Seasonal performance and behaviour of Nellore cattle in integrated crop-livestock systems

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Abstract

The first objective of this study was to assess Marandu palisade grass in an integrated crop-livestock system (ICL), and two integrated crop-livestock-forest (ICLF) systems. The first ICLF had a single row of eucalyptus (ICLF-1L) and the second had three rows of eucalyptus (ICLF-3L). The second objective was to assess the performance, behaviour, and thermal comfort of Nellore cattle in these systems during summer and winter. The data were collected for two years after the systems were implemented. Sixty Nellore cattle were used. Dry forage mass (DFM) was similar in all systems with a higher percentage of leaves observed in ICLF-3L in winter compared with ICL. Forage in the ICLF-1L and ICLF-3L systems contained more crude protein (CP) than ICL. The radiant thermal load was lower for ICLF-1L and ICLF-3L, with a lower globe temperature in ICLF-3L. The stocking rate was lowest for ICLF-3L. Two years after the systems had been established, the trees provided low density shade and implementing the ICLF-1L and ICLF-3L systems improved the CP content of the grass without changing the levels of DFM and performance of the cattle. The trees reduced the total thermal energy exchanged between the animal and the environment, improving thermal comfort, but did not influence cattle behaviour or their haematological parameters.

Introduction

Concern is growing over the protection of life on Earth, which is critical to the survival of human beings. With each succeeding societal generation, there is greater awareness of the need to produce meat, while considering the importance of socio-economic and environmental sustainability principles. Beef cattle production is usually identified for its key role in causing negative impacts to the environment, particularly in Brazil, where the most common production model is the extensive grazing system (Amaral *et al.,* 2012).

Beef cattle systems need to be adjusted to optimize the production process without damaging the environment. Integrated crop-livestock systems and ICLF are considered innovative tools that take advantage of the synergy between crop, livestock, and forest, thereby increasing livestock productivity and ensuring economic and environmental sustainability (FAO, 2010).

These systems are managed in various ways, such as intercropping, crop succession, and rotation of crops and pasture areas. Forage in ICL systems benefits from residual fertilization from annual crops (Adriano *et al.,* 2015). Consequently, these systems increase production of higher quality forage, which augments meat production per area, in comparison with systems that use conventional pasture (Kichel *et al.,* 2014).

In systems where trees are introduced, competition can occur between key components of the system. This could lead to a reduction in forage growth (Oliveira *et al.,* 2014) and competition for nutrients,

water and light (Soares *et al.,* 2009). However, the trees could modify the microclimate, increasing the thermal comfort of cattle and improving animal welfare (Souza *et al.* 2010b; Giro *et al.,* 2019).

The density and arrangement of trees in ICLF systems determine the conditions of the environment for forage growth and thermal comfort of the cattle, so it is important to evaluate the interaction of system components at the beginning of implementation to determine when and in what ways they interact.

The objective of this study was to assess forage characteristics and the performance, behaviour, thermal comfort, and haematological parameters of Nellore cattle in an ICL system, an ICLF system with a single row of trees and a density of 196 eucalyptus stems ha^{-1}, and an ICLF system with three rows of trees and a density of 448 eucalyptus stems ha^{-1} in summer and winter. Data were collected for this study two years after the systems had been implemented.

Material and Methods

The experiment was conducted according to the ethical principles for animal tests, as determined by the Ethics Committee on the Use of Animals (CEUA) of the College of Agricultural and Technological Sciences, São Paulo State University (UNESP) (Protocol No. 26/2014). The experiment was conducted at São Paulo State Agribusiness Technology Agency, in Andradina (Latitude: -20° 53' 26.99" South, Longitude: -51° 22' 26.39" West) at an altitude of 400 m in São Paulo State.

The regional climate is Aw, according to the Köppen system, that is, tropical savanna with dry winters (Alvares *et al.,* 2013). The experiment was designed in the first half of 2012, when treatments and paddock divisions were set up. The study area soil was classified as dystrophic Red-Yellow Argisol (Santos *et al.,* 2013). The average ground slope was 6%.

In July 2012, soil samples from 0 to 20 cm depth were analysed and had these attributes: pH (calcium chloride) 4.8; organic matter 16 g dm^{-3}; phosphorus 3 mg dm^{-3}; the cations potassium 1.9; calcium 7; magnesium 5, and aluminium hydride 20 mmolc dm^{-3}; sulfate 1 mg dm^{-3}; and base saturation was 42 g/kg^{-1}. The soil was composed of clay 107 g/kg^{-1}, silt 113 g/kg^{-1}, and sand 780 g/kg^{-1}.

Trees (eucalyptus clone I-224) were planted between December 2012 and March 2013, following contours in the area. Soya beans (BMX Potência) were grown in December 2012 and harvested in May 2013. Maize (DKB 390 maize hybrid) with palisade grass (*Urochloa brizantha* syn*. Brachiaria brizantha* Marandu) were grown in December 2013 and harvested for silage in March 2014. The crops were used for pasture recovery in the three systems.

In December 2014, the forage was standardized with mechanical cutting 15 cm from the soil, followed by nitrogen fertilization with 40 kg/ha^{-1} N in the form of urea. The evaluation started two years after the system had been established. During the experiment, the height of the eucalyptus was 9.0 ± 1.8 m for ICLF-1L and 9.9 ± 1.7 m for ICLF-3L and the diameter at breast height was 9.6 ± 1.7 cm and 9.3 ± 1.3 cm for ICLF-1L and ICLF-3L, respectively.

The experimental period lasted from December 2014 to March 2015, corresponding to summer, and from June to September 2015, corresponding to winter. The precipitation and average ambient temperature were 332.8 mm and 26.7 °C in summer, and 122.7 mm and 23.8 °C in winter.

The total area used for the experiment was 25.7 ha, and the average paddock area was 2.1 ± 0.3 ha. This was divided into 12 paddocks. The first treatment was ICL. The second treatment of ICLF-1L consisted of integrated crop-livestock-forest with eucalyptus planted in a single row, with 17 to 21 m between rows. The distance between plants was 2 m, and the density was 196 eucalyptus trees per ha^{-1}. Treatment ICLF-3 L consisted of ICLF with eucalyptus planted in three rows. The distance between rows was 3 m. The distance between plants was 2 m, and the distance between the eucalyptus bands was 17 to 21 m. There was a density of 448 eucalyptus trees per ha^{-1}.

The experiment was designed in randomized blocks. The blocks were divided by weight range (244 ±17 kg; 218 ± 13 kg; 200 ± 6.2 kg; and 184 ± 7.4 kg) and were organized in a 3 × 2 split plot design, with three systems (ICL, ICLF-1L, and ICLF-3L) and two seasons (winter and summer). Sixty Nellore cattle were used at an initial age of 16 ± 2.81 months. The initial average weights of the cattle were 217 ± 30.6, 208 ±20.8, 210 ± 29.5 kg for ICL, ICLF-1L, and ICLF-3. There were no statistically significant (*P* >0.05) differences in the initial weights of the cattle between treatments. The animals were divided into the three treatments, with a total of 20 animals per treatment and four replicates (paddocks) per treatment, consisting of five animals per replicate.

At the beginning of the experiment, the animals were vaccinated against clostridiosis and submitted to anthelmintic treatment using levamisole phosphate. This was administered at a dosage of 1 ml for each 40 kg of live weight, according to the manufacturer's instructions. During the experiment, faeces were checked for eggs per gram every 28 days to monitor for endoparasites. The cattle grazed only in the paddocks, and were not fed other forage. They received supplements containing 20 g kg^{-1} crude protein, 68 g kg^{-1} total

digestible nutrients and minerals, with consumption of 0.1 g/kg^{-1} live weight. Consumption was monitored once a day, and the supplement and leftovers were weighed. The cattle were castrated at 22 months old.

The grazing method consisted of continuous stocking with a variable stocking rate, using the 'put and take' technique (Mott & Lucas, 1952). Six tester animals and a variable number of regulators were used in each paddock in line with the need to adjust the stocking rate to maintain the management target, with an average pasture height of 30 cm (Euclides *et al.,* 2014). The pasture management target was monitored with a graduated ruler at 90 random points in each plot. This was undertaken at average intervals of 14 days during each season. Stocking rate was adjusted according to pasture height.

Forage was collected every 28 days. The total dry mass of the forage was evaluated by cutting all material inside a 1 × 0.5 m (0.5 m²) metal frame at nine representative points in each plot to 5 cm from the ground, using a STIHL® electric brush cutter and a model HL-KM 145° pruning bar.

The cut forage was packed into plastic bags and then weighed. The sample was homogenized, and a subsample was removed, weighed, packed in a paper bag, and placed in an oven with forced air circulation at 65 °C until it reached a constant weight. The subsamples were weighed again to determine the weight of the partially dry matter. 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 DM of forage was expressed in kg/ha^{-1}.

To evaluate the morphological composition of the forage, a second subsample was extracted from the field sample before drying and weighing to determine the forage mass. This was then manually separated into leaf, stem/pseudostem, and senescent material. Subsequently, each component was placed in a paper bag and weighed. The bagged material was then placed in a forced air ventilation oven at 65 °C until a constant weight was reached. It was then weighed again to calculate DM. The morphological components were expressed as a percentage of the forage mass and converted to the DM of each component, which was expressed as kg/ha^{-1}.

Bromatological analysis of the forage was conducted every 28 days, using a grazing simulation technique. This involved identifying localities and plant parts that the cattle preferred in various areas of the plot. The process of harvesting the forage was simulated manually according to Johnson (1978). The collected material was dried in a forced air circulation oven at 65 °C until it reached a constant weight. It was then stored for chemical analysis. The DM content at 105 °C and CP level were analysed according to the AOAC (1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest (1991), as adapted by Mertens (2002). The mineral matter (MM) content was obtained by burning the samples in a muffle furnace at 600 °C. These analyses were performed at the Bromatology Laboratory of the College of Veterinary Medicine and Animal Science (UNESP).

The cattle were fasted for 16 hours before each monthly weigh in. They were weighed every 28 days on a model VF-B digital electronic scale by Valfran®, with an accuracy of 1 kg. The difference between the final and initial weights was divided by the number of days to evaluate the average daily weight gain (ADG). The weight gain per area (WGA) was calculated by multiplying the ADG by the average number of animals per hectare and the number of grazing days. The stocking rate was calculated as the sum of the weight of the grazing tester and regulator animals, divided by the plot area, with one animal unit (AU) corresponding to 450 kg of live weight.

Cattle behaviour was assessed according to Martin & Bateson (1986), who used continuous monitoring with focal sampling at 10-minute intervals. Sampling took place for 12 hours (06h00 to 18h00). All cattle in the paddocks were observed. Behavioural assessments were conducted in summer (28 February 2015) and winter (15 August 2015).

The behavioural types that were observed included grazing, ruminating while standing and lying, resting while standing and lying, and other activities, interacting with other animals, drinking water, licking salt, urinating and defaecating, and displacement. The evaluators remained at a distance that did not interfere with the animal's behaviour. The results of the behavioural data were presented as frequencies in relation to the percentage of the total evaluation period for each season.

Grazing time was defined as the time spent searching for and picking forage. Rumination time was defined as the time that the animal was chewing not grazing. This was characterized by repetitive and cyclic mandibular movements. Resting behaviour was defined as the time that the animal did not perform other activities.

During the behavioural assessment, black globe temperature, air temperature, and relative humidity were measured and recorded using Onset HOBO® U12-012 data logger. This equipment was placed at a height of 1.4 m, simulating the height of the centre of mass of large ruminants. Wind speed (WS) was measured every hour by a trained evaluator using a low-cost LM-81AM digital anemometer (Lutron®).

In the ICL system, the equipment was placed in the sun. In the ICLF systems the equipment was placed in the sun and in the shade. After the evaluation, several thermal comfort indices were determined. The temperature and humidity index (THI) was calculated using the equation proposed by Thom (1959):

$$THI = AT + 0.36DPT + 41.5$$

where AT = ambient temperature and DPT = dew point temperature. The black globe temperature and humidity index (BGHI) were calculated according to Buffington *et al.* (1981):

$$BGHI = BGT + 0.36DPT + 41.5$$

where BGT = black globe temperature and DPT = dew point temperature. The radiant thermal load (RTL) was calculated using the equation proposed by Bond *et al.* (1954) and Esmay (1969):

$$RTL = \sigma MRT^4$$

where σ = Stefan–Boltzmann constant (5.67 x 10^{-8} W m^{-2} K^{-4}) and MRT = mean radiant temperature, calculated as:

$$MRT = 100\sqrt[4]{2.51\sqrt{ws}(BGT - AT) + \left(\frac{BGT}{100}\right)^4}$$

where ws = wind speed, BGT = black globe temperature, and AT = ambient temperature.

To record the microclimate variables, dataloggers were placed in the shade and in the sun. The position and luminosity therefore determined the treatments, which were divided into ICL, ICLF-1L in the sun and in the shade, and ICLF-3L in the sun and in the shade, totalling five treatments.

The day after behaviour assessment, blood samples were collected to analyse haematological parameters. The samples were collected by puncturing the jugular vein using 5 mL Vacutainer® vacuum tubes with ethylenediaminetetraacetic acid as an anticoagulant. Immediately after collection, refrigerated blood samples were sent to the clinical analysis laboratory of Andradina Veterinary Hospital, Andradina, SP. Haematological analysis was performed with the Poch-100 iv Diff automatic veterinary haematological analyser (Sysmex do Brasil Industria e Comércio Ltda., São Paulo, Brazil), which provided the global counts of leukocytes, erythrocytes, haemoglobin, platelets and lymphocytes, and calculated the globular volume.

In the data analysis, the paddock was considered an experimental unit for all variables. Data were analysed with the R program (R Development Core Team, 2009). This mathematical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ij} + \epsilon_k + \alpha\epsilon_{ik} + \varepsilon_{ijk}$$

where μ = a constant common to all observations, α_i = the effect of the ith level of the system factor, β_j = the effect of the jth block, e_{ij} = the random whole-plot error, ϵ_k = the effect of the kth season, $\alpha\epsilon_{ik}$ = the interaction between system and season, and ε$_{ijk}$ is the random subplot error. Normality of errors 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 system and season main effects, and to study the simple effects of interactions. *P* <0.05, was considered significant, and the presence of trends was discussed if *P* was > 0.05 and ≤ 0.10.

Results and Discussion

Table 1 shows the DFM and morphological composition of the palisade grass *Urochloa brizantha* (syn. *Brachiaria brizantha*) in an ICL system, and in ICL system and ICLF systems in summer and winter. No differences were found between the variables in the treatments (*P* >0.05). However, differences were observed for dry forage mass (DFM) per hectare and the percentage of stems. These variables showed higher values in data collected during summer. In contrast, the percentage of senescent material was higher during winter (*P* <0.05).

Table 1 Dry forage mass and morphological composition of palisade grass (*Urochloa brizantha syn. Brachiaria brizantha*), in an integrated crop-livestock system and integrated crop-livestock-forest systems in

	Dry forage mass, kg ha^{-1}	Stem to leaf ratio, %	Senescent material, %
Treatment			
ICL	5852.7	30.06	42.64
ICLF-1L	5405.2	32.67	40.63
ICLF-3L	5150.9	32.42	42.74
Season			
Summer	6852.3^a	36.84^a	24.63^b
Winter	4086.9^b	26.60^b	59.38^a
P-value			
Treatment	0.3937	0.3007	0.6860
Season	<0.0001	<0.0001	<0.0001
Interaction	0.1738	0.9440	0.1739
Coefficient of variation, %			
Whole plot	17.54	10.55	12.66
Subplot	7.13	13.46	14.58

^{a,b} Within columns, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3L: integrated crop-livestock forest with three rows of eucalyptus

There was an interaction between treatment and season for leaf percentage and leaf-to-stem ratio (*P* <0.05). In the summer, leaf percentages and the leaf-to-stem ratio were higher in the ICL and ICLF-1L systems and lower in the ICLF-3L (*P* <0.05). However, in winter ICLF-3L showed higher percentages of leaves in the leaf-to-stem ratio, with lower values for ICL and ICLF-1L (*P* >0.05) (Table 2).

Table 2 Leaf percentage and leaf to stem ratio of palisade grass (*Urochloa brizantha syn. Brachiaria brizantha*) in integrated crop-livestock system and integrated crop-livestock-forest systems in summer and winter

	ICL	ICLF-1L	ICLF-3L
Leaf, %			
Summer	42.56^{Aa}	39.80^{Aa}	33.21^{Ab}
Winter	12.02^{Bb}	13.60^{Bb}	16.46^{Ba}
Leaf-to-stem ratio			
Summer	1.26^{Aa}	1.15^{Aa}	0.88^{Ab}
Winter	0.51^{Bb}	0.51^{Bb}	0.63^{Ba}

^{A,B} Within columns, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
^{a,b} Within rows, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock forest with three rows of eucalyptus

Regarding the bromatological composition of palisade grass, a significantly higher CP content was observed in the ICLF systems and a lower CP content in the ICL system (*P* <0.05). The other parameters did not differ (*P* >0.05) (Table 3) between treatments. The CP in the ICLF-1L treatment was 1.71% greater than that of ICL and ICLF-3L. However, the CP reading was 1.29 % greater than that of ICL. The percentage of NDF was higher in winter, whereas ADF and mineral matter were lower in winter (*P* <0.05) (Table 3). There was also a tendency toward a lower percentage of CP in winter (*P* =0.08).

Table 3 Bromatological composition of palisade grass (*Urochloa brizantha syn. Brachiaria brizantha*) in integrated crop-livestock and integrated crop-livestock-forest systems, in summer and winter

	DM, %	CP, %	NDF, %	ADF, %	MM, %
Treatment					
ICL	28.53	9.60[b]	74.26	35.41	9.37
ICLF-1L	27.66	11.31[a]	72.03	35.25	9.14
ICLF-3L	27.28	10.89[a]	71.70	35.00	9.22
Season					
Summer	27.80	10.80	70.59[b]	35.70[a]	10.03[a]
Winter	27.85	10.41	74.73[a]	34.73[b]	8.46[b]
P-value					
Treatment	0.4340	0.0106	0.1242	0.3171	0.6826
Season	0.9007	0.0827	0.0004	0.0234	<0.001
Interaction	0.1935	0.1695	0.9534	0.0589	0.5541
Coefficient of variation, %					
Whole plot	6.61	10.79	3.12	1.32	5.46
Subplot	3.89	10.41	2.61	2.48	3.41

[a,b] Within columns, means followed by a common superscript do not differ according to Tukey's test at $P < 0.05$
DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, MM: mineral matter ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock forest with three rows of eucalyptus

There were no significant effects of the interaction between treatment and season on the livestock performance variables reported in Table 4 ($P > 0.05$). No differences were found in ADG and WGA between treatments ($P > 0.05$). Stocking rates, expressed in kg ha^{-1} and animal unit ha^{-1}, were lower in ICLF-3L ($P < 0.05$) and highest in ICL and ICLF-1L ($P > 0.05$). The integrated crop-livestock system and ICLF-1L did not differ from each other. Average daily gain and WGA were higher in summer. Stocking rates were lower in the winter, but these values were not significant ($P = 0.07$).

Table 4 Average daily weight gain of Nellore cattle, stocking rate, and weight gain by area in the integrated crop-livestock system and integrated crop-livestock-forest systems in summer and winter

Treatment	Average daily gain, kg/day	Weight gain, kg	Stocking rate, kg/ha	Stocking rate, AU/ha
ICL	0.453	210.82	738.07[a]	1.64[a]
ICLF-1L	0.394	191.33	698.87[a]	1.55[a]
ICLF-3L	0.389	190.37	601.23[b]	1.34[b]
Season				
Summer	0.702[a]	165.36[a]	706.00	1.57
Winter	0.121[b]	32.70[b]	652.81	1.45
***P*-value**				
Treatment	0.2965	0.6229	0.0142	0.0089
Season	<.0001	<.0001	0.0718	0.0718
Interaction	0.0943	0.4770	0.1453	0.1483
Coefficient of variation, %				
Whole plot	20.07	23.07	9.13	8.85
Subplot	16.61	24.24	9.40	8.94

[a,b] Within columns, means followed by a common superscript do not differ according to Tukey's test at $P < 0.05$
ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock forest with three rows of eucalyptus

There was no interaction between the microclimate variables for treatment and season ($P >0.05$) (Table 5). Significant differences were observed ($P <0.05$) (Table 5) between black globe temperature and RTL treatments. Black globe temperature was lower in the shade of ICLF-3L than in ICL. The GT was 4.83 °C lower in the ICLF-3L shade than in ICL. For AT, there was a trend for lower values in the ICLF-1L shade and ICLF-3L shade than for the other treatments ($P =0.06$). There was a lower BGHI in the ICLF-3L shade in comparison with the other treatments (P 0.09). Radial thermal load was lower in the shade in the ICLF-1L and ICLF-3L systems and higher in the ICL and ICLF-1L sun, whereas the ICLF-1L sun did not differ from the ICL and ICLF-3L sun. The reduction in RTL was 11.8% for ICLF-1 in the shade and 15.3% in ICLF-3 in the shade in comparison with ICL. There was higher ambient temperture, relative humidity, GT, THI, and BGHI during the summer. There were no differences between seasons for WS and RTL (Table 5).

Table 5 Microclimatic variables and thermal comfort indices in integrated crop-livestock and integrated crop-livestock-forest systems in summer and winter

Treatment	AT, ºC	RH, %	BGT, ºC	WS, m/sec	THI	BGHI	RTL
ICL	29.14	53.46	36.87[a]	1.08	77.92	85.67	621.3[a]
ICLF-1L sun	29.05	52.92	35.83[ab]	0.74	78.20	85.41	593.4[ab]
ICLF-1L shade	27.69	57.62	34.35[ab]	0.76	76.51	83.38	547.9[c]
ICLF-3L sun	28.99	54.24	34.91[ab]	0.63	77.87	83.76	573.3[b]
ICLF-3L shade	28.16	56.23	32.04[b]	0.70	77.15	81.05	526.1[c]
Season							
Summer	30.11[a]	64.73[a]	37.00[a]	0.69	81.12[a]	88.28[a]	584.8
Winter	27.11[b]	45.05[b]	36.61[b]	0.86	73.94[b]	79.44[b]	560.0
P-value							
System	0.0583	0.2551	0.0336	0.1481	0.1281	0.0876	0.0062
Season	<.0001	<.0001	0.0004	0.3428	<.0001	<.0001	0.1144
Interaction	0.1119	0.5636	0.5479	0.3997	0.3142	0.6578	0.6880
Coefficient of variation, %							
Whole plot	2.67	6.87	6.02	35.27	1.38	3.14	3.93
Subplot	2.37	7.48	6.76	59.91	1.05	2.81	6.84

[a,b,c] Within columns, means followed by a common superscript do not differ according to Tukey's test at $P <0.05$
AT: ambient temperature, RH: relative humidity, BGT: globe temperature, WS: wind speed, THI: temperature humidity index, BGHI: black globe temperature humidity index, RTL: radiant thermic load, ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock-forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock-forest with three rows of eucalyptus

There was no interaction effect between treatment and season ($P >0.05$) in the behaviour of the Nellore cattle. No differences were observed between the ICL, ICLF-1L, and ICLF-3L systems ($P >0.05$) (Table 6). The exception was the tendency for lower rumination and resting on standing in the ICL system ($P =0.05$ and $P =0.09$, respectively). In summer, the animals spent more time ruminating in comparison with winter, regardless of system. The frequency of other activities was similar for the treatments (Table 6).

Table 6 Behavioral time-budget (%) of Nellore cattle kept in integrated crop-livestock and integrated crop-livestock-forest systems during summer and winter

Treatment	GRAZ	RUWS	RUWL	TR	REWS	REWL	TI	OA
ICL	41.05	9.31	13.89	23.21	18.44	9.96	28.40	7.33
ICLF-1L	43.50	12.19	5.39	17.60	20.55	10.82	31.37	7.55
ICLF-3L	34.65	14.61	6.26	20.87	26.33	7.46	36.78	7.69
Season								
Summer	37.73	12.12	9.90[a]	22.02	25.00	7.81	32.81	7.35
Winter	41.73	11.98	7.14[b]	19.12	20.59	11.01	31.60	7.70
P-value								
Treatment	0.1028	0.0524	0.1666	0.4740	0.0905	0.7504	0.5159	0.8378
Season	0.2962	0.9650	0.0116	0.4948	0.3280	0.1931	0.6671	0.6942
Interaction	0.1487	0.3982	0.7934	0.4773	0.8855	0.6441	0.8218	0.1804
Coefficient of variation, %								
Treatment	13.68	20.67	79.06	35.47	23.85	81.63	28.03	90.48
Season	18.66	67.76	19.17	41.66	51.55	49.18	37.48	40.39

[a,b] Within columns, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
GRAZ, grazing; RUWS, ruminating while standing; RUWL, ruminating while lying; TR, total rumination; REWS, resting while standing; REWL, resting while lying; TI, total resting; OA, other activities, including interaction with other animals, drinking water, licking salt, urinating, defecating, and displacement, ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock forest with three rows of eucalyptus

The haematological parameters of Nellore cattle were not influenced by treatment *(P* >0.05). However, the percentage of lymphocytes was lower in summer than in winter (Table 7). The reference values for cattle indicative of normal health were leukocytes (4-12 x10^3 µl^{-1}) lymphocytes (45-75 %), platelets (100-700 x10^3 µL^{-1}) hemoglobin (8-10 g dL^{-1}) and corpuscular volume (24-46 %) (Schalm *et al.*, 1975). Season did not influence the other haematological parameters.

Table 7 Hematological parameters of Nellore cattle in integrated crop-livestock systems and integrated crop-livestock forest systems during summer and winter

Treatment	RBC, x10^6/µL	HGLB, gd/L	PLTL, 10^3/µl	LFCT, %	CV, %
ICL	11.84	14.53	306.59	47.40	43.00
ICLF-1L	11.61	15.81	285.45	46.75	46.92
ICLF-3L	11.65	14.85	307.91	52.09	45.90
Season					
Summer	12.05	15.10	314.19	41.64[b]	45.80
Winter	11.63	15.03	285.78	55.86[a]	44.75
P-value					
System	0.9173	0.5035	0.7804	0.4916	0.4118
Season	0.2179	0.9111	0.4206	0.0088	0.6490
Interaction	0.4799	0.4275	0.9205	0.9394	0.5154
Coefficient of variation , %					
Whole plot	10.61	14.11	23.38	18.87	12.58
Subplot	11.31	11.80	27.49	21.49	11.97

[a,b] Within columns, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
RBC: red blood cells, HGLB: haemoglobin, PLTL: platelets, LFCT: lymphocytes, CV: corpuscular volume, ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock-forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock forest with three rows of eucalyptus

There was an interaction between treatment and season for leukocyte count *(P* <0.05) (Table 8), which increased in ICLF-1L in summer. However, the other treatments did not differ between seasons. No differences were observed between treatments during summer and winter *(P* >0.05) (Table 8).

Table 8 Number of leukocytes (10^3 μl^{-1}) in Nellore cattle an integrated crop-livestock and integrated crop-livestock-forest systems in summer and winter

Season	ICL	ICLF-1L	ICLF-3L
Summer	14.43[Aa]	15.36[Aa]	14.02[Aa]
Winter	12.88[Aa]	10.15[Ba]	12.80[Aa]

[A,B] Within columns, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
[a,b] Within rows, means followed by a common superscript do not differ according to Tukey's test at *P* <0.05
ICL: integrated crop-livestock, ICLF-1L: integrated crop-livestock forest with a single row of eucalyptus, ICLF-3: integrated crop-livestock-forest with three rows of eucalyptus

The ICL and ICLF systems were examined two years after implementation to investigate the component interactions of these systems. Forage characteristics, animal performance, behaviour, thermal comfort, and haematological parameters were evaluated.

The reduction in DFM was reported by several authors, who described ICL systems with a tree component (Paciullo *et al.,* 2007; Oliveira *et al.,* 2014; Lopes *et al.,* 2017). The decrease in DFM was caused by the reduction in luminosity, which was intercepted by trees. This allowed the penetration of only low-quality radiation with less light coming through the canopy (Soares *et al.,* 2009), in addition to competition between trees and forage for water and nutrients. However, the decrease in DFM was not found in the ICLF systems in relation to the ICL system, probably because the trees were still growing, being 9.0 m and 9.9 m high for ICLF-1L and ICLF-3L. The distance between the eucalypt rows was 17 and 21 m, which allowed light into the system. Therefore, this could minimize the competition between trees and herbaceous forage.

In the summer, the better climatic conditions, higher temperature, higher light intensity, and higher precipitation promoted forage growth (Caminha *et al.,* 2010). This increased the percentage of leaves by forming new tillers and leaf tissue (Domiciano *et al.,* 2018). This could be seen in the ICL and ICLF-1L systems, which had a higher percentage of leaves and a higher leaf-to-stem ratio (Table 2). However, in winter, the percentage of leaves and the leaf-to-stem ratio were higher in the ICLF-3L system (Table 2). This probably resulted from the higher density of trees in this treatment. During periods of low precipitation, moisture evaporated more slowly in systems with higher shading densities (Kichel *et al.,* 2014) and water retention levels in the soil were therefore higher (Silva *et al.,* 2016).

Treatments with a tree component had a higher percentage of protein (Table 3), which was observed by Souza *et al.* (2010a), Paciullo *et al.* (2011), and Lopes *et al.* (2017). This increase could be attributed to greater mineralization because of nitrogen supply from nutrient cycling. Shaded environments (Paciullo *et al.,* 2007; Araújo *et al.,* 2016) promote a more favourable microclimate in the soil (Araújo *et al.,* 2016), higher soil moisture content, and lower temperatures.

The increase in the CP content in shaded plants occurred because of reduced photosynthesis, which increased the efficiency of converting solar energy into chemical energy, thereby increasing the chlorophyll content and consequently the levels of nitrogen and CP (Ribaski *et al.,* 1998).

Higher NDF values observed during winter could be related to the increase in senescent material during this season. Lower water availability and lower temperatures make this a common situation in winter. The low growth of forage during winter resulted in a higher mineral matter content.

The changes observed in the forage did not interfere with the ADG or WGA, which were similar to those reported by Domiciano *et al.* (2018) and Oliveira *et al.* (2014), who evaluated ICL systems both with and without the tree component.

Although no difference was observed in ADG, the stocking rate was lower in ICLF-3L and higher in the ICL and ICLF-1L. The larger area occupied by eucalyptus in the ICLF-3L system reduced the useful pasture area, leading to a reduced stocking rate.

The reduction in precipitation, temperature, and light in winter reduced growth, and forage quality was expected to be lower in winter than in summer. However, there was only a general tendency for reduced stocking rate in winter, perhaps because of the standardization of pasture before the experiment. The stocking rates used in all treatments were therefore low in the early summer to ensure grass growth.

In summer, the better quality of palisade grass improved ADG and WGA. However, in winter, the unfavourable environmental conditions caused a significant decrease in forage production, which could affect animal production (Janusckiewicz *et al.,* 2010).

The data illustrated that ICL systems could contribute to an increase in meat productivity. In Brazil, the average annual production per hectare (ABIEC, 2019) was 67.5 kg with a stocking rate of 0.93 AU/ha. This was well below that reported in the present study, which encompassed only winter and summer.

Shade offers significant beneficial effects in hot climates. These include a reduction in air temperature (Souza *et al.,* 2010b; Giro *et al.,* 2019; Aranha *et al.,* 2019), lower surface temperatures of cattle (Broom, 2017; Giro *et al.* 2019) and improved thermal comfort indices (Souza *et al.,* 2010b; Pezzopane *et al.,* 2019; Giro *et al.,* 2019; Aranha *et al.,* 2019). When assessing thermal comfort, a reduction in RTL and GT was found, and a tendency towards lower ambient temperature and BGHI in treatments with trees. However, there were no differences between other parameters.

Radiant thermal load consists of the total thermal energy exchanged between the individual and the environment. Trees reduce the incidence of solar radiation (Pezzopane *et al.*, 2019), and consequently the RTL. A reduction in this parameter was observed in the shade for the ICLF-1L and ICLF-3L treatments. The black GT encompassed the effects of air temperature, solar radiation, and wind speed, which was lower for ILCL-3L shade, which was the area with the most shade.

The treatments did not influence THI. In contrast, air temperature and BGHI had lower values in the shade. Black globe temperature and humidity index had lower values in the shade of ICLF-3L. In Giro *et al.* (2019), Pezzopane *et al.* (2019), and Souza *et al.* (2010b), in which the height of the trees was 26 m, 25 m, and 18 m, the authors found better indices and temperatures in systems with trees compared with the present study. This could be attributed to the larger trees in those studies which provided greater shade, compared to the smaller tress in the present study.

The influence of trees was not sufficient to significantly affect the behaviour (Table 6) and haematological responses (Table 7) of the cattle. There were only tendencies toward treatment effects for the time spent ruminating while lying and resting while standing. The animal behaviour and haematological responses had been expected to change in systems with trees, according to Souza *et al.* (2010b) and Giro *et al.* (2019).

Another factor that may have led to these results was the adaptation of the Nellore breed to tropical climates (Shiota *et al.,* 2013), because temperatures between 15 °C and 29 °C do not affect the performance of these beef cattle (Scahaw, 2001). The values of temperature observed on behaviour days were within these limits and could have contributed to the lack of change in cattle behaviour during the treatments.

Behaviour was influenced only by season, in which the frequency of rumination in the lying position was higher in summer. This was attributed to the increase in body surface exposed to the ground, thereby increasing the loss of heat to the environment (Vizzotto *et al.,* 2015). The ambient temperature, THI, and BGHI were higher in summer than in winter, contributing to this behaviour.

The higher temperatures and thermal comfort indices in summer contributed to lower lymphocyte percentages. According to Blecha (2000), lymphocyte reduction occurs under stressful conditions.

Conclusion

The ICLF systems improved CP without changing DFM compared with the ICL system. Despite changes in forage, there were no differences in the performance of Nellore cattle and weight gain by area, although the lowest stocking rate was observed in ICLF-3L. Two years after the ICLF systems had been established, the trees were still growing, and their size provided lower shade density. Under these conditions, trees can reduce the total thermal energy exchanged between the animal and the environment, improving thermal comfort. However, this was not sufficient to affect animal behaviour and haematological parameters. The experiment should be repeated after a few years to ascertain whether larger trees have an effect on these parameters. In summer, Nellore cattle show better general performance, regardless of system, which is a consequence of better pasture quality. In contrast, lower levels of thermal comfort and microclimate variables in summer caused a higher occurrence of standing rumination in cattle and a reduction in lymphocytes.

Authors' Contributions

ASA, CA, GCL, GPM, and RF designed the experiments. ASA, CA, GCL, GPM, EARS, ARA, BMSS, LGFB, PRLM, SAM, AACC, and JMFS performed the experiments and collected the data. ASA, CA, EARS, GMP, RF, GCL and ARA analysed and interpreted the data. ASA, CA, GPM, EARS, and BMSS drafted the manuscript, which was reviewed and approved by the co-authors.

Conflict of Interest Declaration

The authors have no conflict of interest to declare.

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