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Methane emissions and milk yields from zebu cows under integrated systems

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HIGHLIGHTS

- One of the first reports of cow CH4 emissions in integrated silvopastoral systems.
- Herbage crude protein content was 35.9% higher on average in CLFI than in the CLI.
- Dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI.
- Milk yield and feed efficiency were similar between systems and seasons.
- Methane emissions were similar between systems and lower in the rainy season.

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ABSTRACT

Integrated systems are technologies that potentially increase animal production and environmental preservation, but the effect of these systems on the efficiency and methane emissions of dairy cows is still unknown. This study aimed to compare enteric methane emissions, dry matter intake and performance of grazing dairy cows in integrated systems in the Brazilian Cerrado biome, i.e., crop-livestock integration (CLI) or crop-livestock-forest integration (CLFI). Eighteen Holstein-Zebu cows were randomly assigned to the two production systems (n = 9 for each system) based on Monbasa pasture (*Megathyrsus maximus* cv. Mombaça; Syn. *Panicum maximum*) under rotational stocking management. Herbage allowance ranged from 12 to 14% body weight, and cows were supplemented with concentrated feed according to milk yield. Herbage samples were collected by simulated grazing to determine nutritional value. Milk yield was determined weekly. Herbage intake was estimated from fecal output and indigestibility of the pasture dry matter. Fecal output was estimated by the external indicator LIPE®, and dry matter digestibility was estimated by the internal indicator NDF_i. Enteric methane emissions were estimated by the SF_6 tracer gas technique. Data were collected in three sampling periods to characterize the rainy season, the transition from the rainy season to the dry season and the dry season. Data were analyzed in split plots, with animals within the system as the plot and seasons as the subplot. Statistical significance was considered at $P < 0.05$. The herbage crude protein content was 35.9% higher on average in the CLFI than in the CLI. *In vitro* dry matter digestibility was 16.7% lower in the CLI than in the CLFI in the rainy season. Milk yield and feed efficiency were similar between systems and seasons. The total dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI. The energy loss, production and yield of methane were 29.8%, 35.0% and 31.3%, respectively, lower in the rainy season than in the other seasons. Enteric methane emissions, milk yield and feed efficiency were similar between the integrated CLI and CLFI systems in the Brazilian Cerrado region.

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1. Introduction

The Cerrado biome (i.e., Brazilian Savannah) of Brazil occupies approximately 204 million hectares (24% of the national territory). Inadequate management in cattle farming, such as unsuitable stocking rates and lack of soil fertility maintenance, can lead to environmental degradation in this biome (Dias-Filho et al., 2014; Cerri et al., 2015). Briefly, integrated systems can be defined as the simultaneous cultivation, in succession or in rotation, of different plant and animal species in the same area. Therefore, integrated systems have been proposed as a strategy to promote the sustainable use of resources, reduce environmental impacts and increase agricultural productivity in this biome (Lemaire et al., 2013).

In 2016, the Brazilian agricultural sector was responsible for the emission of 439,213 Gg of CO_2 equivalents (CO_2 eq.), representing 34% of the national emissions of greenhouse gases (GHG). In the same year, enteric methane (CH_4) emissions represented 56.5% of agricultural emissions (MCTIC, 2020). In the context of agricultural decarbonization in the tropics, the use of integrated systems has been identified as a promising sustainable strategy (Norse, 2012; Figueiredo et al., 2016; Torres et al., 2017). Crop-livestock integration (CLI) and crop-livestock-forest integration (CLFI) are the two forms of integration. CLI is defined as the integration of different crops and animals, whereas CLFI is defined as the integration of crops, animals and forestry. Both integrations are implemented in the same area to explore possible synergism among the components, which would increase system productivity and income outputs (Paciullo et al., 2014; Magalhães et al., 2018; Oliveira et al., 2022). Among the main benefits of this system are greater carbon stock (Almeida et al., 2021), better animal comfort and welfare (Martins et al., 2021; Reis et al., 2021), better herbage nutritional value (Lima et al., 2018) and farm income diversification (Müller et al., 2011). Additionally, according to Liu et al. (2021), by continuously improving production efficiency, livestock can be a short-term solution to mitigate anthropogenic effects on climate change while long-term solutions for carbon emissions from fossil fuel use are developed.

According to Silva et al. (2013), intake by grazing cattle is primarily influenced by sward structure and secondarily by nutritional value. Geremia et al. (2013) showed that silvopastoral systems (SPSs) with moderate shading (49 m between ranks; 338 trees ha^{-1}) provided an intake rate, bite mass and bite rate similar to those of pasture monoculture. In these SPSs with moderate shade, improved herbage nutritional value, especially the increase in protein content (Paciullo et al., 2014; Geremia et al., 2018; Santos et al., 2018), and better thermal comfort during the day (Giro et al., 2019; Martins et al., 2021) can increase intake and improve feed efficiency and cattle performance (Santos et al., 2018). The greater efficiency of nutrient utilization by dairy cows can increase the overall efficiency of the production system (Lemaire et al., 2013; Soussana and Lemaire, 2014). Furthermore, improved comfort caused by shading can also reduce energy use for controlling thermal stress and increase animal efficiency (Schütz et al., 2010; Vizzotto et al., 2015), especially in tropical conditions (Reis et al., 2021).

The main GHG generated in ruminant production systems is enteric CH_4 (Hagemann et al., 2011; O'Brien et al., 2012; Yan et al., 2013). In SPSs, CH_4 emissions can be reduced by improving pasture nutritional value (Pedreira et al., 2009). However, an integrated assessment of animal production and GHG emissions to better characterize the efficiency and sustainability of these animal production systems is lacking. Furthermore, the determination of CH_4 emission factors that are specific to these systems must be developed to improve the accuracy of the GHG emissions inventory.

This study aimed to compare enteric CH_4 emissions, dry matter intake (DMI), milk yield (MY) and feed efficiency of grazing dairy cows in two integrated systems, CLI vs. CLFI, both of which are typical of the Brazilian Cerrado biome. The study spanned the rainy and dry seasons as well as the rainy-to-dry transition. Our first hypothesis was that, regardless of the seasons, the CLFI system would have improved pasture nutritional value compared to that in the CLI system, and this improvement would result in increased feed intake and feed efficiency. Our second hypothesis was that higher DMI increases enteric CH_4 production (in g/day) but reduces CH_4 yield (in g CH_4/kg DMI) and intensity (in g CH_4/kg MY) in dairy cows.

2. Materials and methods

Experimental procedures were approved by the animal use ethics committee of Embrapa Cerrados (protocol n°. 533-2541-1/2017).

2.1. Experimental area and treatments

The study was carried out in the Cerrado biome at the Center of Technology for Dairy Zebu Breeds, located in Brasília, DF, Brazil (15°57′09" S, 48°08′12" W, altitude 998 m). The climate is classified as tropical rainy Awa (A - tropical rainy climate, w - rainy summer, a - hot summer, with average temperature of the hottest month above 22 °C) (Alvares et al., 2013). The Cerrado biome has two well-defined climatic seasons with hot and rainy summers (rainy season; between October and March) and cold and rainless winters (dry season; between April and September). The experimental area's soil is characterized as red ferralsols (WRB, 2006).

The treatments consisted of integrated production systems based on Mombaça grass (*Megathyrsus maximus* Syn. *Panicum maximum* cv. Mombaça) established in succession with soybeans (*Glycine max*) in the CLI and CLFI. Trees in the CLFI were planted in an east−west orientation in 2013 with simple rows of *Eucalyptus urograndis* spaced 25 m apart with a density of 130 trees/ha (which can be considered low density), and pasture was implemented in 2016. At the evaluation times, the trees were approximately 28 m tall. The experiment lasted 95 days from February to May 2019 and comprised three sampling periods as follows: rainy (February), transition (March), and dry (May) seasons.

The soil chemical characteristics in the 0–20 cm layer in the CLFI were pH = 6.2, soil organic matter (SOM) = 33.7 g dm^{-3}, P = 14.1 mg dm^{-3}, K = 202 mg dm^{-3}, Ca = 1.1 cmol dm^{-3}, Mg = 0.7 cmol dm^{-3}, Al = 0.01 cmol dm^{-3}, and H + Al =1.7 cmol dm^{-3}; those in the CLI were pH = 6.1, SOM = 26.6 g dm^{-3}, P = 18.91 mg dm^{-3}, K = 140 mg dm^{-3}, Ca = 2.1 cmol dm^{-3}, Mg = 0.6 cmol dm^{-3}, Al = 0.01 cmol dm^{-3}, and H + Al = 1.8 cmol dm^{-3}. The pasture area was fertilized with urea during the experimental period with two applications of 54 kg ha^{-1} (totaling 108 kg N ha^{-1}) in the CLI and in the CLFI.

2.2. Animal management

Eighteen lactating Holstein-Zebu cows were used as repetitions (test animals). The animals were evenly distributed considering days in milk (DIM), MY and body weight (BW), with nine cows in the CLI (MY = 16.5 ± 4.28 kg/cow.day, DIM = 95.2 ± 49.4 days and BW = 490 ± 51.4 kg) and nine in the CLFI (MY = 18.9 ± 4.74 kg/cow.day, DIM = 98.7 ± 42.8 days and BW 498 ± 72.9 kg). The CLI and CLFI areas contained 8 ha each. Each of these systems' areas was divided into 12 paddocks and managed in rotational grazing with a variable stocking rate, with two to three grazing days and 22 or 33 rest days in the rainy and dry seasons, respectively, to maintain an average herbage allowance of 12–14 kg of dry matter (DM) per 100 kg of BW, according to herbage mass evaluations.

Cows received concentrated feed based on corn (*Zea mays*) and soybeans (180 g/kg of crude protein and 760 g/kg of total digestible nutrients) with the proportion of one kg for every three kg of milk produced (based on individual yield) when cows produced more than eight kg of milk per day. Concentrate was offered during the morning and afternoon milkings. In addition, cows received a water and mineral mixture (80 g/kg phosphorus, 115 g/kg sodium, 30 mg/kg selenium and 3000 mg/kg zinc) *ad libitum*.

2.3. Herbage chemical composition

Herbage samples were manually collected from paddocks during grazing days in both systems during the three seasons. Sampling was carried out by simulated grazing to represent pasture strata grazed by the animals (Aroeira et al., 1999). Samples were dried in an oven at 55 °C for 72 h and processed in a knife mill with 1 mm sieves (Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ, USA). Crude protein contents (Method 976.05; AOAC, 1990) were determined by the Kjeldahl method. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined according to Van Soest et al. (1991) in an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA) with methodology adapted by ANKOM (2021). Neutral detergent fiber residues were submitted to CP analysis to determine neutral detergent insoluble protein (NDIP). *In vitro* dry matter digestibility (IVDMD) was determined by the methodology proposed by Tilley and Terry (1963) with adaptations for execution in the Daisy^{II} digestion apparatus (Ankom Technology, Macedon, NY, USA) described by Mabjeesh et al. (2000).

2.4. Milk yield and feed intake

Milk yield was determined weekly during the experiment. On the day of MY evaluation, individual samples were collected to determine milk fat content. Milk yield was corrected to 4% fat (4% FCM) according to the equation proposed by Gaines (1928):

$$4\%FCM = (0.4\ x\ MY) + [15\ x\ (MFY\ x\ MY\ /\ 100)]$$

where 4% FCM = 4% fat corrected milk yield (kg/cow.day), MY = milk yield (kg/cow.day), and MFY = milk fat yield (kg/cow.day).

Fecal output was estimated using the external indicator LIPE® (isolated, purified and enriched *Eucalyptus grandis* lignin) (Berchielli et al., 2000; Saliba, 2005), and dry matter digestibility was estimated by the internal indicator indigestible neutral detergent fiber (NDF_i) (Casali et al., 2008). The external indicator LIPE® was offered in capsules at a dose of 500 mg per cow/day for six consecutive days in each season (rainy season, transition season and dry season) (Saliba et al., 2013). The protocol used three days of adaptation to the indicator followed by three days of feces collection, carried out directly in rectal ampoules.

Fecal samples were collected twice a day after milking. Samples were dried in an oven at 55 °C for 72 hs and processed in a Wiley knife mill with 1 mm sieves (Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ, EUA). Equal amounts of each sample from each collection were used to form composite samples of each animal by season. Approximately 10 g of each composite sample was used to determine the LIPE® concentration by infrared spectroscopy in a spectrophotometer (Varian 800 FT-IR, Varian Systems - Inc, Palo Alto, CA, USA) with Fourier transform (FT-IR) (Saliba, 2005; Saliba et al., 2013). Fecal output (FO) was estimated by the equation:

$$FO = (ingested\ dose\ of\ LIPE^{®}\ /\ Fecal\ concentration\ of\ LIPE^{®})$$

For NDF_i determination, 0.8 g of feces and herbage samples were weighed into F57 bags (Ankom Technology, Macedon, NY, USA) in triplicate and incubated for 264 h in crossbred steer (3/4 Holstein x Gyr) (Casali et al., 2008). After incubation, F57 bags were washed in water and submitted to NDF analysis according to Van Soest et al. (1991) in an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA) with methodology adapted by ANKOM (2021). Dry matter digestibility (DIG) was determined using the equation:

$$DIG = [1 - (NDFip\ /\ NDFif)]$$

where NDFip = indigestible neutral detergent fiber from herbage and NDFif = indigestible neutral detergent fiber from feces.

Individual intake of herbage and concentrate were determined by the equation:

$$DMI = [FO\ /\ (1 - DIG)]$$

where DMI = dry matter intake (kg/cow.day), FO = fecal output (kg/cow.day), and DIG = dry matter digestibility (% DM).

Total dry matter intake (TDMI) was determined as the sum of herbage and concentrate intake. Herbage intake, concentrate and TDMI were expressed as % BW. The feed efficiency (FE) was determined by the equation:

$$FE = (4\%FCM\ /\ TDMI)$$

where FE = feed efficiency; 4% FCM = 4% fat corrected milk yield (kg/cow.day); and TDMI = total dry matter intake (kg/cow.day).

2.5. Methane emission

The CH_4 emission was estimated using the sulfur hexafluoride trace gas dilution technique (SF_6) (Johnson et al., 1994) for at least four consecutive days per animal in each season (rainy season, transition season and dry season). One cow from the CLI treatment was excluded from this assessment due to its low daily rate of SF_6 capsule emission. Regarding CH_4 emissions, the variables calculated were ruminal CH_4 production (g CH_4/day), intensity (g CH_4/4%FCM.day) and yield (g CH_4/kg DM). These parameters were estimated by the following equations:

$$MP = [(CAF * (MCA - MCC)) / (SCA - SCC)] * 60 * 24$$

where MP = ruminal CH_4 production (g CH_4/day), CAF = capsule average flow (g/min), MCA = CH_4 concentration in the animal's yoke (μg/m^3), MCC = CH_4 concentration in the control's yoke (μg/m^3), SCA = SF_6 concentration in the animal's yoke (μg/m^3), and SCC = SF_6 concentration in the control's yoke (μg/m^3).

$$MEI = (MP\ /\ 4\%FCM)$$

where MEI = CH_4 emission intensity (g CH_4/4%FCM.day), MP = ruminal CH_4 production (g CH_4/day), and 4%FCM = 4% fat corrected milk yield (kg/cow.day).

$$MEY = (MP\ /\ TDMI)$$

where MEY = CH_4 yield (g CH_4/kg DM), MP = ruminal CH_4 production (g CH_4/day), and TDMI = total dry matter intake (kg/cow.day).

After determination of the individual herbage and concentrate intake, herbage and concentrate samples were submitted to combustion in an adiabatic calorimetric pump (Model PARR 2081 - PARR Instrument Company, Moline, IL, USA) to determine feed gross energy. The herbage gross energy intake was determined by multiplying the gross energy and individual herbage intake, the concentrate gross energy intake was determined by multiplying the gross energy and individual concentrate intake, and the total energy intake was determined by adding the herbage and concentrate gross energy intake. The gross energy loss as CH_4 (Ym, %) was estimated by the equation:

$$Ym = [(MP\ *\ 13334)\ /\ GDEI)\ *\ 100]$$

where Ym (%) = gross energy loss as CH_4 (%), MP = ruminal CH_4 production (g CH_4/day), 13334 = CH_4 gross energy concentration (cal/g), and GDEI = gross dietary energy intake (cal/cow.day).

2.6. Statistical analyses

Data were submitted to Shapiro–Wilk's and Barttlet's tests to verify the assumptions of normality and variance homogeneity, respectively. However, no variable needed to be transformed. Data were analyzed by analysis of variance (2-way ANOVA) using a split-plot arrangement with repeated measures over time, with "animals within system" as the plot and "seasons" as the subplot. Production system, season and their interaction were considered fixed effects, and animals were considered random effects. As repeated measures over time are not totally independent (nonzero covariation), Mauchly's test (Mauchly, 1940) was applied to check whether there was a need to correct the analysis of variance. When Mauchly's test was significant ($P < 0.05$), a correction was performed using Greenhouse−Geisser's test (Greenhouse and Geisser, 1959).

Days in milk was tested as a covariate for all of the variables measured in the animals and was incorporated into the model for variables for which DIM had a significant effect ($P < 0.05$). Season means were compared by Tukey's test and systems by Fisher's test ($P < 0.05$). Pearson's correlation analysis was performed between variables ($P < 0.05$). Correlation was considered weak when the correlation coefficient was less than 30%, moderate when the correlation coefficient was between 30% and 70%, and strong when the correlation coefficient was greater than 70%. All analyses were performed in the R Core Team (2019) software.

3. Results

The herbage CP content showed a significant interaction between system and season ($P = 0.007$) (Table 1). The crude protein content in the CLFI was similar between seasons, but in the CLI, it was 29.3% lower in the dry season than in the other seasons. Crude protein was similar between systems in the transition season, but it was 31.4% and 83.0% higher in the CLFI than in the CLI in the rainy and dry seasons, respectively. Neutral detergent fiber and ADL were not influenced by any evaluated factor ($P > 0.05$). Acid detergent fiber was 7.50% higher ($P = 0.05$) in the CLI than in the CLFI and 11.3% lower ($P = 0.004$) in the dry season than in the other seasons.

In vitro dry matter digestibility showed a significant interaction between system and season ($P = 0.028$). *In vitro* dry matter digestibility in the CLFI was similar among seasons, but in the CLI, it was lower in the rainy season than in the other seasons. In the transition and dry seasons, IVDMD was similar between systems, but in the rainy season, IVDMD was 16.7% lower in the CLI than in the CLFI. Neutral detergent insoluble protein was 21.7% higher ($P = 0.034$) in the CLI than in the CLFI. Neutral detergent insoluble protein was lower ($P = 0.013$) in the rainy season, intermediate in the transition season and higher in the dry season.

Milk yield, 4% FCM and milk fat content were not altered by any evaluated factor ($P > 0.05$) (Table 2). Concentrate intake was 24.6% lower ($P = 0.009$) in the dry season than in the other seasons (Table 3). Herbage intake showed a significant interaction between system and season ($P = 0.002$). Herbage intake in the CLFI was 55.7% lower in the dry season than in other seasons, but in the CLI, it was lower in the rainy season, intermediate in the dry season and higher in the transition season. Total dry matter intake showed a significant interaction ($P = 0.003$) between system and season. The total dry matter intake in the CLFI was 50.5% lower in the dry season than in the other seasons. The total dry matter intake in the CLI was higher at the transition station than at the other stations. The total dry matter intake in the rainy season was 34.6% higher in the CLFI than in the CLI, with no differences in the other seasons. Feed efficiency showed an interaction between system and season ($P = 0.045$).

The gross energy losses of CH_4, CH_4 production, and CH_4 yield were 29.8%, 35.0% and 31.3% lower ($P < 0.01$), respectively, in the rainy season than in the other seasons (Table 4). Milk yield corrected to 4% fat showed a moderate positive correlation with concentrate intake and a negative correlation with CH_4 emissions (Fig. 1). Milk yield corrected to 4% fat showed a strong positive correlation with feed efficiency. Concentrate intake showed a moderate positive correlation with TDMI and a negative correlation with CH_4 emissions. Herbage intake was strongly positively correlated with TDMI and moderately negatively correlated with feed efficiency. Total dry matter intake showed a moderate negative correlation with feed efficiency. Feed efficiency showed a moderate negative correlation with CH_4 emissions.

4. Discussion

4.1. Nutritional value

The increase in herbage CP content in SPSs compared to full sun is a result that has been described in previous studies with tropical grasses under shade (Geremia et al., 2018; Lima et al., 2018; Santos et al., 2018; Oliveira et al., 2022). This increase is mainly due to physiological changes in plants in SPSs that allow plant cells to remain younger

Table 1
Crude protein, neutral detergent fiber, acid detergent fiber, acid detergent lignin, *in vitro* dry matter digestibility and neutral detergent insoluble protein (DM basis) of *Megathyrsus maximum* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			Mean	SEM	*P*-valueS	*P*-valueSE	*P* value S * SE
	Rainy	Transition	Dry					
Crude protein (g/kg DM)								
CLFI	116aA	135aA	136aA	-	6.2	<0.001	<0.001	0.007
CLI	88.3aB	122aA	74.3bB	-				
Neutral detergent fiber (g/kg DM)								
CLFI	623	673	615	-	8.8	0.400	0.264	0.105
CLI	679	643	635	-				
Acid detergent fiber (g/kg DM)								
CLFI	322	322	278	307B	6.3	0.050	0.004	0.420
CLI	356	328	305	330A				
Mean	339a	324a	294b					
Acid detergent lignin (g/kg DM)								
CLFI	43.4	57.1	54.3	-	2.05	0.217	0.619	0.087
CLI	49.4	42.5	45.7	-				
In vitro dry matter digestibility (g/kg DM)								
CLFI	651aA	648aA	666aA	-	12.0	0.005	0.028	0.028
CLI	542bB	650aA	626aA	-				
Neutral detergent insoluble protein (g/kg DM)								
CLFI	35.1	36.9	45.6	39.2B	2.45	0.034	0.013	0.295
CLI	35.8	50.8	56.6	47.7A				
Mean	35.4b	42.9ab	52.2a					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value S, *P* value for system effect; *P*-value SE, *P* value for season effect; *P*-value S * SE, *P* value for interaction between system and season effect.

Table 2
Milk yield, 4% fat corrected milk yield and milk fat of crossbred dairy cows grazing *Megathyrsus maximum* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			SEM	*P*-valueCOV	*P*-valueS	*P*-valueSE	*P*-valueS * SE
	Rainy	Transition	Dry					
Milk yield (kg/cow.day)								
CLFI	18.9	16.9	13.1	0.71	< 0.001	0.135	0.207	0.139
CLI	16.6	17.6	13.4					
4% fat corrected milk yield (kg/cow.day)								
CLFI	19.5	17.8	13.9	0.68	< 0.001	0.085	0.574	0.099
CLI	16.6	18.3	13.9					
Milk fat (%)								
CLFI	4.30	4.50	4.60	0.102	0.092	0.517	0.300	0.995#
CLI	4.10	4.30	4.30					

Means followed by different lowercase letters in the line differ by the Tukey' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value COV, *P*-value for covariate days in milk, *P*-value S, *P* value for system effect; *P*-value SE, P value for season effect; *P*-value S * SE, *P* value for interaction between system and season effect; #, *P* value corrected for Greenhouse-Geisser.

Table 3
Concentrate intake, herbage intake, total dry matter intake and feed efficiency of crossbred dairy cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			SEM	*P*-valueCOV	*P*-value S	*P*-value SE	*P*-valueS * SE
	Rainy	Transition	Dry					
Concentrate dry matter intake (% BW)								
CLFI	0.883	0.830	0.680	0.0321	0.180	0.854	0.009	0.367
CLI	0.897	0.800	0.606					
Mean	0.890a	0.816a	0.643b					
Herbage dry matter intake (% BW)								
CLFI	1.90aA	2.21aA	1.32bA	0.068	0.099	0.122	<0.001	0.002
CLI	1.51cB	2.05aA	1.67bA					
Total dry matter intake (% BW)								
CLFI	2.80aA	2.97aA	1.92bA	0.078	0.321	0.229	0.002#	0.003
CLI	2.08bB	2.83aA	2.34bA					
Feed efficiency (kg milk/kg DM)								
CLFI	1.40aA	1.21aA	1.41aA	0.061	< 0.001	0.146	0.038	0.045
CLI	1.66aA	1.31aA	1.12aA					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; *P*-value COV, *P*-value for covariate days in milk, *P*-value S, *P* value for system effect; *P*-value SE, P value for season effect; *P*-value S * SE, P value for interaction between system and season effect; DM, dry matter; #, P value corrected for Greenhouse-Geisser.

Table 4
Enteric CH_4 emissions and gross energy loss as enteric CH_4 of crossbred dairy cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil.

System	Season			SEM	*P*-value COV	*P*-valueS	*P*-value SE	*P*-valueS * SE
	Rainy	Transition	Dry					
Methane production (g CH_4/day)								
CLFI	351	500	451	23.3	0.465	0.743	< 0.001	0.389#
CLI	297	583	471					
Mean	325b	541a	460a					
Emission intensity (g CH_4 /4%FCM.day)								
CLFI	18.5	31.5	32.9	1.68	0.013	0.761	0.087	0.943
CLI	19.8	32.0	35.3					
Methane yield (g CH_4/kg DM)								
CLFI	25.2	34.2	44.4	1.81	0.612	0.512	0.006	0.310#
CLI	29.5	41.2	38.5					
Mean	27.2b	37.7a	41.5a					
Ym (%)								
CLFI	8.33	10.9	13.9	0.58	0.642	0.387	0.009	0.327#
CLI	9.63	13.6	12.4					
Mean	8.94b	12.3a	13.2a					

Means followed by different lowercase letters in the line differ by the Tukey' test and uppercase letter in the column differ by the Fisher' test. CLFI, crop-livestock-forestry integration; CLI, crop-livestock integration; SEM, standard error of mean; #, *p* value corrected for Greenhouse-Geisser; CH_4, methane; DM, dry matter; FCM, 4% fat corrected milk, Ym, gross energy loss as CH_4 (% of ingested). *P*-value COV, *P*-value for covariate days in milk, *P*-value S, *P* value for system effect; *P*-value SE, *P* value for season effect; *P*-value S * SE, *P* value for interaction between system and season effect.

(Guenni et al., 2008; Taiz et al., 2015; Guenni et al., 2018), accumulate fewer fibrous compounds (Geremia et al., 2018; Lima et al., 2018) and proportionally have higher CP. Another factor that may explain the CP increase is the greater soil nitrogen availability (Wilson, 1996; Chatterjee et al., 2018). The more uniform CP content in the CLFI between seasons is due to the lower metabolic stress of plant cells in the CLFI

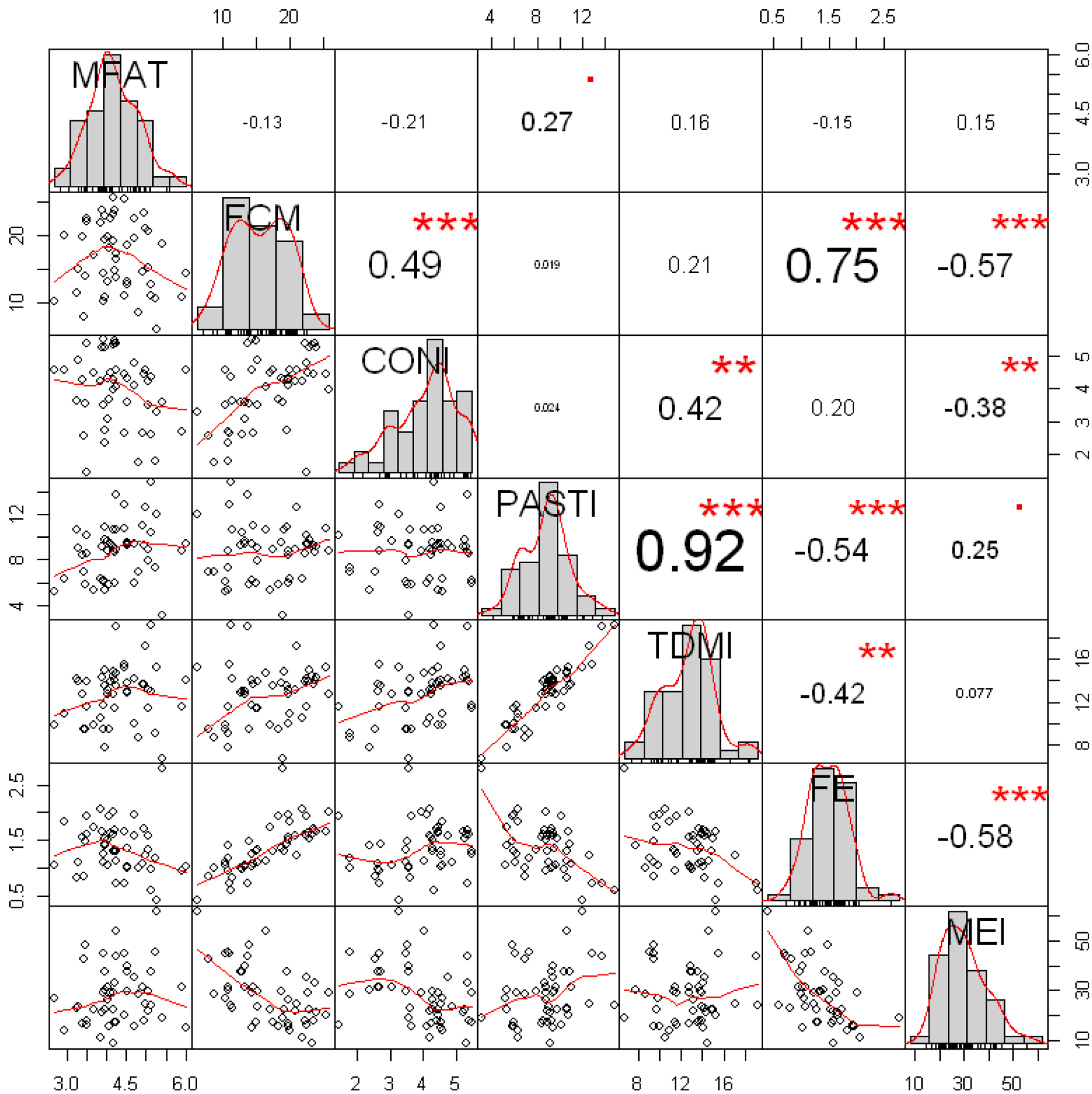

Fig. 1. Matrix of correlation between performance, feed intake and methane emission of crossbred cows grazing *Megathyrsus maximus* cv. Mombaça managed in the CLI and CLFI systems in Brasília, DF, Brazil. MFAT, milk fat; FCM, 4% fat corrected milk yield; CONI, concentrate intake; PASTI, pasture intake; TDMI, total dry matter intake; FE, feed efficiency; MEI, methane emission intensity; values inside the box indicate the coefficient of correlation; *** = P-value < 0.001; ** = P-value < 0.01; * = P-value < 0.05.

compared to the CLI. Lower exposures to UV-B radiation, extreme temperatures and intense light delay the senescence process (Gómez et al., 2012; Taiz et al., 2015; Santiago-Hernández et al., 2016) of plant cells under shade, which explains the CP content maintenance in the CLFI.

Increases in height and stem percentage in plants are factors that can increase NDF. However, the maintenance of cells at a younger stage and a lower senescent material percentage can reduce NDF. Therefore, these factors together explain the equality of NDF between systems (Paciullo et al., 2014; Geremia et al., 2018; Silva et al., 2020). Although NDF and ADL were similar between systems, ADF was higher in the CLI, which may have generated higher IVDMD in the CLFI in the rainy season. This higher herbage IVDMD probably also occurred due to higher CP and lower NDIP contents. These results confirm the hypothesis that CLFI improves herbage nutritional value and indicate that, in the Cerrado region, pastures cultivated in CLFI systems can offer better quality herbage for animals, especially in the rainy season.

Higher NDIP contents in herbage under full sun were also observed by Paciullo et al. (2016), who found 14% lower NDIP in *Panicum* cultivars subjected to 58% shading. These results are important because they indicate that herbage plant cells in CLFIs show fewer chemical bonds between fibrous and protein compounds, which probably increase IVDMD and may increase nutrient supply to animals (Van Soest et al., 1994).

4.2. *Performance and feed intake*

Although herbage had better nutritional value in the CLFI and in the rainy season, cows had similar MY. Martins et al. (2021) and Paciullo et al. (2014) also did not observe any effect of the silvopastoral system on the MY of Holstein-Zebu cows in the Cerrado and Atlantic Forest biomes in Brazil, respectively. These results probably occurred because the animals received concentrate supplementation according to MY, which supplied the nutrients that were deficient in the pasture.

Supplementation with concentrated feed is a management practice normally adopted on farms that produce milk from grazing animals in Brazil. Bottini-Luzardo et al. (2016) also did not observe a difference in the MY of cows in SPSs with *Leucaena leucocephala* and *Cynodon nlemfuensis* compared to full sun. These authors observed greater blood urea nitrogen of cows in SPSs compared to full sun (19.1 vs. 15.3 mg/dL), probably due to the failure in synchronism between ruminal metabolism of protein and carbohydrates.

Bretas et al. (2020) observed higher nitrogen concentrations in the excreta of animals in a CLFI compared to those in full sun, which corroborates the hypothesis of lower efficiency in protein utilization. This failure in synchronism occurs due to the rapid availability of nonprotein herbage nitrogen fractions in the rumen, and this excess nitrogen is excreted as urea (Kolver et al., 1998; Zhang et al., 2020). Milk yield equality of animals grazing herbage with higher CP content in the CLFI may indicate that in commercial farms, the balancing of diets could use lower CP content in concentrated feed and reduce nutrition costs. Therefore, future studies should evaluate different concentrations of protein supplementation for dairy cows in CLFIs, which may indicate greater production efficiency with lower protein supplements.

Another factor that may have generated similar MY between systems is the cows' lactation stage. The cows had already passed the lactation peak, and at this stage, these animals have low productive efficiency because they change their energy metabolism to produce body tissues (Santos et al., 2014; Lage et al., 2021). In addition, cows had medium MY and therefore did not have a very high demand for nutrients. Under these conditions, supplementation with concentrated feed probably met the cows' requirements in the CLI, and there was no limitation of protein and amino acids. This adequate supply of nutrients allowed for similar yields to cows in the CLFI, even though they were consuming a diet with lower CP content. Furthermore, the cows' body weights were not changed during the experiment, which indicates that the animals in the present study had no feed restriction.

As concentrated feed was supplied according to MY, the animals with higher yield also ingested more concentrate, which explains the correlation between 4% FCM and concentrate intake. This result was corroborated by concentrate intake, which was also lower in the dry season than in the other sampling periods. Furthermore, the results showed that the most productive animals were also more efficient and emitted less CH_4. Britt et al. (2003) also observed a positive correlation between feed efficiency and MY (r = 66.4; $P < 0.001$). These results indicate the need to select animals with high productive capacity and lower dry matter intake to increase the productivity efficiency of dairy cows and reduce the environmental impact (Yan et al., 2013) of integrated systems in the Cerrado region.

Herbage intake was higher in the CLFI than the CLI in the rainy season, which also increased total dry matter intake. Wims et al. (2010) also observed higher herbage intake by dairy cows (16.9 vs. 15.4 kg DM/cow.day) in pastures of better quality compared to those in pastures of lower quality. This higher intake occurred due to better herbage nutritional value demonstrated by higher CP content, higher IVDMD and lower NDIP. The intake of grazing cows is mainly influenced by a physical limitation caused by ruminal filling (Allen, 1996; Mertens and Grant, 2020). Therefore, the higher herbage IVDMD and lower NDIP in the CLFI may have increased the flow of digesta through the gastrointestinal tract, reduced physical limitation and increased the intake of cows in the rainy season. In addition, higher temperature and solar radiation in the CLI in the rainy season likely reduced cow comfort and may have reduced grazing time and herbage intake (Karvatte-Júnior et al., 2016; Oliveira et al., 2017; Pezzopane et al., 2019).

Herbage intake in the CLFI was lower in the dry season, probably due to a reduction in nutritional value. In addition, worse herbage structure in the dry period may cause reduced intake (Santos et al., 2016; Santos et al., 2018; Nascimento et al., 2021). Geremia et al. (2018) observed lower bite mass (1.00 vs. 1.20 g DM/bite) and intake rate (45.9 vs. 49.2 g DM/min) in dairy heifers on CLFIs in the rainy season compared to those in the dry season. These results corroborate the lower intake observed in the dry season in the present study due to worse herbage structure.

Although not evaluated in the present study, heat stress probably reduced herbage intake in the rainy season. Similar to the present study, Martins et al. (2021) observed a lower black globe temperature and humidity index (82.4 vs. 88.9), udder temperature (35.3 vs. 37.1°C) and eye temperature (35.4 vs. 36.4°C) in the CLFI than in the CLI, which indicates better animal thermal comfort in the CLFI and may have improved herbage intake. National Research Council (2001) also emphasizes the negative effect of heat stress on intake. In addition, as cows were producing more milk in the rainy season, concentrate intake was also higher and may have reduced replacement herbage intake.

As concentrate intake was not influenced by the systems, the highest TDMI observed in the CLFI in the rainy season was due to higher herbage intake. Sousa et al. (2008) and Santos et al. (2012) observed a TDMI of 2.5 and 2.39% BW, respectively, in Girolando cows grazing and with supplementation similar to that in the present study, which indicates that the adopted methodology was adequate to determine the intake of cows. The total dry matter intake in the present study was slightly lower than that cited by the National Research Council (2001) for mid-lactating cows, probably because those recommendations were developed for Holstein cows.

The results showed that Zebu cows kept on pasture and supplemented with concentrate according to MY showed similar feed efficiency among the integrated systems in the Cerrado region, probably due to the similarity in MY and small change in TDMI. These results reject the hypothesis that better herbage nutritional value in the CLFI improves MY and feed efficiency of dairy cows. The feed efficiency observed in the integrated systems (1.35 kg 4% FCM/kg DM) in the present study can be considered average compared to studies that evaluated the FE of dairy cows (Britt et al., 2003; Arndt et al., 2015; Hurley et al., 2018).

4.3. Methane emission

The lower CH_4 production in the rainy season was probably because the animals were consuming a better-quality diet. In addition to the better herbage nutritional value in the rainy season, the amount of concentrate offered per cow was also higher in the rainy than in the dry season. Digestion of herbage cell wall carbohydrates produces mainly acetate and two molecules of H^{+2}, which is a precursor of CH_4 production by methanogenic bacteria in the rumen (Sejian et al., 2012). On the other hand, digestion of carbohydrates from concentrated feed mainly produces butyrate (a reaction that produces less H^{+2}) and propionate (a reaction that consumes H^{+2}) (Moss et al., 2000; Knapp et al., 2014). Due to the intake of a diet with a higher concentrate proportion in the rainy season, there was probably a lower production of H^{+2}, which explains the lower emission of enteric CH_4.

Furthermore, according to Martin et al. (2010), the intake of younger forages with better nutritional value reduces CH_4 emissions due to the higher concentrations of soluble sugars and linolenic acid. Polyunsaturated fatty acids are toxic to gram-positive bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus albus*, through cell wall disruption (Maia et al., 2007). This mechanism may have helped to reduce CH_4 emissions in the rainy season in the present study, since tropical grasses have less senescent material in the rainy season.

Although the herbage nutritional value in the CLFI was better than in the CLI, cows' CH_4 emissions were similar in both systems, which rejects the hypothesis that the better nutritional value in CLFIs reduces enteric CH_4 emissions. This result was not expected, because several studies have shown a reduction in CH_4 emissions due to improvements in diet nutritional value (Martin et al., 2010; Shibata et al., 2010; Beauchemin et al., 2011). According to Seijan et al. (2012), excessive breakdown of nitrogen compounds in the rumen by microorganisms such as

Ruminococcus spp. and *Butyrivibrio* spp. increase the availability of free carbon skeletons in the rumen, which may have increased CH_4 emissions in the CLFI and generated similar emissions between systems. These compounds can be fermented and increase the production of H^{+2}, which is a precursor to CH_4. This hypothesis is supported in the present study by the higher CP and lower NDIP content in the CLFI, which indicates greater availability and digestibility of nitrogen compounds in the rumen.

The average enteric CH_4 emissions for cattle range from 95.9 to 151 g/animal.day (Sejian et al., 2011; Sejian et al., 2012). However, dairy cows have greater emissions due to more intense rumen metabolism. Emissions above these parameters in dairy cows grazing tropical grasses were observed by Primavesi et al. (2004) (331 g/cow.day), Pedreira et al. (2009) (196 g/cow.day), Alves et al. (2017) (491 g/cow.day), Silva et al. (2017) (260 g/cow.day), Congio et al. (2018) (394 g/cow.day) and Jiménez et al. (2021) (383 g/cow.day). Therefore, the average emission of 442 g/cow.day observed in the present study is within the range observed in dairy cows grazing tropical grasses.

The energy loss as CH_4 indicated by the IPCC for grazing dairy cattle is 6.5% ($\pm$ 1%) (Dong et al., 2006), values that are much lower than those found in the present study. However, the authors emphasized that these parameters need to be improved, especially for animals fed on tropical pastures. According to Kurihara et al. (1999), these emission parameters were established mainly with animals fed temperate forages (Johnson and Ward, 1996). Therefore, due to the lower digestibility, higher fiber content and lower soluble carbohydrate content of tropical forages (Archimède et al., 2011), the emissions factors of animals consuming tropical forages may be higher than those cited by the IPCC.

Values close to those established by the IPCC were observed by Hynes et al. (2016) (Ym = 5.6%), Dall-Orsoletta et al. (2019) (Ym = 7.8%) and Moate et al. (2020) (Ym = 6.07%) with dairy cows on temperate grass pasture, mainly ryegrass (*Lolium multiflorum*). On the other hand, higher emissions factors, as observed in the present study, were observed by Primavesi et al. (2004) (Ym = 10.6%) in Girolando cows grazing on tropical grass. The gross energy loss as CH_4 data for dairy cows grazing on tropical grasses are still scarce, which indicates the need for further studies to determine an emission factor more suitable for this situation.

5. Conclusion

The improvement in the herbage nutritional value in CLFI increased intake only in the rainy season and did not change enteric CH_4 emissions, milk yield or feed efficiency of Holstein-Zebu cows in integrated systems in the Brazilian Cerrado region.

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Roberto Guimarães Júnior: Investigation, Conceptualization, Data curation, Methodology, Formal analysis, Visualization, Writing – review & editing. **Alan Figueiredo de Oliveira:** Investigation, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Isabel Cristina Ferreira:** Investigation, Conceptualization, Data curation, Methodology, Writing – review & editing. **Luiz Gustavo Ribeiro Pereira:** Methodology, Visualization, Writing – review & editing. **Thierry Ribeiro Tomich:** Methodology, Visualization, Writing – review & editing. **Guilherme Lobato Menezes:** Visualization, Data curation, Writing – review & editing. **Lourival Vilela:** Conceptualization, Writing – review & editing. **Ângela Maria Quintão Lana:** Formal analysis, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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