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Effects of the Integrated Crop-Livestock and Crop-Livestock-Forestry systems on forage growth, grazing behaviour, feed intake and performance of Nellore heifers during the dry season in Mato Grosso do Sul, Brazil

Master Thesis

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Hereby declare that I am the sole author of this Master Thesis; no assistance other than that permitted has been used and all quotes and concepts taken from unpublished sources, published literature or the internet in wording or in basic concentration have been identified by footnotes or with precise source citations.

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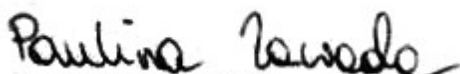
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A handwritten signature in black ink, reading "Paulina Zawada". The signature is written in a cursive style with a horizontal line underneath.

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Abstract

This study aimed at evaluating the effects of Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) systems on grazing behaviour, feed intake and performance of Nellore heifers by altering microclimate and forage morphological and productive characteristics in comparison to continuous pasture (CON) during the dry season. Data were collected in June and July 2019 in Embrapa Beef Cattle Research Corporation in Campo Grande, Brazil. The experimental site was divided into eleven paddocks. CON, sown with *Brachiaria decumbens*, was compared to ICL, sown with *Brachiaria brizantha* cv. BRS Piatã and ICLF with *Eucalyptus urograndis*, sown with *Brachiaria brizantha* cv. BRS Piatã in terms of morphological characteristics and nutritional value of forage. The RumiWatch system was used to assess the grazing behaviour of 36 Nellore heifers, while titanium dioxide (TiO₂) marker method was used to evaluate the organic matter intake (OMI) of the heifers. The average daily gain (ADG) served as a measure to determine the livestock performance. The results revealed that ICL was characterized by the greatest forage biomass, while the forage biomass in ICLF was significantly reduced due to shading. Forage in ICLF presented enhanced nutritional quality in terms of crude protein (CP) and digestibility in comparison to ICL. ICL and ICLF provided heifers with a greater mass of green forage, while the forage in CON consisted mostly of dead plant material. The heifers in CON had a longer grazing time in comparison to ICL, whereas the OMI did not differ between the systems. The heifers achieved a greater ADG in ICL and ICLF, demonstrating that integrated systems have the potential to overcome the constraints of the dry season in Brazilian Cerrado.

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List of Abbreviations

ADF	Acid Detergent Fiber
ADG	Average Daily Gain
BGHI	Black Globe Temperature and Humidity Index
BW	Body Weight
CON	Continuous Pasture
CP	Crude Protein
DM	Dry Matter
DMI	Dry Matter Intake
dOM	Digestibility Organic Matter
FM	Fresh Matter
FO	Fecal Output
GLM	General Linear Model
GHG	Green House Gasses
ICF	Integrated Crop Forestry
ICL	Integrated Crop Livestock
ICLF	Integrated Crop Livestock Forestry
IFL	Integrated Forestry Livestock
IVDOM	<i>in vitro</i> Digestibility Organic Matter
K	Potassium
N	Nitrogen
NDF	Neutral Detergent Fiber
NIRS	Near-infrared Spectroscopy
OM	Organic Matter
OMI	Organic Matter Intake
P	Phosphorus
PAR	Photosynthetically Active Radiation
RH	Relative Humidity
RTL	Radiant Thermal Load
T	Temperature
THI	Temperature Humidity Index
TiO ₂	Titanium Dioxide

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1. Introduction

Brazil, as the second largest meat producer in the world, has a significant impact on the world's food supply and therefore continuous study of the main obstacles and constraints of Brazilian agriculture is required (Kunrath et al., 2020). Approximately 50% of Brazilian beef production is concentrated in the Cerrado biome, which comprises almost one-fourth of the country's territory (Macedo & Araujo, 2014). Low investments, lack of nutrients replenishment to the soils, inadequate planting of pastures, and continuous cattle breeding without an appropriate animal management resulted in the degradation of approximately 70% of pastures, mainly in the North, Central-West (Cerrado) and Northeast areas of Brazil (Kichel et al., 2014; Santos et al., 2016). Pasture degradation is considered a factor which affects sustainable beef production to the greatest extent and leads to a decrease in production efficiency (Macedo & Araujo, 2014). Another challenge that Brazilian farmers face every year is the seasonality of forage production. Brazilian cattle husbandry is based on grazing systems and thus production is almost entirely dependent on grasslands, which are subjected to significant fluctuations in quantity and quality during the dry season (Silva & Carvalho, 2005). When the rainfall is scanty and unevenly distributed, the pastures demonstrate poor nutritional quality and diminished growth. Reduced availability of nutritious forage contributes to poor livestock productivity during the dry season. Over the long run, it compromises farm's profitability and food security (Lamy et al., 2012). Since the 1980s, Brazilian research institutions have started to step up the creation of strategies aimed at recovering grazing areas and addressing the issue of production seasonality. This has contributed to the growing adoption of integrated systems in Brazil (Kichel et al., 2014). According to the definition provided by Balbino et al. (2011) integrated systems are the "systems which incorporate crop, livestock and forestry components in a temporal and/or spatial framework aiming at sustainability of the farm through synergies between the components". However, these farming systems are arranged in various ways, differing in rotation and species sequences, as well as implementation details. The introduction of components in the integrated systems is not random. Each component is a strategic tool which must interact and complement with others (Balbino et al., 2014).

It is claimed that the adoption of integrated systems is advantageous due to reduced pasture degradation, increased soil fertility, enhanced cycling of nutrients and better soil aggregation (Balbino et al., 2014). Moreover, greater resilience towards pests, weeds and diseases is accomplished by crop rotation and livestock inclusion; as the life cycles are disrupted. The environmental impact and production costs are reduced, while the economic outcomes increased (Salton et al., 2014). Promotion of biological diversity and enhanced soil quality help to withstand seasonal impacts by providing a higher and more consistent supply of forage with improved quality relative to monosystems (Kichel et al., 2014). Yet, increased complexity creates different risks and challenges, altering the cultivation and living conditions for forage and livestock. Grazing behaviour of cattle, their feed intake, and thus performance is directly related with forage canopy characteristics they are provided with. Usually there is a proportional relationship between the time spent by cattle grazing and the quantity and quality of forage canopy, or more precisely the lower quality of forage, the longer grazing time cattle requires to compensate for it (Fonseca et al., 2012). Inclusion of additional components, such as trees, alters the microclimate for pasture growing in the understorey, by reducing light and creating competition for water between forage grasses and arboreal components. Plants often trigger morphological and physiological alterations to adapt to the new conditions; moreover, their growth can be impaired (Geremia et al., 2018). On the other hand, various studies have shown that shade provided by trees may boost the nutritional value of grasses, in particular the crude protein concentration, which, combined with enhanced thermal comfort of cattle, contributes to increased animal welfare and productivity (Paciullo et al., 2011; Ryschawy et al., 2012; Souza et al., 2010). All in all, constant evaluation, which takes into account the various seasons and locations, is required to assess the factors affecting forage and livestock responses to the alterations of living conditions created by integrated systems; and their ability to address the constraints of Brazilian beef production.

The major aim of this study is to evaluate the effects of living conditions provided by Integrated Crop-Livestock (ICL) and Crop-Livestock-Forestry (ICLF) systems in comparison to continuous pasture (CON) on forage growth and its characteristics, grazing behaviour, feed intake and consequently the average daily gain (ADG) of Nellore heifers during the dry season in Brazilian Cerrado.

Specifically, the objectives are:

- i. To determine differences in forage biomass and canopy characteristics in ICL and ICLF in comparison to CON during the dry season
- ii. To determine differences in behavioural patterns of Nellore heifers grazing in ICL and ICLF in comparison to CON during the dry season
- iii. To evaluate effect of ICL and ICLF on feed intake and ADG of Nellore heifers in comparison to CON during the dry season

Corresponding hypotheses are:

- i. The forage biomass in ICL will be increased in comparison to CON and ICLF. Shading in ICLF will result in decreased forage biomass in comparison to ICL, while the nutritional quality of forage in ICLF, e.g. a crude protein concentration, will be enhanced due to shading. Forage grass in ICLF will present adaptation mechanisms towards shading, e.g. a stem elongation, resulting in increased stem proportion in the forage composition. CON will be characterized by the lowest forage biomass, with a high proportion of dead plant material and a low nutritional value.
- ii. Shading in ICLF will improve the microclimatic conditions for heifers in comparison to ICL and CON, therefore the heifers in ICLF will present the longest grazing from all systems, also as a consequence of low forage biomass resulting from reduced solar radiation. Even with low forage biomass in CON, the heifers will not increase their grazing time due to compromised thermal comfort in comparison to ICLF.

- iii. High forage biomass in ICL will result in a greater feed intake in comparison to ICLF and CON. Stem elongation in forage in ICLF will lead to a decreased feed intake in comparison to ICL, with stems as a limiting factor. The longest grazing in ICLF will result in a higher feed intake of the heifers in comparison to CON. Owing to a higher forage biomass in ICL, greater nutritional quality of forage in ICLF and higher feed intake in both integrated systems in comparison to CON, the heifers in ICL and ICLF will achieve higher ADG in comparison to CON.

2. Literature review

2.1 The integration of crop, livestock and forestry as a production system

Due to rising demand for food products and efficient production, higher production costs and a more competitive market, but also due to growing awareness of animal welfare and the environmental impact of agriculture, a significant number of studies have been undertaken in recent years to assess integrated farming systems in terms of their potential to increase the yields, quality of products and profitability of the system, without harming the environment. There is a general consensus that integrated systems bring various benefits throughout the synergies created between their components, but also further challenges arising from their sophistication.

Balbino et al. (2014) outlined four major classes of integrated systems in Brazil:

- i. Integrated Crop-Livestock: a production system that includes the crop and livestock in rotation, succession or combined at the same time in the same area
- ii. Integrated Forestry- Livestock (IFL): a production system applied in the areas where it is difficult to grow crops; integrates livestock and arboreal components in association
- iii. Integrated Crop-Forestry (ICF): a production system that integrates tree species with annual or perennial crops
- iv. Integrated Crop-Livestock-Forestry: a production system that includes the crop, livestock and arboreal components in rotation, succession or combined in the same area. The most popular combination in cattle ranching areas in Brazil is *Eucalyptus* with soybeans or maize crop.

The integrated production systems gather several production systems into one within many different combinations depending on local conditions and needs.

2.2 Main advantages of ICL and ICLF systems

With the implementation of integrated systems in addition to land use intensification, other environmental benefits are generated.

Salton et al. (2014) conducting a long-term study (from 1995) at the Embrapa Western Agricultural station in Dourados in the state of Mato Grosso do Sul, Brazil, confirmed their initial hypothesis that rotation of crops and pastures in ICL is more efficient and environmentally beneficial than traditional monosystems. Over the years, forage yields have been less affected by frost and drought, indicating a greater resilience due to improved soil conditions in terms of fertility, physical structure, nutrient use by plants, accumulation of organic matter and increased biological activity and diversity, leading the authors to the conclusion that complexity of the systems has resulted in enhanced production efficiency.

The concept of eco-efficiency was introduced by Wilkins (2008), who outlined the effects of crop rotation on soil fertility and nutrient use efficiency with an aim of sustainable use of resources in the farm production. There is no straightforward definition of eco-efficiency, however the term is linked to “ecology” and “economy”. Sustainable use of resources and land management with the minimization of environmental impact are the key concerns that affect eco-efficiency. Eco-efficiency increases as a certain level of productivity is achieved with less resources and fewer effects on the environment, but also without a loss in the productive capacity and economic output of the farm. As one of the approaches to increase the eco-efficiency Wilkins (2008) pointed out alteration of land-use system in terms of crop rotations. According to the author, crop rotation has an important role in conservation of nutrients, improvement of soil fertility, management of soil-borne diseases, control of weeds and pests. Moreover, incorporation of livestock in the system provides nutrients in manure and enables to use a wider range of crops, including grassland. The use of grass in rotation is crucial in the areas with soil structural problems. Grass improves the structure of soil by increasing proportion of water stable aggregates resulting in better water infiltration. Improved soil structure with complete year-round cover in integrated systems gives the grassland ability to resist erosion.

Also, Vilela et al. (2001) have reported promising findings on soil properties after implementation of crop rotation. The authors conducting study at Santa Terezinha farm in the state of Minas Gerais, Brazil, observed an increase in aggregate stability in pastures planted after soybean, demonstrating a major role of forage grasses root system for the aggregation of soil particles. The organic matter concentration of soil was also increased from 0.84-0.94% under continuous system to 1.23% under rotation.

Villa Alves et al. (2017) provided a technical report on another property of ICLF, which is carbon accumulation. Trees in ICLF during their growth absorb ambient CO₂ and accumulate it. This characteristic may serve as a solution for carbon sequestration and mitigation of greenhouse gas (GHG) emissions. Implementation of ICLF was presented by the Brazilian Government in 2010 as one of the strategies to mitigate GHG from agriculture in Plano ABC, a low-carbon emissions agricultural plan providing credits for projects adopting this system. “Carbon Neutral Brazilian Beef” is a concept developed in 2015 by Embrapa, representing beef cattle raised in the system with forestry under certain requirements. The arboreal component must be managed in such a way that part of the wood produced is used for products with a long shelf-life, such as furniture and building wood, ensuring longer carbon immobilization and neutralization of GHG from livestock (Villa Alves et al., 2017).

Additionally, some other advantages of ICL and ICLF were pointed out by Balbino et al. (2014) including: more efficient control of pests, diseases and weeds, resulting in lower pesticide use; reduced pressure for deforestation and clearing of natural vegetation areas; supporting biodiversity protection; intensification of nutrient cycling and recovery through litter and decaying plant residues resulting in increased soil organic matter; reduced erosion due to increased soil cover from crops and forage grasses residues; enhanced microclimatic conditions and thermal comfort for cattle leading to increased animal well-being.

Last but not least, several studies available in the literature have shown that ICL and ICLF implementation offers economic prosperity and benefits to farmers. Cobucci et al. (2007), Muniz (2007) and Martha Junior et al. (2011) have identified advantages in several economic viability indicators of integrated systems via profit diversification and more stable production throughout the year in comparison to continuous traditional systems. However, the present study did not evaluate

financial profitability of integrated systems, therefore this aspect is not explored in detail.

2.3 Main challenges of ICL and ICLF systems

Besides the above-mentioned advantages, integrated systems pose a range of challenges arising from their complex construction and the impact of the components on each other.

Santos et al. (2018) complementing their previous work (Santos et al., 2016) evaluated Nellore heifers' performance and Piatã grass quality characteristics in ICLF with *Eucalyptus urograndis* in Brazilian Cerrado. The forage dry mass of Piatã grass in the system with arboreal component was reduced in comparison to the treeless system. This is one of the main concerns about the forage productivity in the systems with trees particularly during the dry season when forage grasses are vulnerable and do not seem to cope well with the competition with trees (Silva & Carvalho, 2005). The research of Santos et al. (2018) emphasized the importance of the photosynthetically active radiation specifically for C4 grasses, such as *Brachiaria*, in order to avoid restrictions of forage growth.

The results from the study of Santos et al. (2018) were consistent with the Oliveira et al. (2014) study, who also observed diminished forage growth in the case of ICLF. Similarly, the research evaluated production and nutritive value of Piatã grass and performance of Nellore heifers in ICLF with *Eucalyptus urograndis*. The experiment was carried out in the state of Mato Grosso do Sul, part of the Cerrado biome in Brazil. The findings confirmed that pasture growing under the shade of trees is subjected to reduced solar radiation and responds with diminished growth of dry matter.

In addition to lack of light, some scholars addressed another potential cause contributing to a decline in forage production, which is an alteration in soil characteristics and competition for water and nutrients. However, some of the findings are contradictory. On the one hand, Santos et al. (2016) observed a reduction in the cumulative dry mass and accumulation rate of Piatã grass grown under *Eucalyptus* trees in ICLF in contrast to a treeless area in the rainy and dry season in Brazilian Cerrado and therefore concluded that due to deep root exploration in ICLF, there is a significant soil moisture removal and competition for

nutrients between trees and forage components. On the other hand, according to Sousa et al. (2015), this aforementioned deep-rooted soil exploration is an advantage that makes nutrients more available to grasses, thus improving yield and quality of forage. Although the various studies have shown that the forage yield is rather reduced in ICLF in comparison to treeless systems, the nutritional value of forage grown under the shade may indeed benefit from this condition (Paciullo et al., 2011; Souza et al., 2010).

Despite certain obstacles relevant with the presence of arboreal components in ICLF, both systems: ICL as well as ICLF demand higher qualification and commitment from people involved in their implementation, as the additional components, such as crops, may bring new risks to the production, for instance various, previously unknown pests. Moreover, according to Balbino et al. (2014), the traditionalism of farmers and their resistance to emerging technologies is one of the main challenges facing the adoption of integrated systems. Secondly, integrated systems involve higher financial investments, while the returns are achieved in the medium and long-term. Lastly, the inclusion of an additional component, such as crops, requires the availability of infrastructure, knowledge and new skills of farmers in order to avoid production losses (Balbino et al., 2014).

2.4 The impact of ICL and ICLF on forage grasses and livestock

The aforementioned advantages, as well as the challenges of ICL and ICLF implementation, have a direct impact on the forage characteristics and the responses of livestock raised in these production systems.

For instance, Barros et al. (2018) evaluating nutritional characteristics of Piatã grass in integrated systems under different densities of *Eucalyptus urograndis* in Mato Grosso do Sul, Brazil, found out that swards in ICLF with higher tree density and therefore higher shade percentage showed increased concentration of crude protein, and lower value of neutral detergent fiber. The *in vitro* digestibility of organic matter of leaf blade in ICLF was also greater in comparison to full sun environment.

Growing under limited solar radiation and modified microclimate, forage grasses are able to partially adapt to new conditions, mainly through phenotypical adjustments such as changes in leaf area arrangements or alterations in the vertical profile of the plant. Geremia et al. (2018) evaluated the effect of shade on the vertical sward

structure of Piatã grass cultivated with maize and *Eucalyptus urograndis* in ICLF in Mato Grosso, Brazil. Three different shading intensities were compared, showing that under intense shading, the swards were the tallest and pasture had the largest proportion of stems, which proves that forage plants are trying to compensate for light reduction by excessive elongation of stems and positioning leaves higher on the sward. Similarly to other studies, the authors found the greatest concentration of crude proteins in forage grown under intense shading.

Since the sward morphological composition directly influences the intake behaviour of cattle and therefore also their performance in the same study, Geremia et al. (2018) aimed at identifying the relationship between sward vertical structure and feed intake of cattle. The authors observed that the dairy heifers decreased their bite mass and intake rate while grazing the tallest swards under intense shading. It was concluded that the percentage of stems in the forage is negatively correlated with the feed intake of cattle and interferes with their grazing behaviour. Additionally, it has been also demonstrated that increased intake was found in the systems with the highest proportion of leaves in the forage mass.

Since the intake of dry matter in ruminants is one of the main variables affecting their performance, grazing behaviour in relation to the characteristics of swards is of interest to many researchers. Grazing behaviour is a significant parameter representing the abundance and quality of the forage. In the pasture-based systems, it is important to balance herd demand with the feed offered to maximise grass utilisation with a focus on increasing animal intake (Werner et al., 2018). Benvenuti et al. (2008) evaluated the effect of stem density of tropical swards and age of Droughtmaster steers (1-year-old and 3 years old) on their foraging behaviour in Australia. Artificial microswards representing *Panicum maximum* have been used. The authors observed a significant negative effect of the stem density on the bite area, the bite mass and the intake rate, particularly for mature steers. As a consequence, stems were perceived to be an obstacle that interfere with the natural movements of tongue to gather plant material. As the stem density increased, more time was needed for the steers to find the leaves and thus, the number of manipulative mouth movements increased. All in all, the intake rate decreased with a greater stem density due to a negative effect on bite mass and time per bite. The research has also shown that this limitation has greater effect on mature steers,

which consequently may prevent them to fulfil their daily requirements of forage intake.

Solenberger & Burns (n.d.) summarized findings about the grazing systems based on C4 grasses and the various relationships between grazing behaviour, canopy characteristics and living environment in relation to forage intake. The authors emphasized that it is difficult to evaluate the independent effect of the canopy structure, as in the natural swards the variables are very often strongly correlated with each other. Moreover, there are challenges relevant to the methodology, for instance the sampling site may not be a proper representation of the grazed area. In addition, there is also an influence of non-canopy factors such as grazing environment. Grazing behaviour often reflects the relationship between internal state of the animal, such as nutritional requirements, and their living environment, such as climate (Rombach et al., 2018). The ruminants adapt to the living conditions and adjust their behaviour to achieve a certain level of consumption and meet their nutritional needs (Baliscei et al., 2012).

The assessment of the effects of living conditions on the behaviour of Canchim cattle in the tropical climate was carried out by Giro et al. (2019) who conducted their research in two integrated systems, ICL and ICLF, in Sao Carlos-SP, Brazil. The difference between the systems was the presence of trees and the provision of shade, thereby altering the microclimate in ICLF. Variations in behaviour between the systems were observed in the morning, when the beef cows ruminated longer in the shaded area and spent less time resting in comparison to the full sun environment. The grazing time (min) differed only in April, when cattle grazed for longer time in ICL during the morning hours due to milder environmental conditions. The authors also observed that cattle preferred to remain in the shade during all activities. This behavioural change, along with lower back and trunk temperatures recorded in cattle in ICLF, suggests that the incorporation of trees has been effective in reducing heat load and provided more favourable microclimate for the animals, resulting in more evenly distributed activities throughout the day, regardless of radiation strength.

The effects of shade on beef cattle behaviour and feed intake were also evaluated in the study of Souza et al. (2010). System without shade was compared to two ICLF systems with different heights of *Eucalyptus* trees (8 m and 18 m) by visual

observation of behavioural patterns of Nellore heifers. Grazing, rumination and idling in the sun and shade were recorded. First of all, the authors noted that, on average, 47% of all available time the heifers remained under the shade. Secondly, the animals which were provided with shade, regardless of the height of the tree, grazed longer in the afternoon than in the morning, although the environmental conditions were more severe. It shows that their grazing time did not depend on milder environmental conditions, but the trees provided such thermal comfort that the heifers were enabled to graze according to their preferences. On the other hand, in the treeless system, the frequency of grazing was lower in the afternoon and the heifers rested more. In view of the more severe environmental conditions in the afternoon, the authors concluded that, in treeless systems, the animals sought to graze more during the time of the day when the environmental conditions were more favourable (morning) and to rest more when the conditions were severe (afternoon). Rumination frequency differed between the systems, however, according to the researchers, not attributable to an altered microclimate in shaded areas, but to a particular canopy structure. The authors therefore concluded that the presence of trees has an impact on the time and frequency of grazing and idle but does not have an impact on rumination activity.

In her work, Villa Alves (2014), reviewed the available researches and outlined the importance and impact of compromised thermal comfort on the feed intake behaviour of beef cattle. Studies supporting the negative relationship between grazing and high temperatures were provided by the scientist. For instance, Ferreira (2010) evaluating the physiological and behavioural responses of cattle subjected to different shade levels, observed that the animals had reduced their grazing time when they had no access to shade. Further, Oliveira et al. (2012) assessing daily behaviour of Nellore calves in integrated systems, showed that beef cattle, if offered a choice, tend to graze under the tree shade in the integrated system instead of full sun environment. Blackshaw & Blackshaw (1994) termed this behavioural change “shade seeking” and referred to it as one of the strategies for coping with a hot environment. The authors examined the effects of heat stress on livestock production and behaviour of cattle and they correlated a decrease in feed intake with immediate reaction to heat stress. The authors also recorded the study of Daly (1984) in which more behavioural alterations were documented. The Shorthorn cows kept in a dry, hot climate avoided cud chewing during the hottest hours of the

day. Conversely, the animals protected by the shade continued this activity. On the basis of that result, Blackshaw & Blackshaw (1994) concluded that the provision of shade allows for a distribution of rumination throughout the day according to the behavioural needs of the animals. Thermal comfort of grazing animals is therefore an important requirement for optimal and efficient livestock production. Brazil has a high incidence of solar radiation all year long owing it to its position in the intertropical zone. Indeed, Navarini et al. (2009) found out that in tropical conditions, Nellore cattle have suffered thermal discomfort. The authors pointed out that even if thermal stress does not lead to physiological problems in healthy animals, it can definitely compromise and diminish the weight gain of cattle. Cattle as homeothermic animals have the ability to control the temperature of their bodies. When, along with high relative humidity, the animals are exposed to extreme temperatures, they undergo into state known as heat stress. To cope with this physiological condition, they trigger mechanisms to re-establish thermal neutrality (Oliveira et al., 2017). When thermal comfort is guaranteed, the energy is used to convert feed into meat instead of regulating body temperature, thus increasing productivity (Vieira Junior et al., 2019). Already more than 60 years ago, the researches were evaluating shade as one of the ways to increase the efficiency of livestock production. McDaniel & Roark (1956) conducted a 4-year-study at West Louisiana Experiment Station on Hereford (60% of examined cattle) and Angus (40%) cows with calves grazing either shaded pastures or areas without shade. The treatments were divided into abundant natural shade, scanty natural shade, artificial shade and treatment without shade. The natural shade was provided by trees such as gum, oak, bay and pay trees, whereas the artificial shade was created by roofs made of hay and straw. The cows protected with natural shade spent about half an hour more on grazing and had greater gains than those grazing areas without shade protection. Interestingly, this pattern was not observed in herds kept in artificial shade treatment. This finding has been explained by a very limited area of shade per animal unit. Calves, on the other hand, achieved significantly greater gains in all three shade treatments than those in full sun environment. Apart from the information that the pastures were equal to each other, no other pasture data was provided, it is therefore difficult to conclude that increased productivity was solely due to shade effect.

Surprising results come from a more recent study of Ainsworth et al. (2012) evaluating the impact of tree shade on the productivity of dual-purpose cows, predominantly Brahman or Brahman crosses, in silvopastoral farms in the Rivas province of Nicaragua. The productivity was assessed by the measurement of milk yield and the individual body score. Shade did not have a major impact on milk production, while the body condition was negatively affected by shade. The authors hypothesized that understorey vegetation could have been negatively impacted by shade resulting in a decline in the quality and quantity of forage, thus altering grazing behaviour and feed intake of cattle. Nevertheless, no such analysis has been carried out in the research. This ambiguity highlights the importance of complex studies, including a pasture analysis, cattle behaviour and feed intake in relation to pasture shade, in order to link potential alterations in animal behavioural patterns and responses to living conditions and farm management. The purpose of the following study is to provide this knowledge.

3. Material and methods

3.1 Experimental site

The study was conducted at the Brazilian Agricultural Research Corporation – Embrapa Beef Cattle, located in Campo Grande, in the state of Mato Grosso do Sul (54°37'W, 20°27'S, 530 m of altitude) which, according to Köppen's classification, is located in the Cerrado biome, in the transition between Humid tropical zone with hot summer (Cfa) and Tropical zone with dry winter (AW). The average annual temperature is 23°C and the average annual precipitation is 1560 mm. During the dry season, which falls on the coldest months from May to August, 30% of the total annual rainfall may occur (Oliveira et al., 2017). The data collection took place in June and July 2019, corresponding to the dry season. During this period, the average air temperature was 21°C, with a peak of 31°C and the minimum temperature of 16°C. The precipitation during the experiment was 64 mm (n= 40 days).

The dominant soil type in the study region is classified as Ferralsol, covering 45% of the Cerrado region. These soils are characterized by high iron (ferrum) and aluminium concentration, predominant acidity (pH= 4.8-5.1), low cation-exchange capacity and poor nutrient concentration. Yet, their physical properties are suitable for mechanized agriculture (Genuario et al., 2019). Figure 1 presents the geographical location of Embrapa Beef Cattle research station and the location of Cerrado biome in Brazil.



Figure 1. Cerrado biome, the black star indicates the geographical location of Embrapa research station in the state of Mato Grosso do Sul and the dark grey color indicates the location of Cerrado biome in Brazil (Modified from <http://wildbrazil.com.br/cerrado>).

3.2 Grazing systems

Three grazing systems namely: CON, ICL and ICLF were analysed and compared with each other. The experiment site covered 13 hectares and was divided into eleven paddocks. CON was represented by four paddocks of 0.8 ha each, ICL was represented by three paddocks of 1.4 ha each and ICLF was represented by four paddocks of 1.4 ha each.

3.2.1 CON with *Brachiaria decumbens*

This grazing system was established in 1993 with *Brachiaria decumbens* and no fertilizers or lime were applied over the years. The pasture was renewed at the end of 2017 with the following steps: the soil was prepared using a harrow disk and application of 2000 kg of lime/ha; 800 kg of gypsum/ha were firstly broadcasted over

the pasture canopy and further incorporated into the soil; phosphorus (P) and potassium (K) in a form of N-P-K 0-20-20 were spread over the pasture canopy in a quantity of 543 kg/ha before seeding which occurred in November of 2017. Lastly, 45 kg of nitrogen (N)/ha in a form of urea was applied in April 2018. No fertilizers or lime were applied afterwards. CON was managed in the same manner as the common grazing system in Brazilian Cerrado, with continuous and set stocking of two Nellore heifers/paddock throughout the year. One of the paddocks representing CON pasture can be seen in Figure 2.



Figure 2. Continuous pasture (CON) with *Brachiaria decumbens* (own photo).

3.2.2 Integrated systems

Both integrated systems (ICL- without trees and ICLF- with trees) were implemented in 2008 aiming at recovering the degraded traditional *Brachiaria decumbens* pasture characterized by low forage biomass, strong presence of weeds and high sensitivity to spittlebug attacks. N fertilizer was applied in a form of urea at a rate of 75 kg N/ha in March 2019. The experiment was carried out in the second year of pasture establishment in the third rotation cycle between crop and livestock farming. The establishment of the integrated systems was previously described by Oliveira et al (2014).

- ICL without trees, with *Brachiaria brizantha* cv. BRS Piatã pasture

ICL was sown with *Brachiaria brizantha* cv. BRS Piatã in rotation with soybean (*Glycine max*) in a no-tillage system. The crop rotation looked as follows: one year of soybean crop cultivation was followed by harvest and further three years of solely sown pasture (*B. brizantha*) grazed by Nellore heifers. The stocking was kept continuous and set for the specific time of these measurements, with four Nellore heifers/paddock. The method is put-and-take stocking across the year.

- ICLF with *Eucalyptus urograndis* and *Brachiaria brizantha* cv. BRS Piatã pasture

ICLF was sown with *Brachiaria brizantha* cv. BRS Piatã in rotation with soybean (*Glycine max*), in no-tillage system. Similarly to ICL, one year of soybean crop cultivation was followed by harvest and further three years of solely sown pasture (*B. brizantha*) and livestock farming with Nellore heifers, 2 heifers/paddock. Initially, *Eucalyptus urograndis* (*Eucalyptus grandis* x *Eucalyptus urophylla*; H13 clone) was planted in single lines oriented at $-20,41^\circ$ south e $-54,71^\circ$ west in relation to the east-west axis, with 22 m distance between the rows and 2 m distance between trees within the row, resulting in 227 trees/ha. In 2017 the *Eucalyptus* trees were thinned by removing every second tree. The final arrangement looked as follows: 22 m x 4 m, resulting in 113 trees/ha throughout the course of the present experiment. The average height of the trees' trunk was on average 30.3 ± 2.8 m while the diameter at breast height was on average 29.9 ± 2.8 mm. One of the paddocks representing ICLF can be seen in Figure 3.



Figure 3. Integrated Crop-Livestock-Forestry (ICLF) with *Eucalyptus urograndis* and *Brachiaria brizantha* cv. BRS Piatã (own photo).

3.3 Experimental design

All procedures were approved by the Ethics and Animal Use Commission of the Embrapa Beef Cattle under protocol nº 014/2014.

A total of 96 21-months-old animals were randomly allocated into the grazing systems and into an additional area with *Brachiaria* sp. pasture for the management of stocking density, in November 2018. Two heifers per paddock were allocated in CON, whereas in ICL and ICLF the stocking was managed according to the put-and-take stocking method, in which a variable number of animals is used to maintain the required management criteria, for instance a desired quantity of forage (Allen et al., 2011). In the present study, 36 heifers with initial body weight (BW) of 343.1 ± 44.4 kg were evaluated. The BW of heifers was measured with an individual balance on a monthly basis from April to July, after 16 hours period of feed withdrawal. Commercial mineral supplement and water were provided *ad libitum*. All heifers were vaccinated and dewormed prior to the start of the experiment, according to the procedure recommended by the National Beef Cattle Research Centre.

The feed intake and grazing behaviour data collection were divided into three periods of 15 days each. Within one period of the aforementioned measurements, two paddocks represented CON, one paddock represented ICL and two paddocks

represented ICLF. This design resulted in evaluation of four heifers per system within one period (n=36). Graphic representation of the experimental design of one measurement period can be seen in Figure 4.

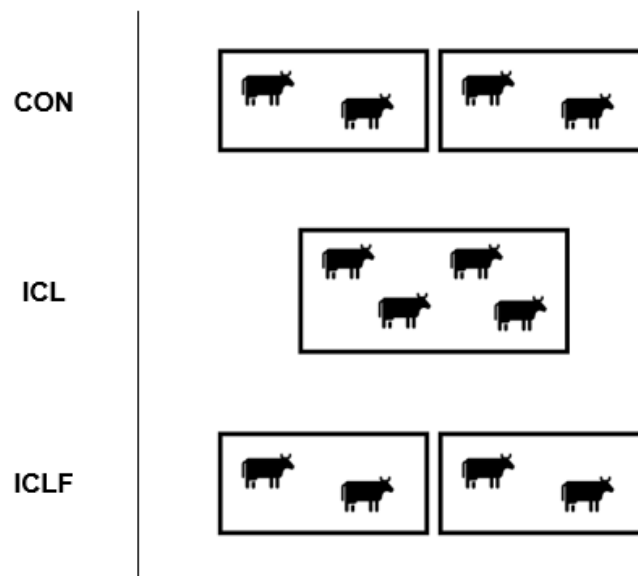


Figure 4. The number of heifers and paddocks included in each period of feed intake and grazing behaviour measurements in three grazing systems (own elaboration). CON- continuous pasture; ICL- Integrated Crop-Livestock; ICLF- Integrated Crop-Livestock-Forestry

The geographical location of the eleven paddocks can be seen in Figure 5. The following paddocks were evaluated in each period of livestock measurements:

1° period: CON- 44, CON- 36; ICL- 9; ICLF- 5, ICLF- 8

2° period: CON- 13, CON- 8; ICL- 10; ICLF- 6, ICLF- 7

3° period: CON- 44, CON- 36; ICL- 11; ICLF- 7, ICLF- 5

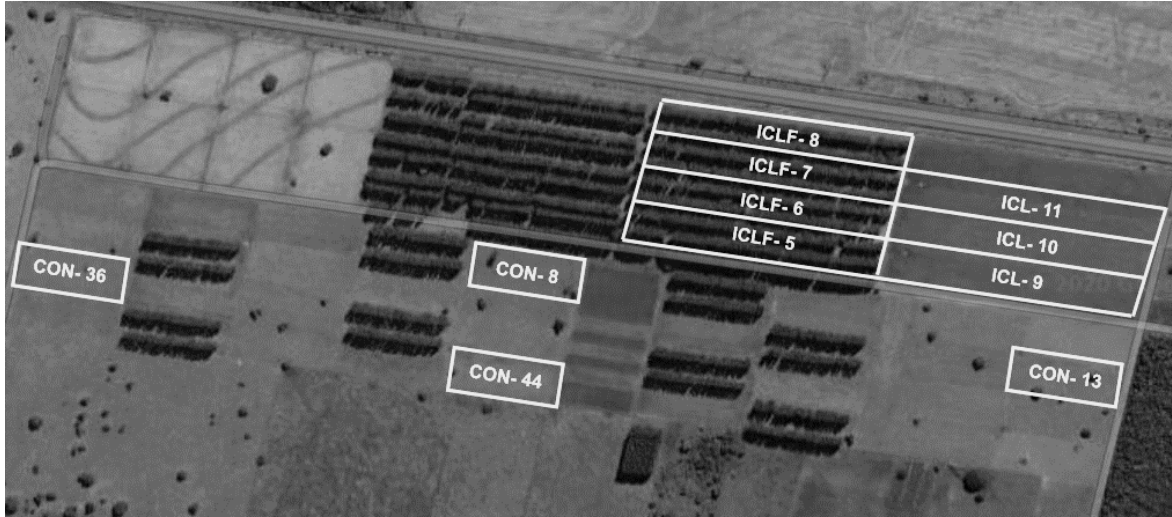


Figure 5. Geographical location of the evaluated paddocks. CON- continuous pasture, ICL- Integrated Crop-Livestock; ICLF- Integrated Crop-Livestock-Forestry

For the forage measurements, all eleven paddocks were evaluated in each period: within one period four paddocks represented CON, three paddocks represented ICL and four paddocks represented ICLF (n=33).

3.3.1 Microclimate data collection

Microclimate parameters, such as ambient air temperature and relative air humidity (RH), were measured daily at one-hour intervals for 58 consecutive days by Tinytag Plus 2 (Gemini Data Loggers, UK). The devices were mounted in one paddock in CON, as a representation of full sun conditions equivalent to ICL, and in one paddock in ICLF, as a representation of shaded environment with trees. In ICLF the device was located in 11 m distance from the tree row. From the microclimate data, the thermal comfort condition of the heifers was determined by the Temperature Humidity Index (THI), which was calculated using the equation from Yousef (1985):

$$THI = T_{db} + 0.36 \times T_{dp} + 41.2$$

where T_{db} - dry bulb temperature, T_{dp} - dew point temperature

Further, the average values of RH and temperature for each day were calculated with the formula provided by the meteorological station of Embrapa:

$$\text{Average RH of the day (\%)} = \frac{RH_{12} + RH_{18} + RH_{24} \times 2}{4}$$

Where RH12- relative humidity at 12:00 GMT time; RH18- relative humidity at 18:00 GMT time; RH24- relative humidity at 24:00 GMT time

$$\text{Average T of the day (}^{\circ}\text{C)} = \frac{T_{12} + T_{24} * 2 + \text{Max T} + \text{Min T}}{5}$$

Where T12- temperature at 12:00 GMT time; T24- temperature at 24:00 GMT time; Max T- maximum temperature over 24 hours, Min T- minimum temperature over 24 hours

As the next step, these values were used to calculate the average RH and average temperature of the 58 days of climate data collection.

The photosynthetically active radiation (PAR, $\mu\text{mol}/\text{m}^2\text{s}$) was measured at canopy height in five points of each CON, ICL and ICLF paddocks, and a point under full sun conditions at the beginning and at the end of recordings in ICLF. All the readings were taken in the morning and afternoon on one sunny day every month with Accupar Ceptometer Model LP-80, Meter group Inc., Pullman, USA. The shading percentage was calculated as follows:

$$\text{Shading percentage (\%)} = \frac{\text{average radiation in system } \left(\mu \frac{\text{mol}}{\text{m}^2} \cdot \text{s} \right) * 100\%}{\text{average radiation in full sun } \left(\mu \frac{\text{mol}}{\text{m}^2} \cdot \text{s} \right)}$$

3.3.3 Forage measurements

Metallic frames (1 m x 1 m) were randomly positioned in paddocks to determine the characteristics of the forage canopy. Five frames (sampling points) were allocated per paddock in the integrated systems, while three frames were assigned per paddock in CON due to smaller paddock sizes. The arrangement was made every two weeks from June to July. The canopy height measurements were taken inside the frames with 1-m ruler graduated in cm. The height was measured from the ground to the top surface of the leaf canopy, also referred to as the “leaf horizon”. Afterwards, the forage was harvested at a height of 0.4 m from the ground by a brush cutter. The forage samples were instantly weighted individually on the field and then again in the laboratory to estimate forage biomass. Further, the forage samples from the same paddock were pooled together to create one homogenous representation of the paddock and divided into three sub-samples of approximately 600 g of fresh matter (FM) each. One sub-sample was used for morphological

separation into live leaves, live stems (stems and sheath), and dead plant material and other two sub-samples were analysed for dry matter (DM) concentration. All forage samples, including leaves, stems, and dead plant material, were placed in paper bags in a forced air-circulation oven at 65°C until a constant mass was reached. Thereafter, they were all weighed again to estimate forage biomass (kg DM/ha), leaf biomass (kg DM/ha), stem biomass (kg DM/ha), dead plant material (kg DM/ha).

The mass of forage harvested in each sampling point (g FM/m²) was multiplied by the average DM (g/g FM) concentration of each corresponding paddock, and by 10 to convert into unit of “kg DM/ha”. The overall forage biomass was the average calculated from all sampling points of each paddock. The percentage of each component, such as leaf (g/100 g DM), stem (g/100 g DM) and dead plant material (g/100 g DM) was determined in proportion to the total of forage biomass. The leaf/stem ratio was estimated dividing leaf biomass by stem biomass, while the green/dead plant material ratio was estimated by dividing the sum of leaves and stems by dead plant material concentration. Canopy density (kg DM/m³) was calculated by dividing the forage biomass by the canopy height converted from cm to m³, by multiplying by 10⁴.

Leaves, stems, and dead plant material were grounded using a 1-mm mesh sieve in order to determine their chemical composition including: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *apparent total tract* digestibility of organic matter (OM) as estimated from *in vitro* fermentation (IVDOM) through near-infrared spectroscopy (NIRS). Forage nutritive value was estimated as a weighted average of the chemical composition of live leaves and stems, multiplied by their proportions in the canopy.

3.3.4 Grazing behaviour measurements

The measurements of behavioural patterns were carried out with the RumiWatch system (developed by Itin&Hoch GmbH, Liestal, Switzerland) including two software packages: RumiWatch Manager for managing the sensors and RumiWatch Converter for analysing the data. The measurements included: grazing time (min/day), rumination time (min/day), other activity time (activities that could not be allocated to ruminating or grazing; min/day), rumination chews (n/day), rumination bout duration (min/day), chews per minute (n/min), bolus number (n/h) and bite

frequency (bites/min). The recordings of four heifers per system were taken during one measurement period, as shown in Figure 4. As a result, behaviour of 12 heifers per system was analysed during the entire experiment duration (three measurement periods). The halters were worn for nine continuous days, considering the first day as an adaptation to the halter and the next eight days as data logging phase ($n=234$). Further, the halters were detached, and raw data was obtained from micro SD card. The RumiWatch Converter (V.0.7.4.13) was used to convert the raw data on 24-h basis. After certain data had been removed from the data set, either due to malfunction of the equipment or due to outliers, the total number of observation was 234: 3 systems \times 12 animals \times 8 days. The behavioural evaluation was carried out simultaneously with the feed intake measurements. Nellore heifers wearing RumiWatch halters in CON can be seen in Figure 6.



Figure 6. Nellore heifers wearing RumiWatch halters in continuous pasture (own photo).

3.3.5 Feed intake measurements

The feed intake, given as organic matter intake (OMI, kg OM/day) of heifers was determined on the basis of daily fecal OM excretion (FO, kg OM/day) and digestibility of ingested forage OM (dOM, g/kg OM) based on the equations provided by Lukas et al. (2005). Further, OMI was divided by the average metabolic body weight (BW), defined as BW to the 0.75 power, to present the feed intake as OMI/kg

metabolic BW/day. In order to estimate the FO, an external marker in the form of titanium dioxide (TiO₂) was administered orally twice a day in capsules in dosage of 7 g at 07:00 AM and 7 g at 04:00 PM for ten consecutive days. During the feed intake measurements, the heifers spent approximately 1 h outside the paddock in the morning and in the evening, as the application of the marker took place in the pan. The application of TiO₂ started on the fourth day of grazing behaviour recordings. Since OMI assessments were carried out at the same time as grazing behaviour measurements, the same configuration of the analysed heifers as mentioned above (Figure 4) was used in three consecutive periods. Conjointly to the administration of TiO₂, fecal samples were obtained directly from the rectum every day from the fifth day of the marker applications. The first five days were considered as an adaptation period. Fecal samples from each heifer were frozen at -20°C immediately after sampling. At the end of the sampling period, all fecal samples were defrosted overnight, grouped per animal and season, taking the same quantity of material from each individual sample. The pooled samples were divided into two sub-samples. One fresh sub-sample was analysed in duplicate for N (g/kg DM) by the micro Kjeldahl method and the values of N concentration in faeces were further used for the determination of fecal CP concentration (g/kg DM). The calculation was done by multiplying the concentration of fecal N by 6.25, as protein molecules contain on average 16% of N (1/16 = 6.25). The second sub-sample was dried in the oven at 40 °C for 72 hours and ground to pass a 1-mm sieve. Dried faeces were analysed in duplicate for TiO₂ according to procedures described by Boguhn et al. (2009), for DM by drying at 105°C overnight and for crude ash by ashing at 550°C for 4 hours.

OMI was calculated from equation:

$$\text{OMI (kg OM/day)} = \frac{\text{FO (kg } \frac{\text{OM}}{\text{day}})}{1 - \text{dOM (g/kg OM)}}$$

FO was calculated from equation (Glindemann et al., 2009):

$$\text{FO (kg OM/day)} = \frac{\text{Daily TiO}_2 \text{ administration * purity (} \frac{\text{g}}{\text{day}})}{\text{TiO}_2 \text{ concentration in faeces (} \frac{\text{g}}{\text{kg OM}})}$$

dOM was estimated based on the curvilinear relationship between the CP concentrations in fecal OM and dOM using the equation from Lukas et al. (2005), with the coefficient $a_i = 2$ from Gumpenstein location data:

$$\text{dOM} \left(\frac{\text{g}}{\text{kg OM}} \right) = 72.86 - 107.7e^{(-0.01515 * \text{fecal CP} \left(\frac{\text{g}}{\text{kg OM}} \right))}$$

3.3.5 Animal performance

Average daily gain (ADG, kg BW/animal/day) was calculated as the difference between the initial and final BW of heifers in each month divided by the interval of weighing. One weighing per time point was performed.

3.3.6 Statistical analysis

Forage and animal performance data were analysed by general linear models (GLM) whereas grazing behaviour and feed intake data by mixed models. All statistical analyses were performed by using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). GLMs included effects of the three periods of measurements and systems in the model. Mixed models included “period” and “system” as a fixed effect for grazing behaviour and feed intake. Further, for grazing behaviour “paddock” and “animal” were included as a random effect and “day” as a repeated measurement. For feed intake analysis “paddock” was also included as a random effect, while “animal” was not included in the model.

Statistical significances were tested at $p < 0.05$ and Least Square mean differences analysed by t-test for forage canopy characteristics and animal performance and Tukey test for grazing behaviour and feed intake.

4. Results

4.1 Climate

Table 1 presents data with climatic conditions registered in the experimental site over 58 days during the dry season. The climatic conditions measured in CON were assumed to be equal to those in ICL therefore the results were implemented in the same column. The average temperature in CON/ICL during the measurement period was 21°C, while the average temperature in ICLF was slightly higher, valuing almost 22°C. The greatest extremes in terms of maximum and minimum temperature were noted in CON/ICL. The peak of temperature during the measurement period reached there 31°C, whereas the maximum temperature registered in ICLF was lower by 2°C, valuing 29°C. The minimum temperature registered during that period was 16°C and it was found in CON/ICL. The average RH in CON/ICL was greater by 12% than the average RH registered in ICLF. The average THI in CON/ICL as well as in ICLF was nearly the same and valued 67. The shading in ICLF was 66%, whereas CON/ICL represented the full sun condition, equaling to 0% of shade.

Table 1. Climatic conditions (means \pm standard deviation) of three grazing systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season

Variable	System	
	CON/ICL	ICLF
Average Temperature (°C)	21.1 \pm 3.0	21.7 \pm 3.2
Max Temperature (°C)	31.0 \pm 3.2	29.0 \pm 3.4
Min Temperature (°C)	16.1 \pm 3.3	17.7 \pm 3.6
Average RH (%)	62.0 \pm 8.4	50.0 \pm 8.4
Average THI	67.2 \pm 4.2	66.7 \pm 4.3
Shading (%)	0	66

Note. RH- relative humidity; THI- Temperature Humidity Index

The daily average THI values remained in the thermal comfort zone based on the range presented by Baêta and Souza (2010). The range suggested by the authors looks as follows: THI 0-70 “non-stressful” conditions; 71-78 “alert”; 79-83 “danger” and 83-100 “emergency” situation for Zebu cattle. The average THI in CON/ICL during the daytime hours (7:00-17:00) surpassed the average THI registered in ICLF, what can be seen in Figure 7.

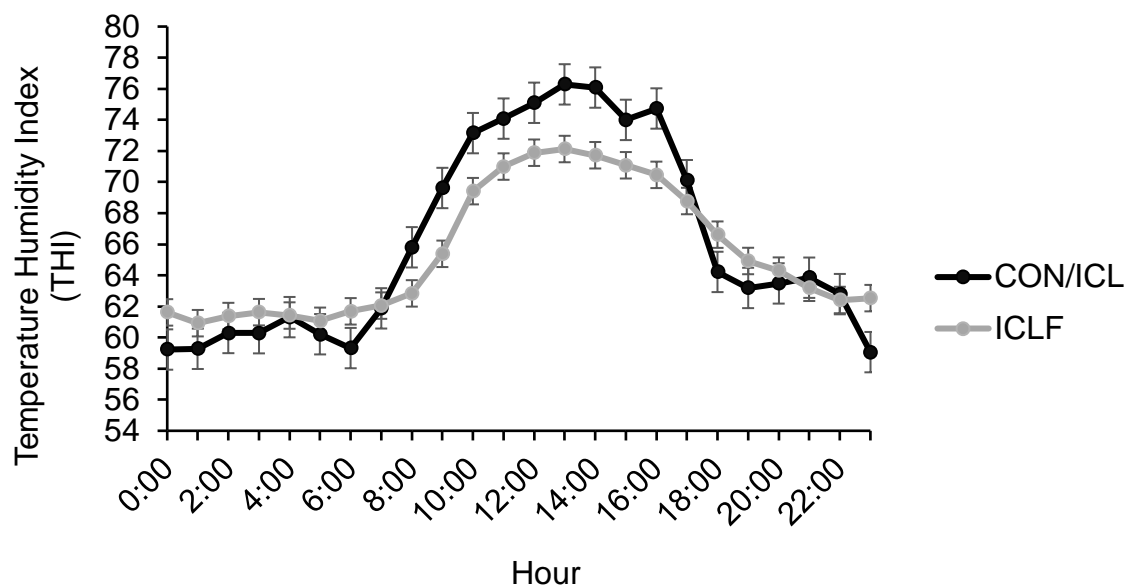


Figure 7. Average hourly analysis of the Temperature Humidity Index (THI) in June and July in the treeless (represented by CON/ICL) and shaded area (represented by ICLF) in Campo Grande, MS, Brazil (local time, GMT-4:00). CON- continuous pasture; ICL- Integrated Crop-Livestock; ICLF- Integrated Crop-Livestock-Forestry

Figure 8 presents the hourly analysis of THI that revealed that during 58 days of data collection, according to the range presented by Baêta and Souza (2010), most of the time THI in all systems was within the range considered as “non-stressful” environment. Further, THI considered as “alert” was prevailing for similar number of hours in treeless systems CON/ICL as well as in ICLF. THI in the range between 79-83, considered as a “danger”, was registered for in total 114 hours during 58 days in CON/ICL, while in ICLF the value was always kept below 79.

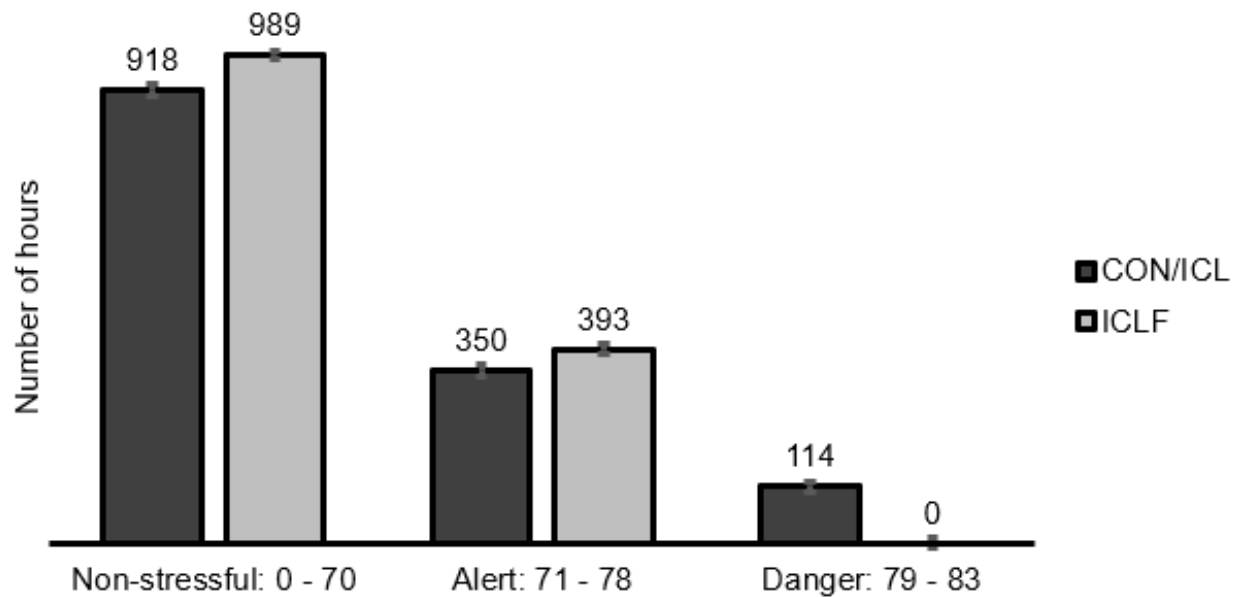


Figure 8. Number of hours spent by Nellore heifers under various thermal conditions in June and July according to the range based on Temperature Humidity Index (THI) presented by Baêta and Souza (2010). CON- continuous pasture; ICL- Integrated Crop-Livestock; ICLF- Integrated Crop-Livestock-Forestry

4.2 Forage canopy characteristics

The forage biomass in the grazing systems differed significantly ($p < 0.001$). As Figure 9 shows the largest total forage biomass was found in ICL, 3853 kg DM/ha, and it was double that of forage biomass in CON ($p < 0.001$), 1901 kg DM/ha. Further, the lowest forage biomass was registered in ICLF ($p < 0.001$), 852 kg DM/ha, and it was less than half of the biomass observed in CON.

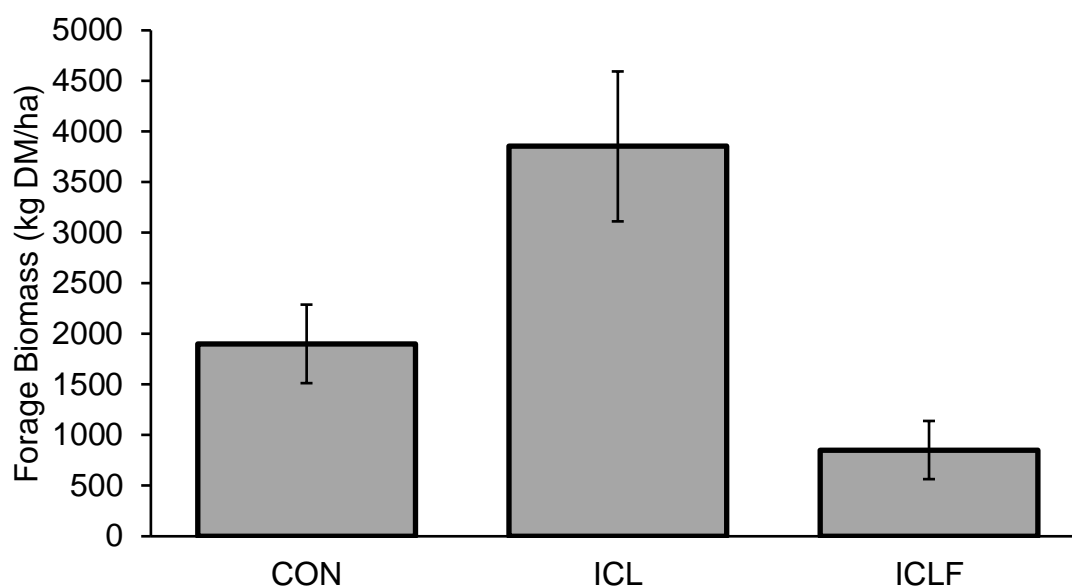


Figure 9. Forage biomass (kg DM/ha, Least Square means \pm standard deviation) in three grazing systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season. DM- dry matter

Figure 10 presents the composition of forage in each system, on total forage biomass basis. Dead plant material, green stems and green leaves were distinguished. As it can be seen, in every system more than half of the canopy consisted of dead plant material. The mass of dead plant material in CON was the greatest ($p < 0.05$) from all systems and valued 72.5 g/100 g DM. The mass of green stems and green leaves in CON was nearly equal, 13.8 g/100 g DM and 13.7 g/100 g DM respectively. The mass of green leaves in CON was lower ($p = 0.014$) than in ICLF and did not differ ($p = 0.633$) from ICL. In comparison to other systems ICL was characterized by the highest ($p < 0.001$) mass of green stems in forage, 27.2 g/100 g DM, whereas the mass of green leaves, 15.3 g/100 g DM did not differ ($p = 0.059$) from ICLF, 22.2 g/100 g DM. The dead plant material mass in ICL, 56.4 g/100 g DM, did not differ ($p = 0.539$) from ICLF, 59.6 g/100 g DM.

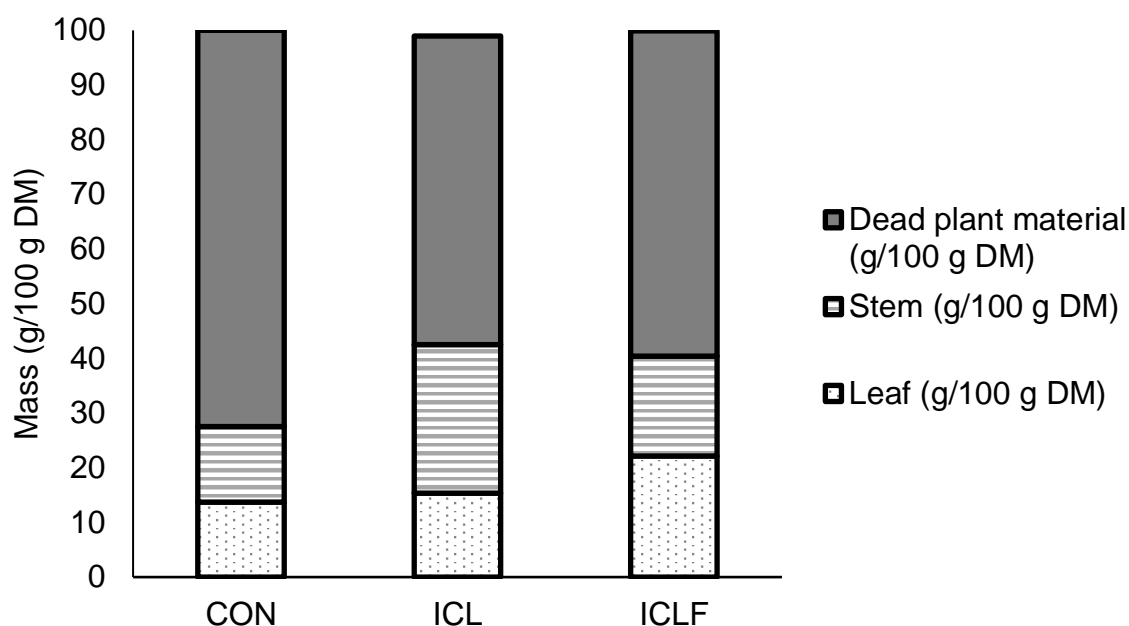


Figure 10. Composition of canopy in grazing systems with the following components (g/100 g DM): dead plant material, stems and leaves. CON- continuous pasture; ICL- Integrated Crop-Livestock; ICLF- Integrated Crop-Livestock-Forestry; DM- dry matter

As it can be seen in Table 2, a large proportion of stems in ICL resulted in the lowest ($p = 0.001$) leaf/stem ratio in that system, while the leaf/stem ratio in CON did not differ from ICLF ($p = 0.526$). CON was distinguished by the lowest green/dead plant material ratio ($p = 0.016$). The green/dead material ratio did not differ between ICL and ICLF ($p = 0.958$). The green matter did not include the inflorescence as Piatã grass has its flowering at the beginning of the year (January, February) and this component was irrelevant in that analysis. The tallest canopy height ($p < 0.001$) was observed in ICL, while the canopy height in CON did not differ significantly ($p = 0.815$) from ICLF. ICL was also distinguished by the greatest ($p < 0.001$) bulk density, whereas the lowest ($p < 0.001$) bulk density was found in forage in ICLF.

Table 2. Forage canopy characteristics (Least Square means \pm standard deviation) in three grazing systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season.

Variable	System			<i>P-value</i>
	CON	ICL	ICLF	
Height (cm)	29.9 ^b \pm 3.3	41.4 ^a \pm 3.9	30.4 ^b \pm 9.0	<0.001
Bulk density*	64.4 ^b \pm 14.4	93.8 ^a \pm 21.1	27.8 ^c \pm 6.0	<0.001
Leaf/stem ratio	1.07 ^a \pm 0.3	0.56 ^b \pm 0.1	1.15 ^a \pm 0.4	0.001
G/D ratio	0.39 ^b \pm 0.2	0.83 ^a \pm 0.4	0.82 ^a \pm 0.6	0.016

Note. Means followed by the same letter in the line do not differ at 5% significance by t-test. DM- dry matter, G/D- green to dead plant material ratio. * kg DM/m³

Regarding the nutritive value of forage, ICL was characterized by the greatest OM concentration ($p < 0.001$), the lowest CP concentration ($p < 0.001$) and the greatest concentration of NDF ($p < 0.001$) as well as ADF ($p < 0.001$) from all systems. Forage in CON consisted of the lowest concentration of OM ($p < 0.001$), while the CP concentration in forage in CON did not differ from CP concentration in forage in ICLF ($p = 0.417$). Also, the composition of forage in CON did not differ from ICLF in terms of NDF concentration ($p = 0.694$) or ADF concentration ($p = 0.699$). Forage grown in CON contained the highest concentration of lignin ($p < 0.001$), while forage in ICLF presented a greater lignin content than forage grown in ICL ($p = 0.003$). Forage in CON and ICLF was characterized by the greatest IVDOM and did not differ significantly between each other ($p = 0.277$). Forage in ICL presented the lowest IVDOM from all systems ($p < 0.001$). The forage nutritional value can be seen in Table 3.

Table 3. Forage nutritional value (Least Square means \pm standard deviation) in three grazing systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season.

Variable	System			<i>P</i> -value
	CON	ICL	ICLF	
OM (g/kg DM)	821.4 ^c \pm 15.6	862.7 ^a \pm 6.3	831.1 ^b \pm 9.3	< 0.001
CP (g/kg DM)	92.3 ^a \pm 20.4	59.5 ^b \pm 8.8	97.2 ^a \pm 9.3	< 0.001
NDF (g/kg DM)	638.0 ^b \pm 29.0	712.4 ^a \pm 8.1	634.5 ^b \pm 17.4	< 0.001
ADF (g/kg DM)	314.4 ^b \pm 34.9	401.0 ^a \pm 8.6	318.5 ^b \pm 16.7	< 0.001
Lignin (g/kg DM)	44.3 ^a \pm 8.4	18.2 ^c \pm 5.5	31.9 ^b \pm 15.4	< 0.001
IVDOM*	746.8 ^a \pm 58.5	605.5 ^b \pm 5.8	727.9 ^a \pm 38.8	< 0.001

Note. Means followed by the same letter in the line do not differ at 5% significance by t-test. OM- organic matter; DM- dry matter; CP- crude protein; NDF- neutral detergent fiber; ADF- acid detergent fiber; IVDOM- *in vitro* digestibility organic matter. * g/kg OM

4.3 Grazing behaviour

Grazing, ruminating and other activities of the heifers in the systems are displayed in Figure 11. Overall, in all systems the activity that the heifers spent most of their time was grazing (min/day): CON- 658; ICL- 601; ICLF- 614. The heifers kept in CON grazed for significantly longer time than the heifers in ICL ($p = 0.017$). Grazing time in ICLF did not differ neither from CON ($p = 0.077$) nor from ICL ($p = 0.786$). The registered rumination time (min/day) in the systems was as follows: CON- 413; ICL- 378; ICLF- 409. No differences in ruminating time among the systems were found ($p = 0.385$). Other activity time (min/day): CON- 372; ICL- 452; ICLF- 410, was significantly longer in ICL than in CON ($p = 0.003$), while the other activity time in ICLF did not differ from CON ($p = 0.249$) neither from ICL ($p = 0.172$).

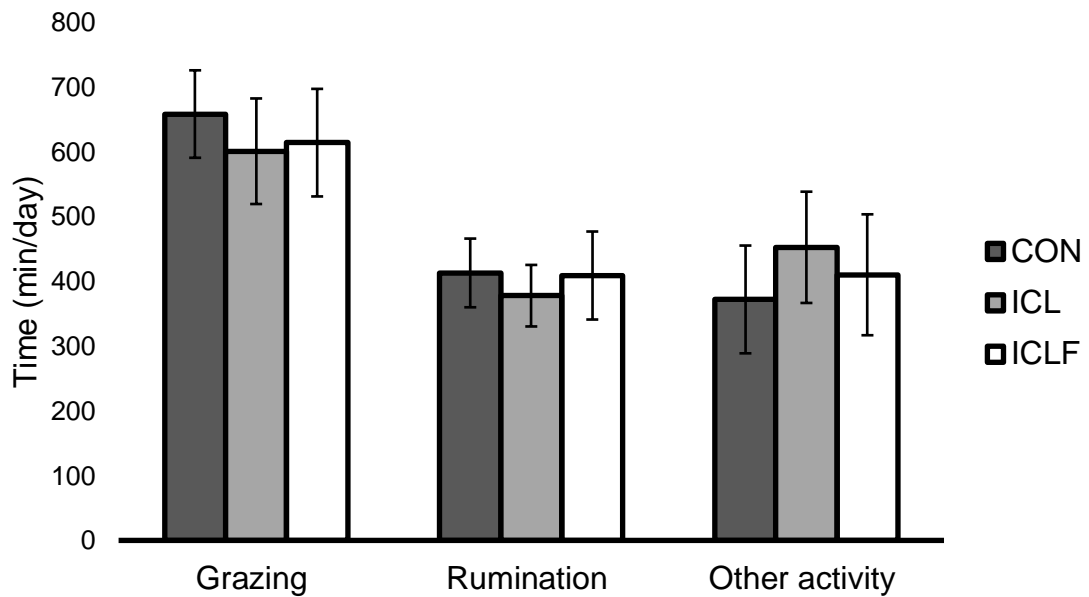


Figure 11. Time spent on various activities by Nellore heifers grazing in three systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF).

The number of rumination chews ($p = 0.373$) and duration of rumination bouts ($p = 0.126$) did not differ between the systems (Table 4). The evaluated systems also did not differ in the number of chews per bolus ($p = 0.153$) and in the number of boluses ($p = 0.058$), however the heifers in CON presented a strong tendency to greater number of boluses than the heifers in ICL ($p = 0.056$). The bite frequency (bites/min) in ICLF was lower than the bite frequency registered in CON ($p = 0.002$) and the bite frequency registered in ICL ($p = 0.008$).

Table 4. Grazing patterns (Least Square means \pm standard deviation) of Nellore heifers in continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season.

Variable	System			<i>P</i> -value
	CON	ICL	ICLF	
Rumination chews*	23333 \pm 3983.5	20705 \pm 3775.0	21055 \pm 4331.6	0.373
Rumination bout duration (min/day)	409.3 \pm 54.9	382.4 \pm 45.1	413.9 \pm 71.1	0.126
Chews per bolus**	53.0 \pm 6.1	55.2 \pm 7.3	50.0 \pm 5.0	0.153
Bolus (n/h)	440.0 \pm 78.3	378.8 \pm 63.4	423.4 \pm 84.6	0.058
Bite frequency***	42.0 ^a \pm 8.3	40.6 ^a \pm 7.2	31.7 ^b \pm 9.0	0.001

Note. Means followed by the same letter in the line do not differ at 5% significance by Tukey test. * n/day, ** n/bolus, *** bites/min

4.4 Feed intake and animal performance

The results of fecal analysis, OMI and ADG of heifers are summarized in Table 5. No significant difference was found in FO ($p= 0.998$) and fecal OM concentration ($p= 0.184$) between the three grazing systems. The greatest fecal CP concentration ($p < 0.001$) and the highest dOM of diet ($p < 0.001$) were observed in ICLF. CON did not differ from ICL in fecal CP ($p= 0.346$) or in dOM of diet ($p= 0.484$). There was no difference found in OMI between the systems ($p= 0.071$), however the heifers in CON tend to have a greater OMI than the heifers grazing in ICL ($p= 0.059$). The heifers in ICL presented significantly greater ADG than the heifers in CON ($p= 0.008$), the heifers in ICLF presented significantly greater ADG than the heifers in CON ($p= 0.030$), while the ADG registered in ICL did not differ from the gains observed in ICLF ($p= 0.249$).

Table 5. Fecal excretion and composition, organic matter intake (OMI) and average daily gain (ADG) of Nellore heifers grazing in three systems: continuous pasture (CON), Integrated Crop-Livestock (ICL) and Integrated Crop-Livestock-Forestry (ICLF) during the dry season (Least Square means \pm standard deviation).

Variable	System			<i>P</i> - value
	CON	ICL	ICLF	
FO (kg OM/day)	1.9 \pm 0.4	1.9 \pm 0.4	1.9 \pm 0.3	0.998
Fecal OM (g/100 g DM)	85.1 \pm 1.1	84.7 \pm 2.3	86.5 \pm 1.3	0.184
Fecal CP (g/kg DM)	96.8 ^b \pm 6.2	92.9 ^b \pm 7.1	110.1 ^a \pm 6.6	<0.001
dOM of diet (g/kg OM)	534.8 ^b \pm 21.8	522.6 ^b \pm 29.8	571.0 ^a \pm 19.8	<0.001
OMI*	56.2 \pm 9.4	44.8 \pm 7.7	51.9 \pm 9.8	0.071
ADG (kg/animal/day)	0.05 ^b \pm 0.3	0.3 ^a \pm 0.1	0.2 ^a \pm 0.1	0.017

Note. Means followed by the same letter in the line do not differ at 5% significance by Tukey test and t-test for ADG. FO- fecal output; OM- organic matter; DM- dry matter; CP- crude protein; dOM- digestibility organic matter; BW- body weight. * g/kg metabolic BW/day

5. Discussion

5.1 Climate

We expected to find more moderate microclimatic conditions in ICLF in comparison to treeless systems CON and ICL in terms of air temperature, RH and THI, as there is a large volume of published studies documenting the role of the trees in providing improved living conditions for grazing livestock (Baliscei et al., 2012; Karvatte Junior et al., 2016; Souza et al., 2017; Oliveira et al., 2017). Arboreal components do not only establish shade and protect against extensive heat load throughout the day, but also provide the shelter against strong wind and rain. However, the average temperature between the systems did not differ significantly. The average temperature in all systems was within the range considered by Baêta & Souza (2010) as the best climate conditions for Zebu cattle, which is a temperature between 10°C and 27°C. The lack of difference between ICLF and CON and ICL in terms of average temperature can be explained by the fact that the experiment was performed during the Brazilian winter and the coldest months of the year, when the weather conditions are much milder in comparison to summer. Nevertheless, Oliveira et al. (2017) conducting study in the same location as the present experiment, recorded dry bulb temperature above 35°C during the winter. The authors reported the occurrence of atypical days for the dry season and therefore emphasized the need to provide the animals with means to alleviate heat stress even during winter, as these unusual conditions can occur more frequently. The microclimatic conditions in ICLF in the present study were more moderate than those observed in full sun environment in CON and ICL in terms of extreme temperatures, as the maximum (31°C) and minimum (16°C) temperature were registered in CON and ICL. The maximum temperature registered in all systems exceeded the favorable range of 10°C to 27°C for Zebu cattle, however all values were below 35°C, which is a critical temperature for Nellore cattle (Baêta & Souza, 2010).

Biotic components such as trees tend to increase the RH of the surroundings, because their canopies reduce the wind speed and solar radiation; and thus, they shield the pasture from moisture depletion (Souza et al., 2017). Therefore, we

predicted to find a greater RH in ICLF in comparison to CON and ICL. However, contrary to the expectations, the greater RH was registered in treeless systems CON and ICL (62%) in comparison to ICLF (50%). This inconsistency may be attributed to the specificity of the dry season, when the strong water competition between trees and forage affects the entire water dynamic. Moreover, the lower RH in ICLF in comparison to CON and ICL may be also correlated with less amount of rain reaching the ground in the system due to tree canopies that create a barrier. It should be noted that the RH is highly affected by wind and temperature fluctuations during the day, and therefore sometimes it is difficult to obtain accurate results (Vieira Junior et al., 2019). All in all, a lower RH contributes to improved thermal comfort as high humidity impedes the heat dissipation of the ruminants (Sousa et al., 2015).

The ICLF system was expected to provide milder microclimatic conditions that would ensure a lower THI in comparison to CON and ICL. Indeed, the Nellore heifers grazing in a treeless environment of CON and ICL were subjected to THI of 79-83, considered as “danger” conditions by Baêta and Souza (2010), for 114 hours during 58 days of data collection, that equals to approximately 2 hours per day under thermal “danger”, while THI in ICLF was always kept below 79. Nevertheless, the average THI in all systems remained as “non-stressful environment” ($THI < 70$) for Nellore heifers according to the range given by Baêta and Souza (2010). In the same manner as recorded temperature, the factor responsible for the absence of differences in average THI between the systems may have been the season when the experiment took place, June and July, two coldest months of the year in Brazil. Another aspect that should also be emphasized is the suitability of THI as a thermal comfort index in the intertropical zones, such as Brazil, that present a high incidence of solar radiation all year long. For instance, Giro et al. (2019) conducting their research in the tropical climate of Sao Carlos-SP, Brazil also did not find a difference in THI between ICL and ICLF. However, the authors reported that the black globe temperature and humidity index (BGHI) and radiant thermal load (RTL) were always higher in ICL in the afternoons. This indicates that THI does not necessarily reflect the real conditions experienced by the animals, as THI does not consider radiant heat load which is a significant factor in the environments characterized by intense solar radiation. According to Domiciano et al. (2018) and Dikmen & Hansen (2009) inclusion of black globe temperature into assessments expresses to the greatest

extent thermal conditions in the locations characterized by intense solar radiation, since this parameter provides information of combined effects of radiation, air temperature and wind speed. Each of these components has a specific impact on thermoregulation of cattle (Domiciano et al., 2018).

5.2 Forage canopy characteristics

Since the inclusion of crops into rotations increases the retention of nutrients and therefore the soil fertility, enhances the management of soil-borne diseases, weeds and pests (Wilkins 2008) we expected to find a greater forage growth in ICL in comparison to CON due to the aforementioned features promoted by rotation with soybean. On the other hand, ICLF was predicted to present diminished forage growth in comparison to ICL due to reduced light incidence on the forage canopy. As expected, the forage in ICL was characterized by the largest biomass and the greatest bulk density in comparison to two other systems. The Piatã biomass found in ICL in the present study was greater than the Piatã biomass in ICL during the dry season reported by Gamarra et al. (2017) in the experiment performed in the same location as the present study. On the other hand, the same authors presented higher Piatã biomass found in two ICLF systems in comparison to Piatã biomass in ICLF in our experiment. One of the factors affecting growth of forage grasses in the systems with arboreal components is the tree density, as the greater the number of trees in the system, the stronger the competition between the trees and forage grasses for natural resources, such as water or radiation, and lower growth of forage (Garcia & Couto, 1997). However, this factor does not explain the lower growth of Piatã in our experiment, as both ICLF systems in the study of Gamarra et al. (2017) were characterized by a greater tree density in comparison to ICLF in our research. Therefore, another reason for these differences may be the lower *Eucalyptus* height in the study of Gamarra et al. (2017), as the authors reported that during the experimental period the trees had a height ranging between 25 to 27 m, while in our experiment 30 m. All in all, Gamarra et al. (2017) observed a decrease in forage growth in the shaded systems when compared to full sun condition. The findings of Gamarra et al. (2017) and the lowest total forage biomass and the lowest bulk density found in ICLF in comparison to CON and ICL in the present experiment confirm our initial expectations and indicate the limiting effect of shade on forage growth and plant's response to shading in a form of reduced tillering and prioritized

maintenance of expended leaves (Gamarra et al., 2017; Pezzopane et al., 2015). Reduced sunlight incidence decreases the photosynthetic rate of plants and diminishes the forage accumulation rate (Santos et al., 2016). *Brachiaria*, as a C4 grass, requires sufficient levels of radiation to allow for full biomass production (Oliveira et al., 2014). The findings of the present study are also consistent with those of Geremia et al. (2018) who concluded that increased shade intensity leads to decreased forage mass. However, several studies have argued that shade in ICLF does not always have the limiting effect on forage growth (Paciullo et al., 2014; Sousa et al., 2015). According to Paciullo et al. (2014), shade levels up to 40% do not reduce the forage growth of tropical grasses due to various morphological and physiological adaptations; nevertheless, our experimental site was characterized by intense shading of 66% which resulted in notably reduced forage biomass in ICLF.

We expected to observe one of the aforementioned adaptations to reduced sunlight in forage in ICLF, namely stem elongation. It was reported, for instance, in the research of Pezzopane et al. (2019) in canopies grown under restricted access to sunlight. The authors explained this phenomenon with the fact that plants allocate their energy in such a way to grow in height and thus to reach scanty but essential light as much as possible. Following on from that, Geremia et al. (2018) found out that forage cultivated under limited access to sunlight appears to have increased proportion of stems in the composition and consequently decreased leaf/stem ratio of canopy. However, contrary to our predictions, in the present study neither the plant height nor the stem proportion in canopy composition in ICLF under shading was higher than in full sun environment in CON and ICL. Firstly, this can be justified by the fact that the heifers in ICLF have been supplied with low forage biomass, which indisposed them to consume only preferable leaves but also compelled to ingest stems, thus preventing the excessive stem elongation under the shade. Furthermore, this inconsistency with the pattern presented by other researchers can be also explained in part by the low precipitation during the dry season which is likely to have a major negative impact on forage growth and development (Domiciano et al., 2018). Direct competition between forage grasses and trees for water and solar radiation not only reduced forage biomass but likely also abated the canopy in ICLF from the excessive stem elongation. Santos et al. (2016) suggested that when there is a water constraint, as in the dry season, water restriction is a more determining factor than the radiation, as a result of which the

plants do not trigger structural adaptations such as those aiming at increase in available radiation. Like in the present research, also Santos et al. (2016) did not note any effect of shade in ICLF system on the canopy height. The authors reported the height of Piatã grass during the dry season in ICLF of 40 cm, thus 10 cm taller than Piatã canopies in ICLF in the present experiment. Euclides et al. (2016) noted the Piatã heights of 31.9 cm, 20.7 cm and 26.5 cm over 3 dry seasons in the same experimental location as the present study, which are the heights comparable to the values registered in our experiment. Gomes et al. (2020) reported different forage adaptive mechanism to reduced sunlight, namely enhanced development of leaves to boost the efficiency of light utilization and to maintain photosynthetic ability. While the proportion of leaves observed in ICLF was the largest from all evaluated systems, 22.2 g/100 g DM, it still remained consistent with the value presented by Euclides et al. (2016) who detected 21.7g/100 g DM of leaves in Piatã pastures during the dry season. Consequently, the development of leaves in ICLF in the present study, although enhanced in comparison to CON and ICL, cannot be perceived as excessive. Low precipitation combined with an attack of spittlebug observed on *B. decumbens* pasture in CON resulted in the greatest proportion of dead plant material in forage composition in comparison to ICL and ICLF. This was expected to occur because *B. decumbens* is characterized by a higher susceptibility to spittlebug attacks than *B. brizantha*, which was one of the reasons why *B. brizantha* gradually replaced *B. decumbens* in the Brazilian pastures (Jank et al., 2014). Moreover, Salton et al. (2014) emphasized that, due to better soil conditions provided by complex synergies created between the components, the forage grasses growing in integrated systems, such as ICL and ICLF, are characterized by a greater resilience towards unfavorable conditions, for instance pest attack or drought, in comparison to monoculture. This finding is of great importance as seasonality of forage production and spittlebug attacks are one of the bottlenecks for pasture-based cattle production in Brazil.

Much of the available literature claims enhanced CP concentrations in the canopies grown in the shaded areas with arboreal component (Geremia et al., 2018; Oliveira et al., 2014; Paciullo et al., 2014; Pezopanne et al., 2019). Therefore, we expected to find a greater CP concentration in forage grown in ICLF in comparison to full sun environment in CON and ICL. In accordance with our predictions, a higher CP concentration was found in the ICLF pasture in comparison to ICL. This

phenomenon may be explained by the fact that lower soil temperature in the shaded areas is favorable for bacterial and fungal growth, thus these species may degrade larger amounts of OM and boost N recycling in the system with trees (Gamarra et al., 2017). Moreover, Sousa et al. (2015) suggested that the trees in ICLF take up nutrients from deeper soil layers, thus making them available in the accessible layers of soil for the forage grasses. Another aspect that explains the greater CP concentration in ICLF than in ICL is that the maturity and development of pasture cultivated under reduced sunlight is delayed, thereby canopies retain in their vegetative stage longer and they are characterized by a greater concentration of CP (Santos et al., 2018). Once canopies are exposed to high luminosity conditions, they contain a high proportion of sclerenchyma tissues with thick walls, consequently they contain a higher NDF concentration (Deinum et al., 1996). Shaded plants, on the other hand, have limited supply of photoassimilates for secondary wall growth, hence the number of supporting tissues and the thickness of the cell wall are decreased (Gamarra et al., 2017). These features are also responsible for lower IVDOM and lower CP concentration in forage in ICL in comparison to ICLF, because the high rates of fibrous carbohydrate synthesis dilute the protein concentration inside the dry matter (Taiz & Zeiger, 2013). Last but not least, larger proportion of leaves and therefore greater leaf/stem ratio registered in ICLF in comparison to ICL may have also contributed to greater CP concentration in forage in ICLF than in ICL, as leaves contain more CP in comparison to stems (Oliveira et al., 2014). Because the concentration of CP is also dependent on nutrient retention and soil fertility (Pezzopane et al., 2019), we expected to find a greater CP concentration in forage grown in ICL in comparison to CON, as the systems with crop rotation are claimed to have enhanced soil fertility relative to monocultures (Wilkins, 2008). Nevertheless, contrary to the expectations, forage grown in ICL was characterized by lower CP concentration than forage in CON. The largest stem proportion in forage composition in ICL might have contributed to low CP and high concentration of NDF and ADF in evaluated pasture. In mature forages, stems are composed of large quantity of fibers and therefore their quality is inferior in comparison to leaves (Oliveira et al., 2014). Dim et al. (2015) working with Piatã grass observed, that the NDF concentration is positively correlated with the height of the canopies, namely the taller plant is, the greater NDF concentration it contains. This corroborates well with the results from the present research, where the greatest concentration of NDF

was found in the tallest canopies in ICL. The greatest proportion of stems found in ICL may be related to grazing, as the forage biomass in ICL was the highest from all evaluated systems, there was much better scope to select and consume preferable leaves, leaving the stems unconsumed. The existence of a grazing selectivity in favor of leaves was confirmed, among others, in the study of Euclides et al. (2000), evaluating voluntary intake of Nellore steers. The authors found, relying on extrusas obtained from oesophageal fistulated animals, that steers preferred to choose leaves over stems and green over dead plant material, and even with low availability of these fractions on pastures, the leaf proportion in the diet was predominant. In general, the CP concentration of 59.5 g/kg DM found in green plant material in forage in ICL can be considered too low to provide a proper supply of nitrogenous compounds for the heifers as, according to Lazzarini et al. (2009), at least 7 g/100 g DM, is essential in the diet for the ruminal microorganisms to be able to grow and to digest roughages to the end products that can be utilized by the ruminant.

Contrary to the expectations, no statistical difference in CP concentration was found between pasture grown in CON and ICLF. The location of one of the paddocks representing CON is one of the potential explanations. Owing to its proximity to ICLF and the position of the sun, the shadow of the *Eucalyptus* trees dropped and obscured, according to the visual observation of the researchers, approximately 80% of one of the CON paddocks after 1:00 PM onwards. We assume that it had an effect on morphological and chemical composition of forage from that paddock. In fact, the CP concentration of forage from that individual paddock was higher than that of the other paddocks representing CON. Therefore, the features affected by shade (such as CP concentration) cannot be taken as completely unbiased when referring to the CON system. As the Tinytag Plus 2 device, which measured microclimatic parameters, was placed in a different, more representative paddock, this shade effect was not captured in the climate data. The discussed weakness in the methodology of the present experiment demonstrates the importance of careful analysis of confounding factors, such as the movement of the sun, resulting in unforeseen implications for the experiment results.

5.3 Grazing behaviour

In the behavioural analysis, we predicted that the heifers in all systems will allocate most of their time into grazing, as according to Kilgour et al. (2012) cattle spend most of their time on this activity. As expected, within all registered activities the heifers in the present research grazed for the longest time and this pattern was observed in all systems. In each system grazing time exceeded 540 min/day which, according to Hodgson (1990), is a duration indicating a reduced supply of forage. Different range was provided by Souza et al. (2010) claiming that the grazing time is usually 8 hours and during extreme situations can reach up to 16 hours per day, while Euclides et al. (2000) suggested the range of 7 to 12 hours per day. Overall, the grazing time in all systems lies within both aforementioned ranges.

We expected to find an impact of shade in ICLF in comparison to CON and ICL on grazing behaviour, precisely we predicted to observe prolonged grazing in ICLF, as a result of improved thermal comfort and low forage biomass. Nevertheless, grazing time in ICLF statistically did not vary neither from CON nor from ICL. Several experiments linked prolonged grazing time with enhanced thermal comfort in the systems with arboreal components. For instance, Souza et al. (2017) observed that heifers grazed 10% longer in the shade than when exposed to full sun environment, thereby the authors attributed increased grazing time with enhanced thermal comfort of cattle. As the differences in microclimatic conditions between ICLF and CON and ICL were rather negligible; and the heifers in all systems were most of the time under THI considered as a non-stressful environment, in the present study grazing time seems to be more determined by forage characteristics than by thermal conditions. It is supported by the fact that the grazing time did not differ between CON representing full sun environment and shaded ICLF.

The longer grazing time registered in CON in comparison to ICL may be attributed to the characteristic of the forage. The lower proportion of green plant material in CON imposed heifers to adjust their behaviour and they required prolonged time to find preferable sward fraction. The time of grazing in CON (658 min/day) is similar to that reported in the study of Baliscai et al. (2012) assessing the behaviour of Nellore steers during winter (627 min/day) and greater than grazing time reported by Euclides et al. (2000) evaluating Nellore steers grazing *B. decumbens* during the dry season (565 min/day). According to Manteca & Smith (1994) in order to sustain

their normal daily intake of forage, ruminants have to graze longer as the quality of forage deteriorates. Presence of leaves, the components with the highest nutrient concentration, and live green forage, are the essential factors in fulfilling the nutritional needs of grazing animals. Various researches proved that selective behaviour contributes to prolonged grazing (Baliscei et al., 2012; Domiciano et al., 2018; Geremia et al., 2018).

Since prior studies have noted the importance of shade and thermal comfort for increased rumination time (Domiciano et al., 2018, Giro et al., 2019; Paciullo et al., 2014) longer rumination in ICLF in comparison to CON and ICL could have been expected. However, in the present study rumination time did not differ between shaded and treeless area, indicating that during the dry season when the temperature is considerably lower than in summer, Nellore heifers are tolerant to prevailing weather conditions and the rumination is not impacted by the thermal discomfort. Duration of rumination in every system in the present study lies within the range claimed by Hodgson (1990), 6-8 hours per day, being closer to the lower limit of six hours. Another factor that raises the rumination time is the high concentration of fiber in forage. In view of the results of the chemical composition of the forage, increased rumination time could be anticipated in the heifers grazing in ICL. The lack of difference between ICL and the two other systems could be linked to the largest forage biomass found in ICL enabling the avoidance of stems, as these parts of the plants are high in fiber. Considering that ICL provided canopies with the highest bulk density, access to leaves in the upper layers of the plant could have been facilitated, which also contributes to the results verified for ADG of heifers in ICL in the further section. McLeod & Smith (1989) evaluating rumination behaviour of cattle consuming forages with different levels of fiber found that even when the cattle given high fiber diet did not modify their rumination time, at least the chews per bolus increased and each bolus was subjected to more chews, as the animals require more chewing effort for the stem fractions than for the leaf fractions. In the present study, no statistical difference was reported in the number of rumination chews and chews per bolus between the systems which supports the concept of the possible avoidance of stems by the heifers grazing in ICL. The higher bite frequency registered in heifers grazing CON and ICL compared to ICLF indicate a smaller bite mass in those systems. It seems that such a decrease in bite mass could be related to greater selectivity to boost the nutritional quality of the diet (Mezzalana et al., 2014).

The applicability of this claim is supported by the large quantity of dead plant material in CON and the high proportion of stems in forage in ICL in comparison to ICLF pasture. As seen by Ungar et al. (2006), the intake rate is the combination of bite weight and bite rate. To maintain sufficient feed intake, the heifers have to manipulate variables such as bite weight, bite rate and grazing time according to the quality of pasture they are provided with.

5.4 Feed intake and animal performance

According to Euclides et al. (2000) the voluntary intake of *B. decumbens* and *B. brizantha* by Nellore steers amounts to 1.98% of BW in *B. decumbens* pasture and 2.00% of BW in *B. brizantha* pasture during the dry season. These values are within the expected range of 1.20-1.90% of BW, of feed intake under conditions of the present study.

Taking into account the highest forage biomass in ICL, we expected to observe the greatest OMI of heifers grazing in that system. Yet, as a consequence of the longest grazing, the heifers in CON presented a strong tendency to a greater OMI in comparison to ICL. Reduced feed intake in ICL relative to CON may be explained by the greatest proportion of stems in forage composition, which according to various authors, is a reducing factor for feed intake (Geremia et al., 2018; Mezzalana et al., 2014). Secondly, the highest levels of NDF and ADF could have contributed to lower digestion rates and slower passage of forage resulting in lower OMI than anticipated. Voluntary feed consumption is restricted by rumen fill; thus, the least digestible forage can be ingested in limited amounts (Teller et al., 1993). Also, low CP concentration in forage in ICL may have been a factor decreasing OMI in ICL, as according to Euclides et al. (2000), a CP concentration of less than 6-7 g/100 g DM can drastically reduce feed consumption.

Several researches have demonstrated the beneficial effect of shade and enhanced thermal comfort on the feed intake of ruminants therefore we expected to register a greater feed intake of the heifers grazing in ICLF in comparison to CON. For instance, Baumer (1991) stated that if the animals are protected from heat and excessive solar radiation, they tend to increase their feed intake. Sousa et al. (2015) endorsed this concept and confirmed that shading increases the forage intake of the grazing heifers. Yet, as no increase in grazing time due to shading in ICLF was

observed, we also did not register any OMI increase in ICLF in comparison to full sun environment in CON. No positive effect of shade in ICLF on OMI can be explained by the fact, that the forage growth in that system was negatively affected by significantly reduced sunlight and therefore the forage allowance was decreased.

ICL and ICLF are claimed to provide improved forage quality and enhanced living conditions for cattle (Balbino et al., 2014), therefore the greater daily gains of heifers in ICL and ICLF in comparison to CON were predicted. In fact, a significant difference between the grazing systems was found for ADG, precisely the heifers kept in ICL and ICLF presented the greater daily gains than those grazing in CON, while ICL did not differ from ICLF. Similar findings were reported by Gamarra et al. (2017), evaluating ADG of Nellore heifers grazing Piatã grass in ICL and ICLF in the same location as the present study during the dry season. The authors reported an ADG of 0.296 kg/animal/day in ICL and ICLF, despite the lower forage allowance in the systems with trees. An ADG of 0.3 kg/animal/day in ICL in the present study lies also in line with the result presented by Oliveira et al. (2014) who reported ADG of 0.28 kg/animal/day of Nellore heifers grazing Piatã grass in the dry season in ICL in the same location as the present experimental site. The ADG of 0.2 kg/animal/day observed in ICLF in our research is slightly higher than those presented by the authors, 0.15 and 0.16 kg/animal/day in ICLF, depending on the tree density in the systems. There are few factors contributing to enhanced performance of the heifers in ICL and ICLF. Firstly, the nutritional quality of forage. While there were no differences found in CP concentration and IVDOM of the forage cultivated in CON and ICLF, it should be pointed out that the nutritional value of forage was determined on the basis of green plant material assuming that the heifers did not consume dead plant material. Nevertheless, in view of the fact that pasture in CON consisted of more than 70 g/100 g DM of dead plant material, it cannot be excluded that dead plant material was also partly ingested, and the poor quality of consumed forage contributed to diminished performance in CON in comparison to ICL and ICLF. Further, in ICL the heifers had access to the highest forage biomass which may have facilitated enhanced selection of diet regardless of the high concentration of fibers and low digestibility. This claim can be supported by findings brought by Domiciano et al. (2018) who evaluated the performance of Nellore steers in ICL and IFL in comparison to CON. The authors reported the highest ADG in ICL and associated it with the highest forage biomass provided by the system through the

integration with crops. According to Euclides et al. (2016) and Walker (1995), diet selection is one of the main factors influencing the nutritional status of animals; therefore, the pasture accessibility and ease of the leaf harvest may significantly impact animal performance. Euclides et al. (2000) found a positive correlation between ADG, forage biomass and green/dead plant material ratio in pasture. Furthermore, even though ICL produced forage with the highest concentration of NDF and ADF, it consisted of the lowest proportion of lignin, while pasture in CON was characterized by the greatest concentration of the aforementioned component. Lignin, as an indigestible non-carbohydrate that restricts the digestion of structural polysaccharides, is considered as an anti-quality component that decreases the nutritional availability of plant fiber. It acts as a barrier and the microbial enzymes are unable to reach their target fibrous polysaccharides in the rumen. The energy concentration is hardly accessible for ruminants which consequently impacts the digestible energy value of forage (Moore & Jung, 2001). According to Gomes et al. (2011), lignin is a dominant factor limiting the nutritive value of tropical forages. The highest concentration of lignin in CON also partially explains the finding that although ICLF had the lowest biomass and nutritional quality comparable to pasture in CON, the heifers still gained more weight in ICLF in comparison to CON. The major challenge associated with the assessment of forage quality is the accurate collection of representative feed samples, which were actually consumed by the heifers grazing heterogeneous pasture. Besides forage collection on pasture and further chemical analysis, the positive relationship between fecal CP concentration and OM digestibility can serve as an assessment of diet quality, particularly the energy value (Lukas et al., 2005). In the present research, the calculation of diet OM digestibility based on feces analysis revealed that forage in ICLF had a higher dOM than CON, despite similar IVDOM values previously reported in forage analysis. This contributed to the enhanced quality of diet in ICLF thus increasing the gains of heifers regardless of the limited forage biomass. Another important aspect that must be discussed is that the heifers in ICL and ICLF were grazing Piatã grass, while CON was sown with *B. decumbens*. Piatã grass stands out among other cultivars as a pasture providing good animal performance mainly during the dry periods (Gamarra et al., 2017). Euclides et al. (2009) evaluating forage nutritive value and animal production in *B. brizantha* pastures confirmed that Piatã grass is characterized by high nutritive value resulting in high animal performance. In

another study, Euclides et al. (2016) observed the ADG of Nellore steers grazing Piatã grass in CON of 0.215 kg/animal/day, while the heifers in the present study in CON presented much lower ADG of 0.05 kg/animal/day grazing *B. decumbens*. Clearly our study differed with regard to the gender of evaluated cattle, however as the ADG observed by the aforementioned authors was equal to ADG observed in the present research in ICLF, the question arises to what extent the greater gains found in the integrated systems than in CON were due to the impact of the systems as a whole, or solely the effect of Piatã grass in comparison to *B. decumbens*. Thus, the importance of usage of the same grass species in the evaluations of grazing systems must be emphasized.

Various studies available in the literature point out the significant impact of thermal comfort on cattle performance. When cattle are exposed to excessive solar radiation and heat, they distribute their energy resources to maintain the core internal temperature and homeostasis of body (Oliveira et al., 2017). Accordingly, when heifers have the access to shade, they minimize the energy utilization for thermoregulation and redirect it to growth, thus improving their performance efficiency (Blain & Nsahlai, 2011). Nellore heifers are considered to be resistant to high temperatures and intense solar radiation, however according to Domiciano et al. (2018), the production efficiency losses can occur. While the heifers in CON presented a low ADG of 0.05 kg/animal/day and the individual weight losses were observed, there were no records of heifers with weight loss problems in ICL therefore we can assume that during the dry season when THI is kept within the thermal comfort zone for most of the time, the aforementioned issue is less severe.

5.5 Evaluation of ICL and ICLF systems as a contribution towards combating rural poverty

One of the reasons responsible for poor beef cattle production in Brazil is inadequate nutrition of cattle, resulting mainly from the seasonality of forage production. Any attempt to improve the supply of high quality forage during the dry season contributes significantly to improving cattle farming productivity and therefore profitability of the farm (Euclides et al., 2016).

The results of this study proved numerous advantages of ICL and ICLF in comparison to CON, but also revealed distinct constraints of these systems during the dry season. The common and traditional method of managing the grazing systems in Brazilian Cerrado, with continuous and set stocking, represented by CON, showed a low forage growth with a high proportion of dead plant material. It resulted in the lowest ADG of the heifers, with the weight loss problems registered in that system. ICL and ICLF were managed in a different way, with the adjustment of the stocking density to forage mass on offer to maintain a similar forage allowance. While both integrated systems, ICL as well as ICLF, were distinguished by similar ADG of heifers, the growth of forage in ICLF was greatly affected by the shade created by *Eucalyptus* trees in the system. Such competition between forage grasses and trees seems to be harmful to the pasture during the dry season, and results in misusing of the system's potential to improve the cattle performance, as the animals are unable to increase their feed intake due to diminished forage biomass. As it has been seen in several studies, ICLF can be highly beneficial if the proper density and spatial distribution of trees is applied, for instance the East-West orientation of tree rows is recommended to ensure greater sunlight incidence into understory (Pereira Serra et al., 2014). The adequate implementation of the ICLF is essential, as the subsequent management problems are often irreversible. Overall, ICL appears to be superior from all evaluated systems during the dry season, as it provided the greatest forage biomass and ADG of heifers comparable to ICLF, while the number of animals grazing on the paddock of the same size as paddock in ICLF was double.

6. Conclusion

The study aimed at a combined approach contributing to a better understanding of the potential of more sophisticated farming systems to overcome the challenges of the dry season in the Cerrado region.

During the dry season ICL provides a greater forage biomass than ICLF and CON. Shading in ICLF leads to reduction in forage biomass in comparison to ICL and CON, while the nutritional quality in terms of CP and IVDOM is improved when compared to ICL. Forage biomass in CON ranges at an intermediate level between ICL and ICLF. The forage quality in CON is inferior in comparison to ICL and ICLF in terms of high proportion of dead plant material and high concentration of lignin.

Shading in ICLF does not improve significantly the microclimatic conditions for heifers during the dry season and thus does not increase the grazing time of heifers in that system. Forage characteristics is a more determining factor during the dry season for grazing behaviour. In order to compensate for the poor quality of forage in CON, heifers prolong their grazing in comparison to ICL.

A high proportion of dead plant material in CON, a large proportion of stems in ICL and low forage biomass in ICLF are constraints that limit the feed intake of Nellore heifers during the dry season. However, due to high forage biomass in ICL, and high proportion of green leaves in ICLF, heifers grazing in the integrated systems are able to achieve greater ADG in comparison to CON.

All in all, regardless of their drawbacks, ICL and ICLF perform better in terms of overcoming the challenges emerging from the dry season, as they ensure improved forage growth and quality resulting in a greater ADG of Nellore heifers in comparison to traditional CON.

7. References

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