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Prediction of voluntary intake and enteric methane emission by dairy heifers in integrated systems

Predição de ingestão voluntária e emissão entérica de metano por novilhas leiteiras em sistemas integrados

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RESUMO: Para comparar as predições de ingestão de matéria seca (IMS) e emissões de metano entérico (CH_4) nos sistemas integrados de lavoura-pecuária (ILP) e lavoura-pecuária-floresta (ILPF), foi realizado um ensaio cruzado 2 × 2 com oito novilhas Girolando com 25 ± 6,8 meses de idade, divididas em dois grupos durante dois períodos experimentais de 30 dias. A IMS foi estimada pela relação entre a digestibilidade *in vitro* da MS e a produção fecal estimada com LIPE®. A emissão de CH_4 foi estimada por equação não linear. As médias diárias de ingestão total de matéria seca (9,66 e 8,44 kg dia^{-1}) e a emissão total de CH_4 entérico (9,99 e 8,79 MJ dia^{-1}; e 186,68 e 164,30 g dia^{-1}) foram semelhantes entre ILP e ILPF, respectivamente. A emissão de CH_4 expressa por unidade de proteína bruta ingerida (PBI) foi menor (P<0,001) no ILPF (8,40 MJ kg CPI^{-1}; 157,00 g kg PBI^{-1}) do que no ILP (10,94 MJ kg PBI^{-1}; 204,37 g kg PBI^{-1}), mas por unidade de ingestão de nutriente digestível total (INDT) ou de carboidrato não fibroso (ICNF) a emissão de CH_4 foi maior (P<0,001) no sistema ILPF. Foram observadas correlações entre digestibilidade da forragem e composição de nutrientes com as emissões de CH_4 por unidade de ingestão de nutrientes. Em síntese, os sistemas ILP e ILPF não diferem em termo de ingestão voluntária e emissão total de metano entérico; no entanto, a emissão de CH_4 por unidade de PB ingerida é maior no sistema sem árvores; e por unidade de energia ingerida, a emissão é maior na pastagem consorciada com eucaliptos.

Palavras-chave: Bovinos de leite. Gases de efeito estufa. Mudanças climáticas. Pecuária sustentável.

ABSTRACT: To compare the predictions of dry matter intake (DMI) and enteric methane (CH_4) emissions in integrated crop-livestock (ICL) and integrated crop-livestock-forestry (ICLF) systems, a 2 × 2 crossover trial was carried out with eight 25 ± 6.8-month-old Girolando heifers divided into two groups during two 30-day experimental periods. The DMI was predicted by relation between *in vitro* DM digestibility and fecal production estimated with LIPE®. The CH_4 emission was predicted by non-linear equation. The daily means of total dry matter intake (9.66 and 8.44 kg day^{-1}) and total enteric CH_4 emission (9.99 and 8.79 MJ day^{-1}; and 186.68 and 164.30 g day^{-1}) were similar between ICL and ICLF, respectively. The CH_4 emission expressed per unit of crude protein intake (CPI) was lower (P<0.001) in the ICLF (8.40 MJ kg CPI^{-1}; 157.00 g kg CPI^{-1}) than in the ICL (10.94 MJ kg CPI^{-1}; 204.37 g kg CPI^{-1}), but per intake unit of total digestible nutrient (TDNI) or non-fiber carbohydrate (NFCI) CH_4 emission was higher (P<0.001) in the ICLF system. Significant correlations between forage digestibility and nutrient composition with CH_4 emissions per unit of nutrient intake were observed. In conclusion, there is no difference between ICL and ICLF systems in terms of voluntary intake and methane emission. However,

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the methane emission per unit of CP ingested is higher in the system without trees; and, per unit of energy intake, it is higher in the pasture integrated with eucalyptus.

Keywords: Climate change. Dairy cattle. Sustainable livestock. Greenhouse gases.

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INTRODUCTION

Agriculture plays a key role in food production worldwide and it is a major component of the gross domestic product of several countries, including Brazil, where agribusiness is the main sector of the economy (CEPEA, 2020). The environmental impacts of livestock production have been examined, and methane emission (CH_4) from enteric fermentation has been targeted as a substantial greenhouse gas (GHG) source. This is because CH_4 is a highly potent GHG and is considered a major driver of climate change along with other GHGs (IPCC, 2014). Of the various anthropogenic activities, ruminants are the major source of CH_4 emissions (Albuquerque *et al*., 2020). The enteric CH_4 emissions from ruminal fermentation contribute to approximately 17% of the total global anthropogenic CH_4 emissions (Knapp *et al*. 2014).

Environmental challenges such as climate change and increasing competition for natural resources, the projected growth of the livestock sector in the coming decades places significant pressure on livestock stakeholders to adopt sustainable development practices (FAO, 2020). However, despite the potential benefits of crop-livestock integration to agroecosystem efficiency and resilience (Peterson *et al*., 2018), little is known about GHG mitigation opportunities in integrated crop-livestock systems (ICLS), especially CH_4 emissions from dairy cattle.

The ICLS with trees presents opportunities for important contributions to global food security and sustainable livelihoods. The trees assist in stabilizing the microclimate (Pezzopane *et al*., 2015) and protect animals from extreme climate changes. In addition to providing thermal comfort, tree shading affects the production and nutritive value of grasses, especially because plants can exhibit alterations in anatomy and physiology to compensate for the lower photosynthetic radiation in the forage canopy (Oliveira *et al*., 2017; Guimarães *et al*., 2018). These responses lead to differences in grazing behavior (Souza *et al*., 2019), which might affect the quantity and quality of the pasture dry matter intake and thus alter animal performance and methane (CH_4) emissions (Souza Filho *et al*., 2019).

The quantification of CH_4 emissions from livestock on a global scale relies on prediction models because measurements require specialized equipment, which may be expensive. The information availability on livestock production systems has increased substantially over the years, facilitating the development of more detailed CH_4 prediction models (Ellis *et al*., 2007; Moraes *et al*., 2014; Patra, 2017; Sobrinho *et al*., 2019; Benaouda *et al*., 2020).

This study focuses on forage intake and CH_4 emissions from Girolando dairy heifers grazing on palisade pastures in two integrated crop-livestock systems, with or without trees. We hypothesized that tree shade would provoke differences in forage nutritional value and dry matter intake (DMI), thus altering CH_4 emissions from grazing animals.

2. MATERIAL AND METHODS

The Ethics Committee for Animal Utilization of the Brazilian Agricultural Research Corporation (Embrapa Rondônia) approved all management practices applied to experimental animals (process number 06-2015). Trials were carried out in the experimental field of Embrapa, Porto Velho, Rondônia, Brazil (8° 48' 03.89″ S and 63° 50' 53.08″ W) from September to November 2015. The predominant climate in this region is Am, according to the Köppen classification reported by Alvares *et al*. (2014). This is characterized by a dry season (from May to September) and a rainy season (October to April). The mean annual air temperature and annual rainfall are 26 °C and 2095 mm, respectively.

Eight 25 ± 6.8-month-old Girolando (¾ Holstein × ¼ Gyr) heifers with an initial live weight (LW) of 268 ± 83 kg were used. They were distributed in a 2 × 2 crossover design between the ICLF and ICL systems. There were two 30-day experimental periods, 10 days for adaptation followed by 20 days for data collection, totaling an experimental period of 60 d.

An area of 10 ha was divided into two five ha areas for each of the ICL (n = 4) and ICLF (n = 4) systems. In each area, the pasture of Xaraés palisade grass (*Urochloa brizantha* 'Xaraés' syn *Brachiaria brizantha*) was divided into four paddocks of 1.25 ha and managed by intermittent grazing (10 days of occupation and 30 days of rest) at a stocking rate of 2.5 animal units (AU) per ha. Water and a mineral salt mix were provided *ad libitum* in the center of each pasture system. The ICLF system had seven tiers of four rows each of eucalyptus trees planted in March 2013 at a 3.0 × 3.5 m plant-line spacing. At the beginning of the trial, the trees had an average diameter at breast height of 11.9 ± 2.7 cm, total height of 13.8 ± 2.5 cm, and crown cover of 65%.

In pastures of both systems, Xaraés palisade grass samples were collected by hand-plucking (Prohmann *et al*., 2012) for four consecutive days (from the 3rd to the 6th day of the paddock occupation period). The samples were oven-dried at 55 °C until a constant weight was achieved. Dried 1 mm samples were analyzed for DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL), hemicellulose (HEM), and lignin (LIG), following the methodologies of the National Institute of Science and Technology of Animal Science (INCT-CA) reported by Detmann *et al*. (2012). Total carbohydrate (TC), non-fiber carbohydrate (NFC), and total digestible nutrients (TDN) were determined according to Sniffen *et al*. (1992), Weiss (1999), and Cappelle *et al*. (2001), respectively. The determination of *in vitro* DM digestibility (IVDMD) of the diet was adapted from ANKOM (2017).

The chemical composition and digestibility of Xaraés palisade grass and the forage allowance in pastures of both systems are shown in Table 1.

Table 1. *In vitro* dry matter digestibility (IVDMD), and chemical composition of Xaraés palisade grass in integrated crop-livestock (ICL) and integrated crop-livestock forestry (ICLF) systems.

Variables	Systems	
	ICL	ICLF
IVDMD (%)	75.64	72.83
Dry Matter (DM, %)	32.38	26.02
on DM basis (%)		
Mineral Matter (MM)	4.80	6.12
Organic Matter (OM)	95.20	93.88
Crude Protein (CP)	9.52	12.57
Neutral Detergent Fiber (NDF)	59.91	60.24
Acid Detergent Fiber (ADF)	27.53	28.89
Lignin (LIG)	2.24	2.60
Cellulose (CEL)	25.29	26.29
Hemicellulose (HEM)	32.38	31.35
Total Carbohydrate (TC)[1]	84.14	79.77
Non-fiber Carbohydrate (NFC)[2]	24.23	19.53
Total Digestible Nutrients (TDN)[3]	70.49	68.10

[1]Sniffen *et al*. (1992); [2]Weiss (1999); [3]Cappelle *et al*. (2001).

To estimate the forage allowance, the forage was sampled in a 1-m^2 plot randomly placed in different areas of the paddock before the occupation period. The grass samples were weighed using a portable digital dynamometer (DD2000 Instrutherm®, São Paulo, Brazil).

Grass samples were oven-dried at 55 °C to a constant weight to determine the dry matter (DM) content.

The pasture DMI was estimated using the equation:

$$\text{DMI (kg DM day}^{-1}) = TFP \times (1 - IVDMD)^{-1}, \quad (1)$$

where TFP = total fecal production (kg fecal DM day^{-1}) and IVDMD = *in vitro* DM digestibility (%).

Total fecal production was estimated using the external digestive marker LIPE® (Produtos de Pesquisas Simões e Saliba, Belo Horizonte, MG, Brazil). One dose (500 mg) per animal was provided each morning for seven days, following the manufacturer's recommendations. From the third day, feces were collected directly from the rectum of the animal. Then, fecal samples were oven-dried at 55 °C until a constant weight was achieved, and dried 1 mm samples were sent to the laboratory for analysis.

Prediction of enteric CH_4 emissions was performed using a non-linear model proposed by Patra (2017), who considered the criteria of Bayesian information (CBI) and the biological relevance of the predicted parameters for constructing the ideal non-linear model. According to Patra (2017), the database was constructed with information from dairy and beef cattle herds, typical of tropical feeding systems, based on forage from Brazil and India. The best model showed high precision (0.82) and accuracy (0.97) in the original database, with $R^2 = 0.826$ and root-mean-square error (RMSE) of 30.3. The model is:

$$CH_4 \text{ (MJ day}^{-1}) = 71.47 \times (1 - \exp^{-0.0156 \times DMI}), \quad (2)$$

where CH_4 = enteric methane emission and DMI = dry matter intake (kg day^{-1}). For conversion of the enteric CH_4 emission values from MJ to g, we considered that 1 MJ = 18.680754 g CH_4, based on Patra (2017).

Statistical analysis of the intake and CH_4 emission data was performed using the SAS (Statistical Analysis System Institute Inc., Cary, NC) MIXED procedure model*:*

$$Y_{ij} = \mu + T_i + S_j + E_{ij}, \quad (3)$$

where:

Yij = is the observation of animal j in the system i;

μ = is the overall mean;

Ti = is the fixed effect of production system i (ICL or ICLF);

Sj = is the random effect of animal j with a mean of 0 and variance of $\sigma 2$.

Eij = is the random error with mean 0 and variance $\sigma 2$.

The correlation between pasture nutritional value and enteric CH_4 emissions per nutrient intake was determined using the CORR procedure of SAS.

3 RESULTS

Means of DMI, CP intake (CPI), and NDF intake (NDFI) did not differ (P>0.05) between the ICL and ICLF systems. However, a higher (P<0.05) NFC intake (NFCI) and a tendency (P<0.06) of higher TDN intake (TDNI) were observed in heifers from the ICL system (Table 2).

Table 2. Means (± standard error) of dry matter and nutrient intakes of Girolando heifers grazing Xaraés palisade grass in integrated crop-livestock (ICL) and integrated crop-livestock-forestry (ICL) systems

Variables	Systems		P-value	VC (%)
	ICL	ICLF		
DMI (kg day^{-1})	$9.66_{\pm 1.10}$	$8.44_{\pm 1.73}$	0.128	16.63
DMI (% LW)	$3.12_{\pm 0.80}$	$2.68_{\pm 0.67}$	0.267	26.32
(kg DM day^{-1})				
CPI	$0.92_{\pm 0.15}$	$1.06_{\pm 0.24}$	0.159	18.10
NDFI	$5.79_{\pm 0.65}$	$5.09_{\pm 1.05}$	0.146	16.72
TDNI	$6.81_{\pm 0.78}$	$5.75_{\pm 1.18}$	0.060	16.49
NFCI	$2.34_{\pm 0.27}$	$1.65_{\pm 0.36}$	0.001	16.50

DMI, dry matter intake; CPI, crude protein intake; NDFI, neutral detergent fiber intake; TDNI, total digestible nutrient intake; NFCI, non-fiber carbohydrate intake; LW, live weight; VC, variance coefficient.

The DMI, expressed in kg day^{-1} or % LW, did not differ (P>0.05) between the systems. However, the DMI values in % LW were 30.0% (ICL) and 11.7% (ICLF) higher than the 2.4% LW reported by the National Research Council (NRC 2001) for growing dairy heifers. This might be related to forage availability higher than 12 kg DM 100 kg^{-1} LW, considered DMI threatening (Hodgson 1990). The forage availability in the ICL and ICLF systems were 41.9 and 32.3kg DM 100 kg^{-1} LW, respectively.

In relation to nutrient intakes, in both systems, the heifers consumed TDN and CP (Table 2) above the daily requirements of 3.7 kg of TDN and 678 g of CP per kg of DM reported for growing heifers with an average LW of 250 kg (NRC 2001). This result showed that with adequate management, it is possible to breed Girolando heifers exclusively with pasture during the rainy season.

The intakes of TDN and NFC in the ICLF were 18% and 42%, respectively, higher than those observed in the ICL (Table 2). This high energy intake in combination with high availability of CP in the rumen (Silva *et al*., 2021) may improve the efficiency of use of fermentable substrates and microbial protein synthesis (NRC 2001), reducing nitrogen losses and, consequently, improving food utilization and animal performance.

The daily total CH_4 emissions and CH_4 emissions as a function of LW percentage or DM and NDF intakes, expressed in MJ or grams, did not differ between the systems (Table 3).

Table 3. Daily means (± standard error) of CH_4 emissions, expressed in MJ and grams, as a function of live weight (LW), and nutrient intake by Girolando heifers grazing Xaraés palisade grass in integrated crop-livestock (ICL) and integrated crop-livestock-forestry (ICL) systems

CH_4 emissions	Systems		*P*-value	VC (%)
	ICL	ICLF		
Total				
MJ day^{-1}	$9.99_{\pm 0.37}$	$8.79_{\pm 0.59}$	0.124	15.53
g day^{-1}	$186.68_{\pm 7.00}$	$164.30_{\pm 11.10}$	0.124	15.52
Live Weight				
MJ % LW^{-1}	$3.23_{\pm 0.82}$	$2.80_{\pm 0.68}$	0.286	25.80
g^1 % LW^{-1}	$60.32_{\pm 15.25}$	$52.23_{\pm 12.75}$	0.285	25.79
Nutrient intake				
Dry Matter				
MJ kg DMI^{-1}	$1.03_{\pm 0.00}$	$1.04_{\pm 0.00}$	0.146	1.25
G kg DMI^{-1}	$19.33_{\pm 0.06}$	$19.52_{\pm 0.09}$	0.126	1.14
Neutral Detergent Fiber				
MJ kg $NDFI^{-1}$	$1.73_{\pm 0.00}$	$1.74_{\pm 0.01}$	0.499	1.45
G kg $NDFI^{-1}$	$32.28_{\pm 0.25}$	$32.41_{\pm 0.66}$	0.564	1.37
Crude Protein				
MJ kg CPI^{-1}	$10.94_{\pm 0.32}$	$8.40_{\pm 0.33}$	<0.0001	1.02
g kg CPI^{-1}	$204.37_{\pm 16.94}$	$157.00_{\pm 17.55}$	<0.0001	1.03
Total Digestible Nutrients				
MJ kg de $TDNI^{-1}$	$1.47_{\pm 0.00}$	$1.53_{\pm 0.01}$	<0.0001	1.18
g kg $TDNI^{-1}$	$27.43_{\pm 0.23}$	$28.66_{\pm 0.38}$	<0.0001	1.16
Non-fiber Carbohydrate				
MJ kg $NFCI^{-1}$	$4.27_{\pm 0.02}$	$5.36_{\pm 0.11}$	<0.0001	3.69

g kg NFCI^{-1}	$78.80_{\pm 0.85}$	$100.18_{\pm 5.6}$	<0.0001	3.67

SE, standard error; DMI, dry matter intake; NDFI, neutral detergent fiber intake; CPI, crude protein intake; TDNI, total digestible nutrient intake; NFCI, non-fiber carbohydrate intake; VC, variance coefficient.

This may be related to the similar NDF concentrations between the pastures of both systems and to the fact that the consumption of DM and NDF was similar between the ICL and ICLF systems. Significant and positive correlations (P<0.001) between the concentrations of TC, NFC, TDN, and IVDMD with CH_4 emission in MJ per kg of CPI were observed (Table 4).

Table 4. Correlation between digestibility and nutrient composition of Xaraés palisade grass with CH_4 emission per unit of nutrient intake by Girolando heifers in integrated systems

Xaraés palisade grass	CH_4 emission per nutrient intake			
	MJ kg FDNI^{-1}	MJ kg CPI^{-1}	MJ kg TDNI^{-1}	MJ kg NFCI^{-1}
IVDMD (%)		0.8265***	-0.9035***	-0.9386***
DM (%)		0.4936*	-0.8234***	-0.7838***
On DM basis (%)				
OM		0.5773*	-0.7970***	-0.8686***
CP		-0.9853***	0.7572***	0.9276***
NDF	-0.6218**			
TC		0.9298***	-0.8306***	-0.9821***
NFC		0.8944***	-0.8933***	-0.9940***
TDN		0.8265***	-0.9035***	-0.9386***

IVDMD, *in vitro* dry matter digestibility; DM, dry matter; OM, organic matter; CP, crude protein; TC, total carbohydrate; NFC, non-fiber carbohydrate; TDN, total digestible nutrients; *P<0.05; **P<0.01; ***P<0.001

Therefore, the higher content of these nutrients, as well as the higher IVDMD of Xaraés palisade grass in the ICL system, may have contributed to the higher CH_4 emissions expressed as MJ kg CPI^{-1} in this system. Significant and negative correlations (P<0.001) between the concentrations of DM, OM, TC, NFC, TDN, and IVDMD with CH_4 emission in MJ per kg of TDNI and NFCI were also observed (Table 4). Therefore, the digestibility and nutritional composition of Xaraés palisade grass in the ICLF system, may have contributed to the higher CH_4 emissions expressed per unit of TDNI and NFCI in this system.

4 DISCUSSION

A reason for the elevated DMI in both systems is related to the nutritional characteristics of the pasture. Silva *et al*. (2021) observed an increase of 33.89% in CP content in the grass of the ICLF system compared with that of ICL. In both systems, however, the CP content was above the critical level (7.0%) for proper functioning of the rumen (Van Soest, 1994) and for the efficient use of forage fibrous carbohydrates (Lazzarini *et al*., 2009). An NDF level of approximately 60% and a DM digestibility higher than 70% are not limiting factors for voluntary DM intake (Van Soest, 1994).

The production of enteric CH_4 by ruminants may be related to the animal size, age, and species (Abdalla *et al*., 2012) but is mainly dependent on the nutritional value of the diet available to animals and the intake level (Archimède *et al*., 2011). In our study, the daily emission values of enteric CH_4 were higher than those reported by Bharanidharan *et al*. (2018) in Holstein heifers fed a diet with a 27:73 roughage:concentrate ratio. They also observed that the feeding method also affects the emission of CH_4, reporting 96.1 g day^{-1} when total mixed ration (forage + concentrate) was supplemented and 84.4 g day^{-1} when forage and concentrate were supplied separately.

Higher CH_4 emissions from animals fed pasture diets is observed because of differences in rumen fermentation methods. Dietary characteristics can affect CH_4 production by providing different substrates to microbial populations that are responsible for volatile fatty acid (VFA) production in the rumen. Concentrates contain non-structural carbohydrates, such as starch and sugar, which are rapidly fermented and lead to a reduction in rumen pH and methanogenic bacteria population and, consequently, increase propionate production (Cota *et al*., 2014).

Forages are rich in structural carbohydrates (NDF) that lead to high rumen pH, resulting in the preferential production of acetate over propionate. Thus, the digestibility of components of plant cell walls, such as cellulose and hemicellulose, is highly correlated with CH_4 emission because most ruminal hydrogen derived from carbohydrate fermentation and much of that generated during the conversion of hexoses into acetate or butyrate, via pyruvate, is converted to CH_4. Thus, high concentrations of acetate and butyrate, particularly from high amounts of fiber, and fractions with a low passage rate, result in increased CH_4 emissions (Nussio *et al*., 2011).

Therefore, it is possible to focus on the quality of pastures as a strategy to mitigate GHG emissions in grazing production systems. Comparing a pasture with low (10.5% CP, 62.7% NDF, and 50.3% DM digestibility) to others of a high nutritional value (22.0% CP, 41.7% NDF, and 67.3% DM digestibility) using Hereford heifers, Dini *et al*. (2017) observed higher DMI in the high-quality pasture, resulting in 11% lower emissions of CH_4 expressed per unit of DMI (g CH_4 kg DMI^{-1}).

The daily CH_4 emissions reported by Frota *et al*. (2017) evaluating Curraleiro Pé-duro × Nellore cattle in a pasture of *Megathyrsus maximus* 'Mombasa', in monoculture (192.8 g day^{-}

^{1}) or integrated with babassu palms (*Attalea* spp.) (203.3 g day^{-1}), during the rainy season were higher than our findings. However, they found lower values during the dry season (122.5 ± 4.66 g day^{-1}), which can be justified by the decrease in forage DMI as a consequence of the lower forage DM availability during the dry season. When performing a meta-analysis of studies carried out with dairy and beef cattle in feeding systems predominantly based on tropical climates, Patra (2017) reported average CH_4 emissions expressed in MJ kg DMI^{-1} and g kg DMI^{-1}, similar to those of the current study (1.04 and 19.01, respectively).

Sobrinho *et al*. (2019) found a low correlation between DMI and CH_4 emissions. However, Ellis *et al*. (2007) reported that the use of DMI in prediction equations for CH_4 emissions from cattle resulted in a lower root mean square prediction error than equations developed using metabolizable energy intake. In addition, Huhtanen *et al*. (2019) compared the GreenFeed emission monitoring (GEM), a system based on spot sampling of eructated and exhaled gasses for measurement of enteric CH_4 production, with equations predicting CH_4 production and concluded that equations based on CH_4 and DMI resulted in the smallest errors.

Although no difference between the two integrated systems was observed in terms of CPI ($P>0.05$), the CH_4 emissions per unit of this nutrient intake (MJ kg CPI^{-1}) were higher ($P<0.05$) in ICL than in ICLF. This difference can be explained by the numerically higher CP concentration in grass in the ICLF system, which may have increased microbial efficiency in the use of carbohydrates, with lower formation of enteric CH_4, which was evident by the significant negative correlation between the grass CP concentration and the CH_4 emission per kg CPI (Table 4).

Considering that CP is the nutrient that most impacts the cost of animal diets, the high levels of this nutrient in forage can be considered an interesting alternative for economic viability of the production system, since forage is considered the cheapest food source for ruminant nutrition. Moreover, when considering that the higher grass CP content led to lower CH_4 emissions in MJ kg CPI, the highest CP content in the grass can also be considered to be of environmental benefit.

The emissions of enteric CH_4 between the systems also differed in terms of energy intake. When expressed in MJ kg TDNI^{-1} and MJ kg NFCI^{-1} ($P<0.0001$), with higher values in ICLF in relation to ICL. This could be related to the higher levels of TDN and NFC in the pasture in the ICL system (Table 1), which was confirmed by the negative correlation between these variables and CH_4 emissions (Table 4). This can be attributed to the NFC, which is a rapidly degraded rumen fraction (composed of pectin, starch, and sugars) during fermentation, resulting mainly in propionate and butyrate (Nussio *et al*., 2011).

In general, crude energy losses as CH_4 by animals grazing tropical forages are approximately 6.5% (Frota *et al*., 2017). These losses, in addition to higher GHG emissions, also mean nutritional losses that consequently interfere with the efficiency of the production system. Therefore, the supply of high-quality diets that provide better microbial efficiency can be considered both environmentally and economically preferable.

As suggested by Mombach *et al*. (2016), one of the main opportunities to reduce the effect of CH_4 production by the consumption of tropical forages is through the implementation of management practices that improve the nutritional value of forage in order to generate an increase in animal performance.

The CH_4 amount produced per unit of product is reduced if the animal's production or growth the is increased (Machado *et al*., 2011). Oliveira *et al*. (2020), evaluating the GHG balance and the carbon footprint of cattle production on pasture systems in tropical climates, also showed better results for carbon footprint expressed per kg of LW gain or kg of carcass per ha in a system with intensified pastures than in degraded pastures, while considering the effects of the management practices first.

Although there was no difference in the total emission of CH_4 in g day^{-1} and MJ day^{-1}, it is important to consider that in the ICL system, each animal emitted 22.38 g CH_4 day^{-1} more than in the ICLF system. This difference represents 8,169 g CH_4 per animal per year, an environmental advantage of the ICLF system that should be considered, especially in production systems with large herds. Another point to be considered is that the ICLF system can generate carbon credits due to the trees presence, an advantage of this system, as observed by Figueiredo *et al*. (2017) when modeling three production systems (degraded pasture, well-managed pasture, and the ICLF system).

In addition, the variations observed in nutrient intake and CH_4 emission by nutrient intake between the ICL and ICLF systems are indicative of the need for further studies comparing the different integrated agricultural systems with productive, environmental, and performance variables, which provide technical evidence for the choice of sustainable production system.

5 CONCLUSION

There is no difference between integrated crop-livestock and integrated crop-livestock-forestry systems in terms of voluntary intake and methane emission by Girolando heifers grazing Xaraés palisade grass in the western Amazon. However, the methane emission per unit of crude protein ingested is higher in the system without trees; per unit energy intake is higher in the pasture integrated with eucalyptus. The enteric methane expressed per unit of nutrient intake is related with the nutritional composition of the pasture.

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