopes et al. (2017) affirmed that the increase in CP in shaded plants occurs due to the lower dilution and translocation of the absorbed nitrogen among the aerial parts in relation to the non-shaded plants, which have less forage mass and higher CP levels (Table 1). Although an increase was observed in CP, no differences were observed in IVDMD in the evaluated systems, which is correlated with NDF, ADF, and lignin levels that do not differ between treatments.

The higher CP content of the forage and a TDM reduction in the ILF-1L and ILF-3L systems did not influence the FLW, ADG, or WGA, which were similar in all treatments (Table 2). However, the lower TDM resulted in a lower SR (Tables 1 and 2) in the shaded systems. In addition to the previously mentioned competition between the forage and trees, the eucalyptus occupied part of the paddocks, reducing the useful area of the pasture, which also contributed to the lower SR in the ILF-1L and ILF-3L systems. Another change observed in the forage was in DM, which was lower in the system with a higher density of trees (ILF-3L) because lower WS and solar radiation contribute to maintain higher humidity (Table 4) and decrease the DM.

In the CS, the SR was the same in the winter and the summer (Table 3) due to the adopted management practice that included pasture deferring. This strategy minimizes the effects of seasonality on the production of tropical grasses and allows reserving pasture areas at the end of summer to be used in the winter, a period when forage shortage occurs (Teixeira et al., 2011). According to this strategy, there was forage reserve in the paddocks in the winter, helping to reduce the differences between the winter and summer.

In the ILF-1L and ILF-3L systems, although there was no system × season interaction (P>0.05) for the TDM, the smaller TDM (Table 1) contributed to the lower SR in the winter. The ILF systems also went through the same deferred grazing management practices as the CS, but because winter presents unfavorable conditions for the growth of grasses—that is, less precipitation and light—, it may have increased the competition for water and light between trees and forage, reducing the forage accumulation rate and, consequently, decreasing SR during this season. In the summer, SR was similar for all treatments, probably because the conditions were favorable for the growth of grasses and trees and, hence, did not result in competition between the different components of the system.

The lower TDM (Table 1) found in the ILF-1L and ILF-3L systems decreased SR (Table 3) in relation to the CS, but the presence of trees did not affect WGA. The higher amount of CP in the forage (Table 1), in ILF-1L and ILF-3L, possibly compensated for the lower TDM, contributing to the similar WGA in the tested systems.

Environmental changes also were observed in the systems. The ambient temperature, black globe temperature, and the thermal comfort indexes (THI, BGHI, and RHL) were more favorable for heifers kept in the shade of the ILF-1L and ILF-3L systems (Table 4) than for those in the CS.

Temperature-humidity index is considered alert category to thermal stress by Brown-Brandl et al. (2005), when the values are greater than or equal to 74 and lower than 78. In the systems evaluated (Table 4), the results were in this range for SC and lower values were found for shaded ILF-1L and ILF-3L systems. The BGHI of ≥ 79 is considered as the beginning of thermal stress in cattle (Pezzopane et al., 2019). The BGHI observed in SC was close to this value and BGHI in ILF-1L and ILF-3L system shade were the lowest indexes. The same reduction in RHL was observed in the shade of the ILF-1L and ILF-3L systems (Table 4) in relation to the CS. This thermal comfort index is considered a good predictor for assessing thermal comfort conditions when considering systems that make use of shading, because the shade protects animals from the stress caused by solar radiation (Van laer et al., 2015).

Therefore, shading provides a better microclimate and positively affects the behavior of ½ Angus ½ Nellore heifers. The animals that remained in the ILF-1L and ILF-3L systems performed their activities more frequently in the shade (Table 5 and Figure 1), where there were lower temperatures and better levels of thermal comfort.

During the summer, heifers that were kept in the ILF-1L and ILF-3L systems spent more time on total rumination and less time on rumination in the sun, preferring to perform these activities in the shade (Table 6), because the trees reduce solar radiation, thus protecting the animal from thermal stress (Van laer et al., 2015; Giro et al., 2019). In the winter, when temperatures are milder and the thermal comfort index is more suitable for heifers (Tables 4 and 6), the frequency of this activity was the same for all treatments.

The total resting and resting in the sun behavior was higher for animals that remained in the CS in summer, because temperatures and thermal comfort indexes were higher (Tables 4 and 6) during this season. The lack of shade in this treatment increased the time spent resting due to a strategy aimed at decreasing physical activities that increase heat production, such as walking, grazing, and ruminating (Vizzotto et al., 2015). Cattle use rest during periods of heat as a regulatory mechanism to dissipate latent heat more efficiently (Porto et al., 2018). In the winter, when temperatures are milder (Table 4), there was no difference in the time spent by animals resting in the sun between the evaluated treatments.

Based on the above results, when environmental and climatic conditions are not favorable, the animal remains at rest as a strategy to reduce the use of energy in the thermal regulation process. This phenomenon explains the lower intensity of other activities performed in the sun (interaction with other animals, drinking water, salt licking, urinating, defecating, and displacement) in the CS during the summer (Table 6). In this study, evaluations were made during the day, and it is likely that the animals intensified these activities at night when, as reported by Pezzopane et al. (2019), there is a decrease in temperature and improvement in thermal comfort.

Despite beneficial behavioral changes observed with the introduction of trees in the production systems, heifers did not show an increased ADG in the ILF-1L and ILF-3L systems. This finding is consistent with Oliveira et al. (2014) and Trivelin et al. (2020), who did not observe greater daily average weight gains in Nellore cattle reared in integrated crop-livestock systems with a tree component.

5. Conclusions

The better forage quality in ILF-1L and ILF-3L does not alter the performance of ½ Angus ½ Nellore heifers or weight gain per area, despite the decreased total dry mass of forage and stocking rate. The ILF-1L and ILF-3L systems provide better thermal comfort to animals, and when trees are present, animals prefer to perform activities in the shade, particularly in the summer, resulting in an increase in rumination time and decrease in resting time. Heifers (½ Angus ½ Nellore) that do not have access to shade reduce their activities as a strategy to reduce the use of energy in the thermal regulation process.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.M.F. Santos, C. Andrighetto, G.P. Mateus and G.C. Lupatini. Data curation: J.M.F. Santos, C. Andrighetto, M.S. Daldon, L.P. Lima and P.A. Luz. Formal analysis: J.M.F. Santos, C. Andrighetto and P.A. Luz. Funding acquisition: C. Andrighetto and G.P. Mateus. Investigation: J.M.F. Santos, C. Andrighetto, G.P. Mateus, G.C. Lupatini, M.S. Daldon, L.P. Lima and H.J.S. Bello. Methodology: J.M.F. Santos, C. Andrighetto, G.P. Mateus, G.C. Lupatini, M.S. Daldon, L.P. Lima and H.J.S. Bello. Project administration: J.M.F. Santos, C. Andrighetto and G.P. Mateus. Supervision: J.M.F. Santos and C. Andrighetto. Writing-original draft: J.M.F. Santos, C. Andrighetto and P.A. Luz. Writing-review & editing: J.M.F. Santos, C. Andrighetto, G.P. Mateus and G.C. Lupatini.

Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the support of the research (Processes: 2017/05944-8 and 2019/20039-5) and the scholarship (Process 2016/25385-0), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship.

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