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The influence of the silvopastoral system on physiological, behavior, and health responses of the Purunã breed of cattle

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Abstract: Sustainable livestock systems should provide high production yields while respecting animal welfare. The silvopastoral system (SPS: silvopastoral – trees, pasture, and cattle) offers a more holistic cattle management strategy, than only pasture system (PAS). The SPS incorporates the production of wood and forage, offering protection for the animals while grazing. However, the relationship between animal behavioral indicators in the SPS has been poorly studied in subtropical conditions. The present study was established to investigate the effect of the SPS on the physiological, behavioral, and health responses during one grazing cycle of the Purunã cattle. Thirty heifers were randomly assigned into two groups of 15 animals, either kept on the SPS or under a monoculture system (PAS: pastoral – cattle on pasture). Body weight and body surface temperature, time of grazing, and lying behavior were assessed for both systems during late Autumn (40 days) in a subtropical humid region of Brazil. The data showed a statistically significant influence of the SPS on the reduction of the heifers' average body surface temperature, from 27.7 ºC to 30.3 ºC in PAS ($P = 0.02$). Although there was an important effect of the SPS on decreasing eating behavior (4.77 events/1.5 h), compared to animals kept in the PAS (6.21 events/1.5 h), the differences in forage availability and quality may have played a role in the animals kept in the SPS. Animals in the SPS presented statistically significantly fewer drinking ($P = 0.0001$) events, higher body condition score (BCS) decline ($P = 0.02$), and *Rhipicephalus microplus* (cattle-tick) score ($P = 0.009$), than animals maintained on the PAS. There was an aggregated tick distribution, and infested animals lost (high tick score) significantly more weight ($P = 0.0216$) than non-infested ones (low tick score) under SPS conditions. More studies are required to understand better the influence of multiple factors on Purunã behavior and health, especially when considering distinct agricultural systems, forage availability, geographical and subtropical-humid (Cfb) climate conditions, and the impact of ectoparasites on animal performance.
Keywords: Silvopastoral system; cattle physiology; infrared thermography; body weight; behavioral response; tick score.

1. Introduction

There is a growing concern over animal welfare and the continuing search for more sustainable farming practices. Nowadays, cattle grazing systems are designed to allow the animals to spend more time on pasture with significant benefits for animal health and welfare (Broom et al., 2013; 2018), including the reduction of mastitis (Peterson-Wolfe et al., 2018) and the possibility of expressing their natural behavior (van den Pol-van Dasselaar, 2005). The evaluation of animal resilience can impact bacterial and parasite infections, which may also lead to the reduction of infections without the input of external interventions (Schafaschek et al., 2021). However, exclusive pasture systems may also expose animals to extreme tropical and subtropical climate conditions (heat, wind, and rain) with major adverse effects on welfare. Ominski et al. (2002) showed that exposure to high temperatures affected the respiration rate and increased body temperature in cattle. This factor has also decreased food intake to maintain a reasonable thermal balance (Blackshaw and Blackshaw, 1994). Similarly, heavy rains, sometimes accompanied by strong winds, can significantly reduce food intake, body temperature, and lying time in Holstein-Friesian dairy cows in New Zealand (Schütz et al., 2010).

In addition, at a time when biodiversity is declining and rapid population growth is a major concern, it has become critical that modern agricultural systems provide high yields while respecting the permanent quest for sustainable development, including animal welfare. Silvopastoral systems (SPS) are considered a niche for sustainable farming (Broom et al., 2018; Molento et al., 2004). The combination of forestry, forage/pasture, and animal production, can also improve the overall productivity of the area, and soil quality, increasing the physical and biological interactions between the different elements (Chara et al., 2019; Peri et al., 2016).

Integrated systems offer many advantages, such as maintaining the physical and biological characteristics of the soil against erosion, reducing greenhouse emissions, and increasing plant and animal production (Peri et al., 2016; Webster et al., 2019). Moreover, Broom et al. (2013), explained that forest plantation provides biological protection against wind, rainfall, and shade for animals, as the systems also fulfill the need of the animals to hide from perceived danger. The authors also have indicated that trees may be the new source of animal fodder, as silvopastoral systems are more productive than pasture-exclusive areas. Indeed, it is widely accepted that shelter created by the livestock-forestry system helps to counter the adverse impacts of harsh weather pressures on cattle physiology, improving particularly thermal comfort and weight gain (Holmes et al., 1978; Kubisch et al., 1991; Van Laer et al., 2015).

Nevertheless, few studies have focused on the SPS influence on animal behavior (van Laer et al., 2015), and health issues (Bello et al. 2020; Oliveira et al., 2017; Schafaschek et al., 2021), especially in beef cattle. Yet, the literature emphasizes the importance of studying the behavioral and health components when assessing animal welfare. According to Fraser and Broom (2015), the recognition of positive welfare depends primarily on the absence of physiological and behavioral indicators of negative welfare. Therefore, beyond physiological indicators, assessing the positive influences of farming practices on animal welfare can be linked to an increase in natural behaviors, such as grooming, lying down, feeding, and drinking. Similarly, there may be a decrease in abnormal behavior such as stereotypes, self-mutilation, or abnormal aggression between individuals. Giro et al. (2019), evaluated the effects of the microclimate on integrated crop-livestock-forestry (CLF) and crop-livestock (CL) systems on the behavior of beef cattle in a tropical climate. The animals in CLF exhibited lower surface temperatures of the back and the trunk, confirming that beef cattle can benefit from milder weather, showing higher thermal comfort.

There are few studies demonstrating the influence of parasite challenge infection and their effect on animal behavior and welfare. Schafaschek et al. (2021) reported a study looking at cattle infected with *Haematobia irritans* (Horn flies), which are hematophagous (blood-sucking) flies commonly found in South America. The authors have determined the prevalence of *H. irritans* in 36 Red Angus male calves under livestock (L), CL, livestock-forestry (LF), and the full integration of CLF conditions in Brazil. Horn flies were counted between systems on each animal every week. Animals were treated for *H. irritans* infestation after reaching the threshold abundance of more than 50 flies. After 1008 evaluations, animals in the CLF had significantly ($P < 0.05$) more horn flies than the other systems. The infestation levels were influenced by the production system ($P < 0.002$) and by the month of the year ($P < 0.001$). The density of flies did not influence the weight gain of the calves between the systems. These findings suggest that it is valid to perform fly counts and treat a single animal when it reaches the threshold to control *H. irritans* with no impact on the performance of the animals when using a low transient threshold index of infestation. Martin, (2022) studied the impacts of an integrated livestock production system (SIPA) and the cattle-tick *Riphicephalus microplus* infestation on Angus calves during 2017 and 2020. The animals were evaluated for the infestation based on the SICOPA 1 to 4 score protocol (Molento, 2020), the number of acaricide treatments, and daily weight gain (DWG) under the same systems as in Schafaschek et al. (2021). The tick-score was significantly lower ($P < 0.05$) for the CL and the CLF, compared to the L and LF. The animals at CL and CLF were less treated as well. The DWG was similar among all four systems. However, a linear regression logistic analysis revealed that the chance of losing weight from scores 3 and 4 scores was 13 times higher when compared to the scores 0, 1, and 2. The author suggests that tick infestation must be controlled when animals reach score 2, allowing optimal performance of the animals. The agricultural component of the integration was very efficient in reducing the number of free-living tick larvae, consequently reducing the adult tick counts in the CL and CLF systems (Martin, 2022).

Previous studies have demonstrated that behavior mechanisms governing animal feeding, especially food intake, are also related to pasture structure (Bremm et al., 2012; Mezzalira et al., 2014; Trindade et al., 2016). Understory plants can exhibit alterations in their anatomy and physiology to compensate for low-light quantity and distinct pasture quality (Niinemets et al., 1998; Cavagnaro and Trione, 2007), and consequently increase or decrease herbage yield and nutritive value (Lin et al., 2001; Pontes et al., 2016). Pontes et al. (2016) showed that, in general, perennial C4 species displayed an increase in leaf proportion and crude protein (nutritional value) and a decrease in dry matter yield under trees than in systems without trees. Therefore, changes in pasture structure are expected in silvopastoral systems and consequently changes in the animal's feeding behavior, resulting in higher weight gain (Trindade et al., 2016). The present study aimed to investigate the influence of the SPS and the traditional monoculture pastoral system (PAS) on the physiological, behavioral, and health responses in the Purunã breed of cattle.

2. Materials and Methods

2.1. Animals and experimental conditions

The study was conducted at the Institute for Rural Development of Paraná, IDR-Paraná, located in Ponta Grossa, from April 25th to June 3rd of 2016. The city is located 845 meters above sea level at latitude 25°07'18,4" S and longitude 50°03'16,4" W. Thirty Purunã heifers were selected for the study. The Purunã breed is composed of ¼ Charolais, ¼ Aberdeen Angus, ¼ Caracu, and ¼ Canchin breed. The animals had an average initial body weight of 207 kg (± 26 kg) and were 12 months old. Animals were raised in a herd of 195 individuals, all born at IDR-Paraná during the same season. Before starting and during the experiments, the animals were kept in a 25-ha area grazing on *Hemarthria altissima* cv. Florida (limpograss) with herbage allowance varying from 14.0 to 19.5%, and 9.1% of crude protein on average. They had unrestricted access to water and mineral supplements during all periods. The animals were identified by ear tag numbers and were randomly allocated (n=15) to two different pasture systems: a monoculture pastoral system (control group - PAS) and a silvopastoral system (SPS) integrating trees. Animals were distributed according to their age and coat color, which are traits known to affect susceptibility to heat stress. Each system was divided into three plots of about 1.5 ha, where five individuals were evaluated in each. The shade was provided by 10-year-old trees (*Eucalyptus dunnii, Grevillea robusta*, and *Anadenanthera colubrina*), 3 m apart from each other within lines. The tree lines were 14 m from one another. Both groups were not treated with any antiparasitic drug before the experimental period.

2.2. Forage measurements

Forage availability was estimated before and one month after the beginning of the study, collecting three random samples of forage from an area of 0.25 m² in each plot, using a metal quadrat of 0.5 m x 0.5 m. Samples were placed in an oven at 60 °C for 48h to determine the forage mass (kg dry matter.ha^{-1}). Separation of leaves stems, and dead material was also performed for each sample to determine the proportions of these components since they present different nutritional values (van Soest, 1994). Additionally, pasture height (cm) was measured in each plot by random measures of 50 sites, using a sward stick (Barthram, 1985).

2.3. Climatic measurements

According to Köppen's classification (Alvares et al., 2013), the climate of the region is described as a warm subtropical humid climate (Cfb) with an average annual temperature of 17.6 °C and an average annual precipitation of 1500 mm. To assess the influence of local weather in both systems, air temperature (°C), rainfall (mm), cloud cover (scale 1 to 8), air humidity (decimal form), and solar radiation (Klux) were measured using a portable weather station THDLA-500 (HighMed Sol. Tech. Ltd, São Paulo, Brazil), directly in the grazing areas, during each behavioral observation session at every 10-minute interval, simultaneously.
In the same way, weather data were measured in each treatment before every thermographic session. Air temperature and humidity were converted to the discomfort index, based on the temperature-humidity index (THI) (Thom, 1959), to assess the risk of thermal stress to individuals during the entire period, according to Equation 1 (Eq.1):

$$THI = 0.8 \times T + [RH \times (T - 14.4)] + 46.4 \quad \text{(Eq.1)}$$

where, T = air temperature in °C, and RH = relative air humidity in decimal form (i.e., 0.60, not 60%). Heat stress risk classes were determined by the Livestock Weather Safety Index (LCI, 1970).

2.4. Welfare measurements of Purunã heifers

2.4.1. Evaluation of physiological responses

To assess the effect of SPS on some aspects of the animal's physiology, each individual was weighed at the beginning and the end of the study, using an electronic scale, after fasting from solids and water for approximately 12h. From these two measurements, we calculated the body weight gain for the period (one grazing cycle). During the weightings and because of the prevalence of Babesiosis and Anaplasmosis, important tick-borne diseases, the severity of the cattle-tick *R. microplus* infestation was also evaluated (Guglielmone, 1995; Jonsson et al., 2008). For this, a visual scale of 1 (from none to very few attached ticks: < 5), 2 (moderate), and 3 (abundant: > 20) was used in all animals. Animals with clinical signs (i.e., apathy, high temperatures, anemia, diarrhea – to monitor endoparasite infection) were examined by a clinician, weighed, and treated on the same day. Five days after the beginning of the study, one animal from the SPS group was injured and replaced by another individual with the same age, coat color, and weight. For this reason, it was decided to describe the number and the type of injury, as well as their severity. A body condition score (BCS) was assigned, ranging from 1 to 5 to all animals at each evaluation date, whereas the ideal BCS would range from 3.0 to 3.5 (Eversole et al., 2009).

To shed light on the potential thermal stress differences between treatments, body surface temperature was measured in Celsius degrees (°C) of each individual using the ThermaCAM C2 FLIR infrared camera (Wilsonville, USA) (McManus et al. 2016). The camera had an infrared resolution of 80×60 pixels and a thermal sensitivity of < 0.10 °C. Following previous studies, cattle skin emissivity was fitted to 0.95, and the reflected temperature was set to 20 °C (Gloster et al., 2011; Steletta et al., 2012). Animals were habituated to both the human presence and the thermal camera 10 days before the initial data collection. Behavioral observations were made every day between 12:30 and 13:00h. Images were taken at 2-3 m range with an angle of measurement of 90° laterally on the shady side of the animals. This was necessary as direct sunlight can alter the conductivity and emissivity of the thermal image (Stewart et al., 2005).

2.4.2. Evaluation of behavioral responses

To assess the influence of the SPS on animal behavior, one observer recorded the frequency of eating, ruminating, and drinking, as well as the frequency of lying or standing. The heifers were considered eating if the grass was being ingested or could be seen in their mouth. Animals were considered ruminating if their lower jaw presented regular chewing and in the presence of regurgitation movements without food ingestion, in a standing or lying position. The animals were considered drinking when their mouth was in contact with water in the drinking trough. Cows were considered lying if their body flank was in contact with the ground, and standing was considered as not lying. All these behavioral occurrences were recorded by scan sampling method every 10 minutes, during 01:30h in each plot, to be able to determine short events, such as drinking.

The frequency of agonistic interactions was recorded at all occurrence samplings, watching the whole group and recording each occurrence of aggressive or particular behavior. Agonistic behavior included headbutting (occurs with physical forceful contact of one animal butting the other animal with its forehead, horns, or horn base), displacement (physical contact where one animal is forcing the other animal to give up its position), chasing (one animal makes another animal move without physical contact), kicking (kicking with a hind leg in direction of other cattle), and fighting (two animals push their heads against each other while planting their feet on the ground with both animals exerting a visual force against each other). Observations were conducted concurrently daily from 09:00 to 16:15, taking care to minimize potential disturbances. The observation of the plots was done one by one due to

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their very large size. Four plots were recorded per day four times (09:00 to 10:30 h, 10:45 to 12:15 h, 13:00 to 14:30 h, and 14:45 to 16:15 h) during the entire experimental period. The day and the period of observation of each plot were randomly selected. For the SPS group, the data were also recorded every 10 minutes during each observation period (1.5 h), the position of each individual under the trees (area of 1 to 2 m in circumference) or outside the shaded area. Then, an average was calculated for the number of occurrences for both conditions for each animal.

2.5. Statistical analysis

Results are presented as mean and standard deviation, and the individuals are the statistical units using the Mann-Whitney test for weight and tick index. Statistical analyses were performed using R software, version 3.2.3 (R Core Team, 2015). The significance threshold was set at $P < 0.05$. Results were presented as mean and standard deviation. Forage structure between the two treatments was analyzed by the student t-test regarding pasture height (independent data), and forage mass, as the proportions of pasture constituents were analyzed using the Fisher-Pitman permutation tests. The animal surface temperature, BCS, parasite score, and animal body weight gain between the two treatments were also analyzed by the Fisher-Pitman permutation tests. Each picture of the animal surface temperature was analyzed through FLIR Tools 5.6. A horizontal line was drawn over the entire length of the middle part of the animal body excluding the tail by manual tracing to calculate the average surface temperature (°C) (Figure 1). Correlations between the body surface temperature and the weather variables were performed using the Pearson correlation test.

All variables related to injuries were regrouped into a single variable. This variable was then divided into three distinct categories according to the injury depth, on a scale of 1 (mild injuries), 2 (clear injuries), and 3 (marked injuries). This variable was then compared between the two systems with the Fisher-Pitman permutation test. The frequency of each behavioral data was reduced by a principal component analysis (PCA). The data obtained per individual was then used to analyze the influence of the system on the frequency of standing and lying. Because of unbalanced data, a non-parametric permutation test was used to compare the data obtained from the PCA between the two systems and the behavior variables not included in the PCA. For the SPS, the time spent under the trees and outside this area was analyzed with the student t-test for dependent data, and the link between this time and the THI index was analyzed through Pearson's correlation test.

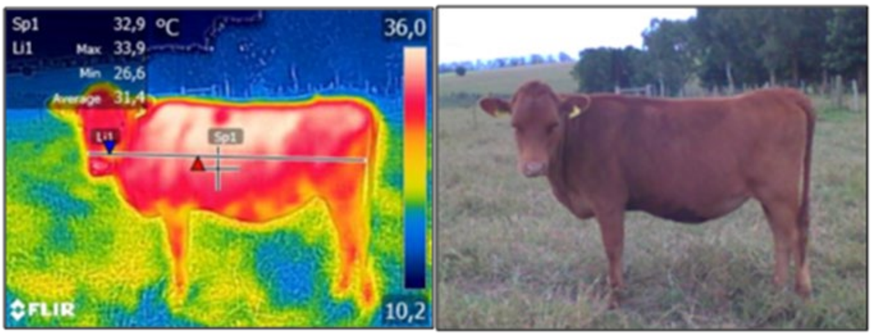


Figure 1 – Thermal-camera imaging of a Purunã heifer, analyzed by FLIR software showing the calculation line and temperature values (left), and a non-thermal picture (right) from the monoculture system at Institute for Rural Development of Paraná, IDR-Paraná, Ponta Grossa, Brazil.

3. Results

3.1. Forage structure

Analyses of the sward structure (Table 1) revealed that the forage mass was 47% lower in the SPS than in the PAS (P = 0.0001) by the end of the grazing cycle. The SPS had a significantly higher (P = 0.045) percentage of leaves (+ 4.6%) when compared to the PAS. Sward height, stem proportion, and the percentage of dead material were not statistically different between the two treatments (Table 1). The animals were not adjusted to account for the differences in forage availability between systems. The season (fall) presented lower temperatures, mild solar radiation, and rainfall than the average for the year, which could have impaired pasture growth, mainly for the SPS.

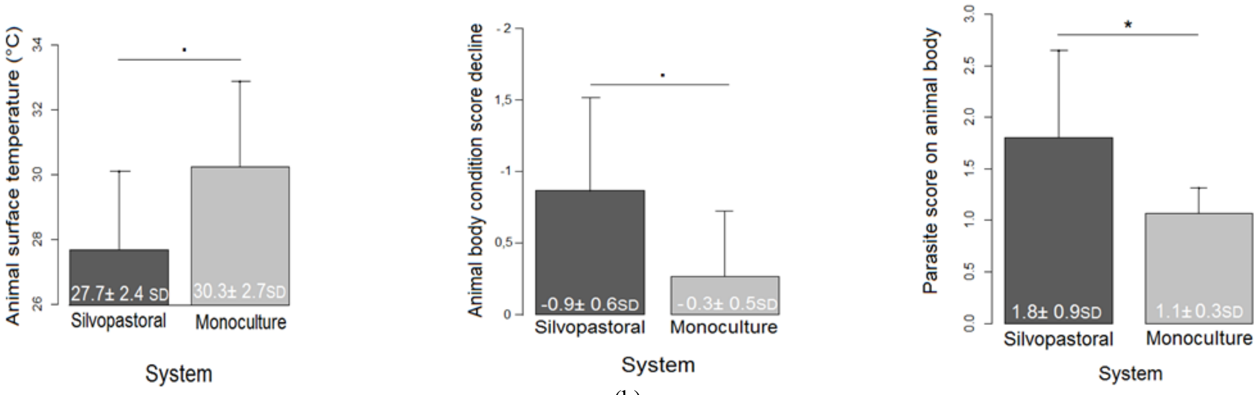
Variable	System		Score (Z)	P-value
	Silvopastoral (mean ± SD)	Monoculture (mean ± SD)		
Forage mass (kg dry matter/ha^{1})	3369 ± 792.7	6331.9 ± 1695.6	-3.15	*0.0001*
Percentage of leaves (%)	11 ± 5.6	6.4 ± 2.6	1.96	*0.045*
Percentage of stems (%)	63.1 ± 9.2	60.6 ± 13.2	0.49	*0.66*
Percentage of dead materials (%)	25.9 ± 11.2	33.0 ± 11.3	-1.30	*0.21*
Sward height (cm)	22.0 ± 5.0	22.0 ± 3.5	t = 0.19	*0.85*

Table 1 – Comparison of different sward structures between silvopastoral (SPS) and monoculture systems (PAS). Foraging samples in each system were harvested on April 26th and May 31st 2016 at the Institute for Rural Development of Paraná, IDR-Paraná, Ponta Grossa, Brazil.

3.2. The influence of the silvopastoral system on beef cattle

3.2.1. Effect on physiological and health responses

Body surface temperature (Figure 2a) was statistically significantly lower (P = 0.02) in animals maintained in the SPS (27.7 °C) than in the PAS (30.3 °C). Even though there was no correlation between body surface temperature and the THI, there was a strong negative correlation between body temperature and cloud cover (r = -0.90). There was a weak but statistically significant correlation between the animal body surface temperature and solar radiation (r = 2.31, P = 0.03). Although animals in the SPS demonstrated a statistically significant BCS decline (-0.9) than animals in PAS (-0.3), after the short period of the experiment (P = 0.02) (Figure 2b), the average BCS for both groups stayed within the ideal range (3.13 for SPS and 3.73 for PAS) for heifers. Moreover, in SPS, animals had a significantly higher tick score (1.8 ± 0.9/moderate count) than animals in PAS (1.1 ± 0.3/low count) (P = 0.009) (Figure 2c). As ticks had an aggregated distribution, it was also determined a statistical difference in the final weight between animals with distinct tick scores (P = 0.0216). The data demonstrated a weight of 200.4 kg (± 35.8) in the low tick score (1) and 173.6 kg (± 12.8) in the tick high scores in the SPS group. No difference was found between the two treatments for the final weight (P = 0.299) since both groups presented similar weight loss of -12.4/± 9.85 and 15.9/± 9.35 for PAS and SPS, respectively, and the number of injuries (P = 0.290).



(a) (b) (c)

Figure 2 – Different physiological responses of Purunã heifers according to the silvopastoral or monoculture system; animal body surface temperature (°C) (a), body condition score decline (b), and parasite/tick score (c) at the Institute for Rural Development of Paraná, IDR-Paraná, Ponta Grossa, Brazil. (*): All group comparisons showed statistically different averages (p<0.05).

3.2.2. Effect on behavior response

The PCA of individual behavior responses demonstrates that the vector frequency of standing (shown as S) and the frequency of lying behavior (L) were mainly represented by axis 1, which explained 42.5% of the sample (Figure 3a). Individuals on the left (negative values) displayed more lying behavior, while animals on the right (positive values) showed more standing behavior. The concentration of the ellipses (Figure 3b) revealed that individuals in the SPS were mainly situated to the left on axis 1, then individuals raised in the PAS. This suggests that animals in SPS spent more time lying down than PAS animals.

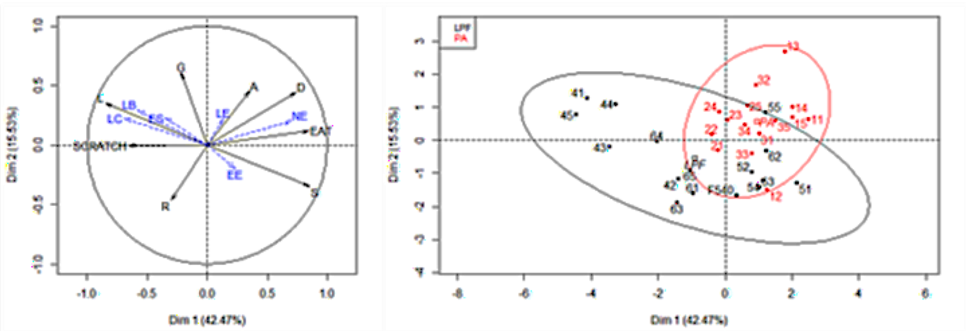


Figure 3 – Principal component analysis of behavior responses. The Variable Factor figure (a) illustrates the total frequency of eating (EAT), standing (S), ruminating (R), scratching (SCRATCH), lying (L), grooming (G), agonistic (A), and drinking (D) behaviors, as active variables. The four quadrants (Axis) are interpreted as positioning indicators. Most of the variables were represented on axis 1 (percentage) than the other axis. Variables in blue lines describe different positions of general behavior, i.e., LB: the animal was lying with her head on its flank. LC: the animal was lying with her body relying on its sternum with a slightly arched neck, and hind legs brought close to the body. LE: the animal was lying on its flank, and its posterior legs were extended. The Concentration Ellipses in Figure (b) represent the individual coordinates of each animal in the silvopastoral (black numbers) and the monoculture system (red numbers) based on the behavior presented in (a) at IDR-Paraná.

The analysis performed on axis 1 demonstrates that individuals in the silvopastoral system had significantly higher frequency (P = 0.004) of lying behavior (2.4 events/h) than individuals in the pasture system (2.2 events/h) (Figure 4a). It was observed that animals reared in the SPS had significantly fewer eating behavior (4.8 events/h) than individuals in the PAS (6.2 events/h) (P < 0.0001) (Figure 4b). Individuals of the SPS demonstrated statistically less drinking behavior (0.2 events/h), than individuals in the pasture system (0.4 events/h) (P < 0.0001) (Figure 4c). Individuals with SPS also demonstrated statistically significantly more ruminating behavior (1.60 events/h) than in monocultures (1.2 events/h) (P = 0.02), (Figure 4d). However, no difference was found between the two groups regarding the mean frequency of agonistic behavior (P > 0.05) or the tick score.

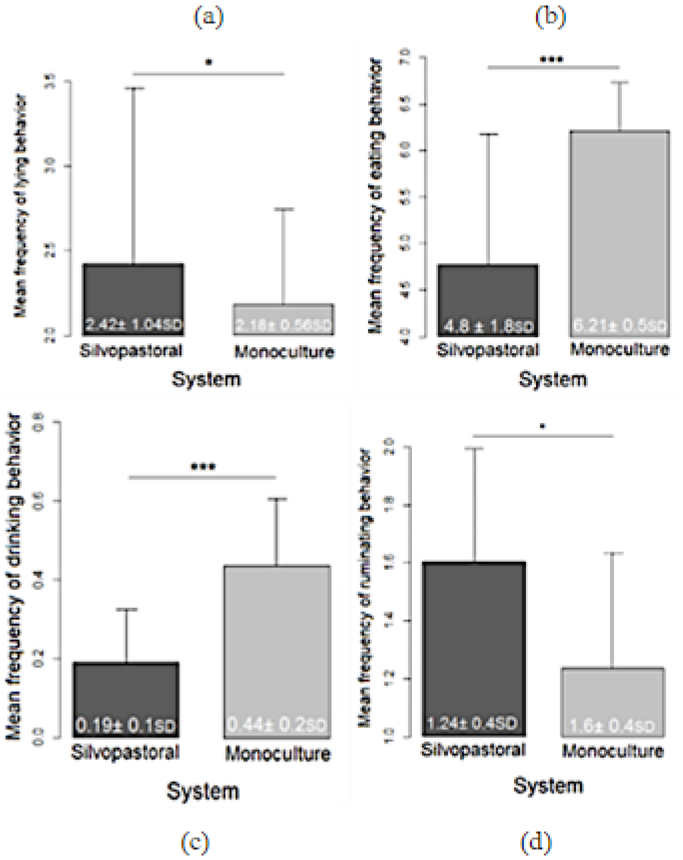


Figure 4 – Average and standard deviation of behavioral responses according to the treatment silvopastoral (SPS) or monoculture system (PAS), the mean frequency of lying (a), eating (b), drinking (c), and ruminating (d) behaviors at the Institute for Rural Development of Paraná, IDR-Paraná, Ponta Grossa, Brazil. (*): significant differences at P < 0.05, and (***) at P < 0.01.

Individuals in the SPS stayed significantly more time outside the trees (5.6 events/1.5 h), which is equivalent to 50 min.; then under trees (4.5 events/1.5 h), which is equivalent to 40.1 min. (P = 0.02). However, between 10:45 and 12:15, animals spent significantly more time under the trees (5.9 events/1.5 h), which is equivalent to 53.1 min., than outside the trees (4.1 events/1.5 h), which is equivalent to 37 min. (P = 0.02). There was no correlation between the presence of the animals under trees and the THI, as temperatures got milder (15 ºC) by the end of the grazing season.

4. Discussion

Although the experimental period was short and happened during a single forage cycle, it was possible to show that Purunã heifers in the SPS had significantly lower body surface temperatures than PAS. Thermal comfort is especially important for animals of European breeds or mixed-bred European and Indicus/Zebu, as they are more sensitive to high temperatures than pure Zebu breeds (Bennett et al., 1985; Kendall et al., 2006). Moreover, the provision of shade created by the trees on pasture can improve the thermal comfort of the animals (Broom et al., 2013, van Laer et al., 2015). However, the THI had never exceeded the normal category (temperature of 18.8 °C and humidity of 78.2%) during the present study. The meteorological data were thermoneutral for cattle (Roenfeldt, 1998), suggesting that the heifers at IDR-Paraná were not subjected to critical thermal stress during the experimental period. Although most of the evaluations of Purunã are still under interpretation, all breeds that compose this breed came from Europe. Caracu and Canchin breeds are more adapted to conditions in Brazil and are now quite tolerant to tick infestation (Fraga et al., 2003; Giglioti et al., 2017). Aberdeen Angus was compared to the indigenous African cattle Nguni and Bonsmara

(European and African mix) breeds and presented higher tick counts. The three breeds had no weight differences (Muchenje et al., 2008).

During the experimental period, individuals in the SPS significantly spent more time outside the trees than in the shade. These results differ from previous studies where the authors measured the use of shades by the cattle during different heat stress periods (van Laer et al., 2014, 2015; Schütz et al., 2008). The present data indicate that the animals may have adopted a different thermoregulatory strategy during the thermoneutral period. The importance of the presence of shaded areas takes place when temperature and solar radiation are high (Schütz et al., 2008, Tucker et al., 2008). Nevertheless, there was no strong association between the use of shade and weather conditions (cloud index) during the present study, suggesting that this factor would not occur when weather variation is in the range of comfort for the animals. The positive association between individual surface temperature and the level of solar radiation found during the experimental period confirms that solar radiation increases body temperature and fur and skin temperature, thus creating an unfavorable environment for the tick larvae (O´Kelly and Spiers, 1983; Verissimo et al., 2019).

Another factor is that young animals may gain and lose heat more easily due to their body volume ratio and faster growth rate (Van Laer et al., 2014). As well as, darker animals may also present lower heat tolerance than whiter animals (Becerril et al., 1993). Additional studies are required to compare the Purunã animals under different weather conditions, imposing higher thermal stress on the individuals, and to assess whether silvopastoral systems could again improve their thermal comfort. The motivation of the animals to use shaded areas rather than to express certain adaptive behaviors may collaborate to explain the present data. It was observed that animals in the SPS presented a reduction in the time budget devoted to feeding as compared to animals in the PAS. Indeed, Shütz et al. (2008) have shown a preference for animals to stay in the shade rather than to spend time lying, even after lying deprivation. Another possibility would be that the SPS group had lower forage available to eat, given the mild temperatures that were experienced throughout the experiment. Thus, the motivation to stay in the shade may be one of the reasons for the decrease in the eating and drinking periods which, in turn, may explain the observed significant decrease in BCS. Data from IDR-Paraná demonstrate that animals selected the areas under the trees during portions of the day, even when in thermal comfort. Thus, the presence of shaded areas seemed to indicate that the option to include trees in the pasture areas is an important condition for the welfare of animals, and warrants further research to test the strength of this condition in different temperatures and geographical regions.

Taking the present scenario, we consider that the reduction in BCS did not affect animal welfare, as the scores stayed within the normal range (Eversole et al., 2009). Further analysis should also be performed to examine the reduction in forage intake in the SPS treatments, focusing on forage availability, forage species, and the time of the year. BCS is regularly used to assess the fitness and nutritional needs of animals, including beef cattle, and is also directly related to reproductive performance and behavior responses (Herd and Sprott, 1998; Tucker et al., 2007). For instance, *Bos taurus* adult cows with low BCS showed a reduction in their reproductive performance and were more susceptible to health problems than animals that had higher BCS (Eversole et al., 2009). Moreover, animals with low BCS differed in time budgets in the expression of specific behavior, such as the reduction in eating (Tucker et al., 2007). According to Ainsworth et al. (2012), the SPS group seemed to have negatively affected the physical condition of the animals, which in turn may have disturbed their physiology (lower surface temperature) and the time budget of eating behavior. It has to be considered that there were differences in the motivation for eating and other behavioral mechanisms, as some environmental differences from both systems may trigger these distinctive stimuli, including the influence of forage availability and quality. Although the SPS showed a forage mass almost twice as low as the PAS, it presented a greater proportion of leaves. As shown before, leaves offer a higher quality food than stems (Van Soest, 1994), giving the animals a higher nutritional fulfillment. It has been found that the improvement of the nutritional value of the forage under shade, mainly through the increase in crude protein content, may contribute to the improved performance of animals on a pasture diet (Hanish et al., 2016; Sousa et al., 2010; Yamamoto et al., 2007). Finally, the results regarding weight gain need further studies during longer periods and thus more sensitive to the effects of the trees and forage availability and quality influencing cattle weight gain.

Pasture quality may also be responsible for the reduction of the time budget spent on eating behavior. It was observed that animals raised under the SPS had an average frequency of eating behavior lower than animals in the PAS. Although Broom et al. (2013), demonstrated that foraging times were reduced by temperature and humidity in monoculture paddocks and not in the silvopastoral paddocks, Tucker et al. (2008), found no difference between animals with access to a shady area and animals that had no access to shadow. These studies had different results regarding grazing time, showing that the effect of shadow in the silvopastoral systems is not very specific and the quality of pasture could be a more significant factor (Tucker et al., 2008). In this line, grazing animals may also explore the environment and adapt their feeding strategies to the prevailing conditions to meet their nutritional requirements (Bailey, 2005). Indeed, pasture structure can therefore directly influence the expenditure of time and energy by the animal in its foraging strategy (Trindade et al., 2016), affecting fitness, performance/production, and other eating behavior. Thus, every bite of an animal in the SPS got a better nutritional value than a bite of an animal in the PAS. Consequently, the animals reduced their foraging time budget while retaining more energy. Despite this possible positive aspect (i.e., forage with a higher nutritive value can reduce foraging time budget), this response was not reflected in the body weight gain and BCS of animals under the trees. In addition, animals in the SPS spent more time resting than animals in the PAS, which is in agreement with previous studies (Broom et al., 2013). As pasture quality varies over time, we still need to determine the optimal period for body development when heifers are eating *Hemarthria* spp. under a warm humid temperate climate at IDR-Paraná.

Lying is an important behavior for cattle and constitutes approximately 50% of their daily time budget (Albright, 1987). Conversely, increased standing is often considered an indication of discomfort or dissatisfaction (Albright, 1987). Cattle can ruminate while standing, but they preferably perform this behavior by lying down, commonly laterally on the left side to optimize the positioning of the rumen (Albright, 1993). Moreover, rumination is subjected to voluntary control, and the animal will therefore cease to ruminate if it is disturbed (Philips, 2007). Any occurrence that gives rise to pain, hunger, maternal anxiety, or illness will also decrease rumination (Fraser, 1980). More lying behavior and higher frequency of ruminating in animals raised in SPS suggest that a higher comfort was experienced, as compared to animals in the PAS. Drinking behavior is a cooling strategy known to be used by animals to promote heat dissipation (Jensen, 2009). Although the data revealed that individuals in the SPS drank less than individuals in the monoculture system, the animals were never confronted with thermal distress during the experimental period. Major factors influencing drinking intake are environmental temperature and humidity, as well as the dry matter content of the herbage.

Beyond some mild behavior preferences, the differences between groups may have been related to the higher tick scores observed in animals in the SPS. Differently from Broom et al. (2013), the SPS seemed to increase the tick population. Salazar-Benjumea et al. (2016) suggested that pasture made of *Leaucena leucocephala* could reduce tick loads on the Colombian creole *Bos taurus* mix, influencing the SPS. So far, we cannot suggest any correlation between pasture cover and tick infestation. The data on weight loss due to tick infestation indicates that the positive effects of SPS on cattle welfare could be impacted, highlighting the need for further studies. However, it can also be noticed that the animals on SPS did not have more skin injuries than the animals raised in the PAS. The current data suggest that strategies to control parasites (preventive or selective-based) are central to offering better welfare conditions to animals once a threshold is reached (Molento et al., 2013). It is important to use efficient acaricides, due to the alarming spread of drug-resistant tick populations against most of the available products (Charlie-Silva et al., 2018; Dolenga et al., 2022). As ticks had an aggregated distribution, it was observed that animals with the highest tick scores within the SPS had weight differences (P = 0.0216), when compared to animals with the lowest tick scores. It was determined that the mild temperature allowed the completion of a third generation of the tick life cycle. Cattle were compared between contrasting production standards and sustainable systems in Africa (Muchenje et al., 2008), Colombia (Salazar-Benjuema et al., 2016), and tropical areas of Brazil (Fraga et al., 2003; Giglioti et al., 2017; Oliveira et al., 2017), looking for differences in tick prevalence. This is the first comparison of SPS and PAS conditions in Purunã cattle and also under a temperate/subtropical environment in Brazil. The shade-tolerant grass *Axonopus catarinensis* Valls, a forage with high protein concentration, has also been suggested for the SPS (Baldissera et al., 2016), and should also be tested at the IDR-Paraná farm to better determine the forage availability and eating behavior.

There was a small decrease in the BCS for both groups (0.9 to SPS and 0.3 to PAS) by the end of the experimental period – possibly due to the reduction of forage quality and ingestion by the SPS group. Nevertheless, these differences did not reflect in weight gain differences between individuals reared in silvopastoral or pasture control plots. Until today, the performance of cattle in SPS has shown certain contradictory results. While specific studies demonstrate the influence of this system on a more consistent weight gain of animals (Broom et al., 2013; Paciullo et al., 2011; Peri et al., 2016), other authors did not find a significant effect or even show a negative impact on weight gain (Kallenbach et al., 2006). These differences should be taken with care as the systems have multiple biotic and abiotic factors, including the production of more than one product (trees/wood) in the SPS, and depend on important aspects, such as soil quality, weather, breed differences, pasture availability, animal density, and local investments (Chara et al., 2017).

The BCS is an easy, inexpensive but subjective method to evaluate the body tissue reserves of the cows. Indeed, BCS alone has been considered a poor indicator of the cow's performance because it is affected by frame size, gastrointestinal infections, and the lack of evaluation of the bioenergetically important tissues (Chagas et al., 2007). The data also revealed no significant difference in body weight of all individuals (SPS= -15.9 kg and PAS= -12.4 kg). However, there were differences when evaluating the BCS. Even though the results showed no difference in the weight among individuals kept on SPS, which had a moderate presence of ticks, and PAS, which had a low presence of ticks; we observed a significant difference in the final weight (-10.6 kg/low tick score vs. -22.2 kg/high tick score) of animals within the SPS (P = 0,0216), when only the 'tick score' was considered. The host-parasite relationship is complex, and a parasite incidence may be correlated to a health/sanitary problem (Molento, 2009). Other factors may have more significant limitations to the animals' performance and behavior (i.e., pasture structure, less eating behavior) as observed in the present study.

The present work suggests that it may be possible to measure individual tick infestation. As seen above, the use of more complex and integrated systems can affect positively or negatively the health of cattle (Oliveira et al., 2017; Schafaschek et al., 2021). In the case of crop-livestock-forestry, the system might increase the horn fly and tick count. Moreover, low parasite count does not affect the welfare of the animals, allowing the animals to express their natural resilience. An important factor is the possibility of having a strong repeatability index (> 60%) of resilient animals, which, in turn, could reduce the number of treatments against ectoparasites. Sustainable health protocols should consider the use of target-selective treatment (TST) as an efficient tool for protecting the environment and human and animal lives (Bello et al., 2020; Molento et al., 2004, Molento et al., 2022). TST can be incorporated into most agroecosystems (Andrade et al., 2022; Molento et al., 2013), integrating ecosystem services and the coexistence of cattle and a diverse number of parasite species.

5. Conclusion

Cattle in the SPS presented lower body surface temperature, even when not confronted with excessive thermal stress. Nevertheless, animals in this system showed a significant BCS decline that may be related to the higher *R. microplus* (tick) index and the associated behavior. The better pasture quality in the SPS might also explain the reduction of time spent on eating. Finally, animals in the SPS spent more time resting and ruminating than animals in the PAS. Future studies, especially under the thermal stress of summer conditions, may assist in the understanding of the importance of trees providing more shade areas to improve animal welfare. Moreover, additional refinements are needed to better determine the relationship between body condition score, weight gain, parasite index, and climate conditions when the SPS and the PAS are compared.

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