

Methane emissions by beef cattle consuming hay of varying quality in the dry forest ecosystem of Costa Rica^{☆, ☆ ☆}



J. Montenegro^{a,*}, E. Barrantes^b, N. DiLorenzo^c

^a Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria (INTA) and Instituto Meteorológico Nacional (IMN), Costa Rica

^b Universidad Técnica Nacional, Sede Atenas, Costa Rica

^c Department of Animal Sciences, North Florida Research and Education Center, University of Florida, Marianna, FL 32446-7906, USA

ARTICLE INFO

Keywords:

Methane
Costa Rica
Beef cattle
Hay quality

ABSTRACT

In livestock production systems, methane (CH₄) is produced and released during the digestive process, representing a loss of energy that can be as high as 12% of total intake. In Costa Rica there are not actual in vivo measurements of methane produced from enteric fermentation in the livestock sector. This research represents the first effort to quantify the CH₄ emitted by growing beef steers fed three different diets during the dry season in the Dry Tropics ecosystem of Costa Rica, using the SF₆ tracer technique. Three diets were evaluated, all of them offered at libitum: 1) Good quality hay of transvala (*Digitaria decumbens*; GOOD). 2) Low quality *Brachiaria tanner* hay (POOR). 3) Low quality hay (*B. tanner*) plus a supplement of 1 kg/d of sugar cane molasses mixed with 46 g/d of urea (POOR+MU). Nine Brahman steers (329 ± 38 kg of body weight) were utilized in a triplicated 3×3 Latin square for a total of 9 replicates/treatment. Variables measured were in vivo CH₄ emissions, feed intake, and apparent total tract digestibility of nutrients using indigestible neutral detergent fiber (NDF) as an internal indigestible marker. Dry matter intake (DMI) was greater in GOOD (7.9 kg/d) compared to the remaining two diets (3.6 and 4.2 kg/d for POOR and POOR+MU, respectively). Enteric CH₄ emission (g/d) was similar ($P > 0.05$) for POOR (110.4) and POOR+MU (125.8) but lower ($P < 0.0001$) than that of GOOD (181.5); when the methane emitted was reported as g of CH₄/kg of DMI, greatest ($P < 0.0001$) emissions were detected with POOR (31.0) and POOR+MU (29.8), and lesser in diet 1 (23.0 ± 1.9). Estimated methane yield (Y_m) for GOOD (6.9) was similar to that suggested by the Intergovernmental Panel on Climate Change (IPCC); however, greater values than those reported by the IPCC were obtained for POOR (9.3) and POOR+MU (9.0). In conclusion, CH₄ emitted by growing Brahman steers fed hay of varying quality was closely related to daily DMI. Furthermore, when CH₄ emission was expressed per unit of DMI, poor quality hay increased emissions intensity, regardless of supplementation with urea and molasses. Supplementing poor quality *B. tanner* hay with urea and molasses increased hay digestibility but did not alter methane emissions. Feeding good quality *D. decumbens* hay decreased CH₄ emissions (in g/kg of DMI) by 30% relative to those by steers receiving poor quality *B. tanner* hay during the dry season in Costa Rica.

1. Introduction

Methane (CH₄) is a greenhouse gas (GHG) of great importance since it has a warming potential 21 times greater than CO₂ (Intergovernmental Panel on Climate Change (IPCC), 2006) and among other sources, it is produced by enteric fermentation in ruminants. Typically, GHG emissions are expressed as CO₂ equivalent, considering the global warming potential of each of the contributing gases relative to CO₂. The release of methane that originates in the gastrointestinal tract of cattle represents a loss of energy, which can be

as high as 12% when fed low-quality forage (Johnson et al., 2007), with a suggested value of 6.5% of the gross energy ingested (IPCC, 2006).

Determining accurate emissions of CH₄, N₂O, and CO₂ in livestock systems, and their relative contribution to global GHG emissions has been a subject of active research. Herrero et al. (2011) estimated that GHG emissions from livestock contribute to between 8% and 51% of total GHG emissions, meanwhile other researchers have reported variable, but more precise values: 16% (Scheehle and Kruger, 2006), 28% (Beauchemin et al., 2008), 32% (Grainger and Beauchemin, 2011) and 18% (FAO, 2006), all expressed as CO₂ equivalent. In this sense, an

[☆] This study was funded by United Nations Program for Development (PNUD) in Costa Rica (project LECB-08317); and FITTACORI (project F010-2011).

^{☆☆} Appreciation is expressed to F. M. Ciriaco, D. D. Henry, L. Rostoll, and Jeffrey Gamboa for their assistance in data collection and sample analysis.

* Corresponding author.

E-mail addresses: jmontenegro@imn.ac.cr (J. Montenegro), ebarrantes@utn.ac.cr (E. Barrantes), ndilorenzo@ufl.edu (N. DiLorenzo).

estimation made by O'Mara (2011) for Latin America, showed that emissions from enteric fermentation in livestock represent around 24% of the global emission. This variation in estimations of the relative contribution of GHG emissions by cattle, reveal a need to obtain reliable and locally estimated measurements of methane from livestock as a basic step prior to implementing mitigation practices.

Because of the importance of livestock systems in food provisioning, and the fact that enteric CH₄ emissions represents a loss in productivity, it is imperative to develop strategies to reduce emissions without decreasing animal productivity (Eckard et al., 2010; Grainger and Beauchemin, 2011).

In Costa Rica, according to the 2010 GHG National Inventory based on IPCC methodology, from the total methane (108 Gg) emitted by the agricultural sector, livestock (and mainly enteric fermentation) is responsible for 86% of it, representing approximately 16% of the national emissions expressed as CO₂ equivalent (Chacón et al., 2014).

Worldwide, over the past years several methods have been developed with the purpose of estimating and measuring methane emissions from ruminants. Some of them include respiration chambers (Muñoz et al., 2012), in vitro systems (Eun et al., 2004; Navarro-Villa et al., 2011), simulation (Cohen et al., 2004), and prediction equations (IPCC, 2006).

The SF₆ tracer gas technique (Johnson et al., 1994) is not only widely used (Lassey, 2007), but is also one of the few techniques available to measure CH₄ emitted by individual animals under grazing or confinement conditions. This technique has been used in different countries: Canada (Chaves et al., 2006), United States (DeRamus et al., 2003; Henry et al., 2015), France (Pinares-Patiño et al., 2003), Australia (Grainger et al., 2007), and New Zealand (Pinares-Patiño et al., 2008 and 2016).

There are relatively few reported experiences in tropical countries measuring CH₄ with the SF₆ tracer technique. In Brazil, Pedreira et al. (2009) measured emissions of 179 g/d of CH₄ in crossbred (Holstein ¾ × Zebu ¼) heifers of 373 kg of body weight (BW) grazing non-fertilized *Brachiaria* spp. grass with an in vitro organic matter digestibility (IVOMD) ranging from 40.9% to 49.8%. Primavesi et al. (2004) determined CH₄ emissions that varied from 182 to 199 g/d in Holstein × Zebu crossbred heifers grazing unfertilized *Brachiaria* grass. Also in Brazil, Moysés do Nascimento (2007) determined emissions of 133, 138 and 134 g of CH₄/d in Nelore steers of 402 kg of BW consuming *Brachiaria brizantha* hay of different regrowth age (15, 45 and 90 days, respectively). In contrast, Neto et al. (2009) reported emissions of 98.5 g of CH₄/d in crossbred cattle (500 kg of BW) fed low quality *Brachiaria brizantha* hay.

In Australia, Brahman cattle fed different tropical grasses had CH₄ emissions ranging from 5.0% to 7.2% of gross energy intake; additionally, the researchers reported that methane yields per unit of dry matter intake (DMI) or digested organic matter were variable across diets, and were related to digestibility and fiber and protein concentrations (Kennedy and Charmley, 2012). These findings are in agreement with those by Chaves et al. (2006) in Canada with Black Angus heifers (380 kg of BW) grazing forages that differed in quality. Chaves et al. (2006) reported lesser emissions (113.5 g of CH₄/d) when forages had in vitro dry matter digestibility (IVDMD) of 58% compared with emissions when forage had an IVDMD of 45.5% (164.8 g CH₄/d). Also in Canada, Boadi and Wittenberg (2002) reported CH₄ emissions of 204, 207, and 145 g/d in beef Charolais-Simmental heifers (310 ± 15 kg of BW) offered high (61.5% IVOMD), medium (50.7% IVOMD) or low (38.5% IVOMD) quality diets, respectively.

Thus, great variations in the amounts of enteric CH₄ produced by livestock can be expected. Some of the variables that explain this variation are breed differences, animal characteristics such as ruminal volume, feed selection capacity, ruminal retention time of feed particles, and associations of factors linked to digestion capacity of fiber fractions in feeds (Lassey et al., 2002). Undoubtedly, based on the literature reviewed, feed characteristics play a major role in the

variations in enteric CH₄ emissions reported.

In Costa Rica there have not been actual in vivo measures of methane produced by enteric fermentation in the livestock sector. This study represents the first effort in quantifying the CH₄ emitted by beef cattle under typical feeding conditions during the dry season in Costa Rica.

Hay feeding is a common practice in beef production systems during the dry season months (December to May) in Costa Rica and other tropical regions with similar agroecosystems. As evidenced by the literature reviewed, changes in the quality of hay offered can greatly influence methane emissions. The development of national inventories of GHG emitted by the agricultural sector is part Costa Rica's commitment to report back to the United Nations Framework Convention on Climate Change. Data generated in this study will be used in future greenhouse gas inventories and prediction models, reducing the need to rely on models developed under different conditions of cattle breeds and climate that may not match those found in the tropical regions of Central America. Thus, the objective of the present study is to assess methane emissions and nutrient digestibility in Brahman steers consuming hay of varying quality, in beef production systems that match the conditions of the Dry Tropics ecosystem in Costa Rica during the dry season.

2. Materials and methods

This study was performed following the standard procedure of scientific ethics, including the use and care of experimental animals in compliance with the Animal Welfare Law No 7451 from the National Service of Animal Health (SENASA) of Costa Rica.

2.1. Experimental design and treatments

The study was conducted during July and August of 2014 at the Universidad Técnica Nacional, Sede Atenas, Costa Rica (464 m a.s.l., 9.96°N, 84.37°W). The climate at the research site was typical of a dry forest ecosystem, with an annual average rainfall of 1200 mm (concentrated from May to mid-November) and 25 °C of average temperature. The experiment involved 9 two-yr-old growing Brahman steers (average of 329 ± 38 kg of BW) in a 3×3 triplicated Latin square design with 3 experimental periods and 3 treatments. During a pre experimental phase, which lasted 8 wk, 12 steers were trained to achieve the required animal docility to be able to measure enteric methane using the SF₆ tracer technique, and 9 were finally selected to enroll in the study based on their docility. During the entire experiment the 9 steers enrolled in the study were housed in individual pens in a 20×15 m concrete-floored open barn.

Three experimental diets were offered at libitum: 1) Good quality transvala hay (*Digitaria decumbens*) with no more than 60 d of regrowth, produced under irrigation and associated with 27% perennial peanut (*Arachis pintoii*) on a total dry matter (DM) basis, as assessed by manual separation of species in two random bale samples taken during each experimental period (GOOD); 2) Poor quality Tanner hay (*Brachiaria arrecta*) produced in dryland conditions and harvested at approximately 90 d of regrowth, as typically done in Costa Rica at the end of the rainy season (POOR); and 3) POOR (same hay source used in previous treatment) plus a daily supplement of 1 kg of sugar cane molasses (as is) mixed with 46 g of urea diluted in 1 L of water and hand mixed with the hay, divided in two equal offerings (POOR +MU). Steers were fed twice daily (0800 and 1500 h), and offered hay and refusals were weighed and sampled prior to each feed offering to analyze for nutrient composition to calculate DM and nutrient intake. Each experimental period consisted of 10 d of adaptation to the diet and 4 d of sample collection to determine feed intake, apparent total tract digestibility of nutrients and in vivo methane emissions. Steers were weighed at the end of each experimental period.

2.2. Digestibility measurements

Beginning on d 10 and d 11, feed and fecal samples were collected, respectively, for 4 consecutive days to determine apparent total tract digestibility of DM, organic matter (OM), crude protein (CP), NDF, and acid detergent fiber (ADF). Feed samples were collected twice daily immediately after each hay delivery, and orts were collected prior to each feeding. All samples were stored frozen at -20°C . Fecal samples were collected twice daily at 0800 h and 1600 h from the ground, inside the pen, immediately after the animal defecated. After collection, fecal samples were stored frozen at -20°C . At the end of the experiment, hay and fecal samples were thawed and dried at 60°C for 48 h in a forced-air oven, ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm screen, and pooled within steer for further determination of nutrient content and digestibility marker concentration. Indigestible NDF (iNDF) was used as an internal indigestible marker (Cole et al., 2011; Krizsan and Huhtanen, 2013). For determination of sample DM and OM, approximately 0.5 g of hay and feces were weighed in duplicate, dried in a forced-air oven at 100°C for 24 h, and subsequently ashed at 550°C for 3 h. Approximately 0.5 g of hay and feces were weighed in duplicate inside of F57 bags (Ankom Technology Corp., Macedon, NY) and analyzed for NDF, using heat-stable α -amylase and sodium sulfite, and subsequent ADF as described by Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). Concentrations of CP in feed and feces were determined by rapid combustion using a macro elemental N analyzer (Vario Max CN, Elementar Americas Inc., Mt. Laurel, NJ) following official method 992.15 (AOAC, 1995). Concentration of iNDF in feed and feces was determined as described by Ciriaco et al. (2015), after 288 h of ruminal in situ incubation using AnkomF57 filter bags (Ankom Technology Corp.) and subsequent NDF analyses as described above.

2.3. Methane measurements

For the direct determination of produced methane the sulfur hexafluoride technique (SF_6 , Johnson et al., 1994) was implemented. Brass permeation tubes were loaded with of SF_6 gas at liquid nitrogen temperature, and incubated at 39°C to determine release rates of SF_6 by weight loss over a period of 6 weeks prior to rumen insertion. The average SF_6 release rate was 2935 ± 169 ng/min. A gas sample from the mouth was continuously collected in a polyvinyl chloride canister placed on the neck of the steers, and connected via a capillary tube connected to the mouth/nostrials area. After daily removal of the canister during the CH_4 collection period, the remaining pressure was registered, the canister was pressurized with nitrogen, and two subsamples were taken from each canister for SF_6 and CH_4 concentrations analyses. Gas samples were analyzed at the Soil Laboratory of the Instituto Nacional de Innovación y Transferencia en Tecnología Agropecuaria (INTA), using a gas chromatograph (GC, Agilent Model 7890 A; Santa Clara, CA, United States) fitted with an electron capture detector (ECD) and a flame ionization detector (FID) to determine SF_6 and CH_4 , respectively. With the known release rate of SF_6 in permeation tubes, and the measured concentrations of CH_4 and SF_6 in the canister, the CH_4 emission for each animal was calculated as the product of the permeation tube emission rate of SF_6 and the ratio of CH_4 and SF_6 in the sample.

Each collection period of CH_4 consisted of four consecutive 24-h periods (Monday through Friday) with canisters exchanged at the same time each subsequent day. Air samples were taken on a daily basis from the main corridor, located between the pens where the steers were housed, to monitor background levels of CH_4 and SF_6 during each sampling period. At the end of each experimental period, upon completion of the methane measurement phase, steers were weighed.

All nutrient content and iNDF concentration analyses in feed and feces were performed at the Animal Nutrition Laboratory located in the University of Florida - North Florida Research and Education Center.

2.4. Calculations and statistical analysis

Apparent total tract digestibility of DM, OM, CP, NDF, and ADF were calculated as follows:

$$100 - 100 \times \left[\left(\frac{\text{marker concentration in feed}}{\text{marker concentration in feces}} \right) \times \left(\frac{\text{nutrient concentration in feces}}{\text{nutrient concentration in feed}} \right) \right]$$

Data were analyzed as a triplicated 3×3 Latin square with nine replications/treatment using the MIXED Procedure of SAS (SAS Institute Inc., Cary, NC). Animal was considered the experimental unit and the model included the fixed effects of treatment, square, period within square, and animal within square. Comparisons of means were made using the PDIF option of SAS. Unless otherwise stated, results are presented as means and standard error (\pm SE). Significance was declared at $P \leq 0.05$.

3. Results

3.1. Diet chemical composition, feed intake and digestibility

Analyzed nutrient content of hay used in the experimental diets is shown in Table 1. Concentrations of NDF and ADF were decreased in GOOD compared with POOR hay, reflecting the greater nutritional quality of hay from transvala (*D. decumbens*) pastures consociated with *A. pintoi*, when compared with Tanner hay (*B. arrecta*) grown in dryland conditions. The differences in concentrations of ADF between the two types of hay used in this study was particularly contrasting (Table 1). Concentration of ADF is typically negatively correlated with potential digestibility, and this was evidenced by total tract digestibility analyses of nutrients. Again comparing hay types, the effects of the presence of 27% of DM from *A. pintoi* in the hay used in the GOOD treatment, shows the impact that a consociation of legumes and grasses can have in the overall CP concentrations of the hay (7.7 vs. 3.3 for GOOD and POOR, respectively; Table 1).

Intake of DM (either as kg/d or as % of BW), OM, NDF and ADF was greater ($P < 0.05$) for GOOD compared with the other treatments, which did not differ ($P > 0.05$) between them (Table 2). Intake of CP differed across all treatments ($P < 0.05$) and was greatest for GOOD and least for POOR (Table 2). Apparent total tract digestibility of DM was greatest ($P < 0.05$) for GOOD (63.78%) and least ($P < 0.05$) for POOR (39.29%), with POOR+MU showing intermediate values (53.26%; Table 2). Apparent total tract digestibility of OM, CP, and ADF was greater for GOOD and POOR+MU when compared with POOR ($P < 0.05$; Table 2). Digestibility of NDF differed among all three treatments ($P < 0.05$) and was greatest for GOOD (65.04%), followed by POOR+MU (56.15%) and POOR (41.86%).

Table 1
Chemical composition of the hay used in the experimental diets (DM basis).

Item	Hay type	
	GOOD ^a	POOR ^b
DM, %	86.2	85.8
OM, %	92.9	92.2
CP, %	7.7	3.3
NDF, %	62.8	72.0
ADF, %	33.3	38.4

^a Transvala hay (*Digitaria decumbens*) with no more than 60 d of regrowth, produced under irrigation and associated with 27% perennial peanut (*Arachis pintoi*) on a DM basis.

^b Tanner hay (*Brachiaria arrecta*) produced in dryland conditions.

Table 2
Effects of hay quality and supplementation with urea and molasses on dry matter and nutrient intake, and apparent total tract digestibility by Brahman steers.

Item	Treatment			SEM ⁴	P-value
	GOOD ¹	POOR ²	POOR+MU ³		
<i>Intake</i> ⁵					
DM, kg/d	7.89 ^b	3.56 ^a	4.22 ^a	0.19	< 0.001
OM, kg/d	7.12 ^b	3.32 ^a	3.93 ^a	0.18	< 0.001
CP, kg/d	0.61 ^c	0.12 ^a	0.24 ^b	0.03	< 0.001
NDF, kg/d	4.96 ^b	2.57 ^a	3.04 ^a	0.17	< 0.001
ADF, kg/d	2.63 ^b	1.37 ^a	1.63 ^a	0.09	< 0.001
DM, % of body weight	2.40 ^b	1.08 ^a	1.28 ^a	0.08	< 0.050
<i>Digestibility, %</i>					
DM	63.78 ^c	39.29 ^a	53.26 ^b	2.76	< 0.001
OM	64.62 ^b	38.28 ^a	55.14 ^b	2.79	< 0.001
CP	61.28 ^b	12.99 ^a	41.04 ^b	7.40	< 0.001
NDF	65.04 ^c	41.86 ^a	56.15 ^b	2.27	< 0.001
ADF	59.99 ^b	37.00 ^a	52.52 ^b	2.42	< 0.001

^{a,b,c}Within a row, means with different superscripts differ ($P \leq 0.05$).

¹Steers fed ad libitum amounts of transvala hay (*Digitaria decumbens*) with no more than 60 d of regrowth, produced under irrigation and associated with 27% perennial peanut (*Arachis pintoi*) on a DM basis.

²Steers fed ad libitum amounts of tanner hay (*Brachiaria arrecta*) produced in dryland conditions.

³Steers fed ad libitum amounts of tanner hay (*Brachiaria arrecta*) produced in dryland conditions plus a daily supplement of 1 kg of molasses (as is) mixed with 46 g of urea diluted in 1 L of water and hand mixed with the hay, divided in two equal offerings.

⁴Pooled standard error of treatment means, n=9 steers/treatment.

⁵Intake measured during the digestibility measurement period of the experiment.

3.2. Methane emissions

Daily enteric CH₄ emissions (g/d) were similar ($P > 0.05$) for steers consuming POOR and POOR+MU, but lesser ($P < 0.001$) than those from steers consuming GOOD hay (Table 3). When CH₄ emissions were reported relative to DMI, the inverse situation was evidenced, where the greatest ($P < 0.001$) emissions were detected in steers consuming POOR and POOR+MU vs. GOOD (Table 3). The same difference among treatments was maintained when CH₄ emissions were expressed as g/kg of DM digested.

An alternative manner to express enteric emissions is using the dimensionless “methane conversion factor” which is also known as “methane yield” (Y_m). This is a ratio that takes into consideration the methane emitted per unit of feed intake when both variables are expressed in terms of energy of combustion, and is expressed as a percentage of the gross energy (GE) consumed (Lassey, 2007). As an example, a typical Y_m value of 6.5% was suggested by the IPCC (2006)

Table 3
Effects of hay quality and supplementation with urea and molasses on methane emitted by Brahman steers.

Item	Treatment			SEM ⁴	P-value
	GOOD ¹	POOR ²	POOR+MU ³		
CH ₄ , g/d	181.5 ^b	110.4 ^a	125.8 ^a	5.6	< 0.050
CH ₄ , g/kg DMI	23.0 ^a	31.0 ^b	29.8 ^b	1.9	< 0.050
CH ₄ , g/kg DM digested	36.1 ^a	78.9 ^b	56.0 ^c	4.0	< 0.050
CH ₄ , % of GE intake (Y _m)	6.9 ^a	9.3 ^b	9.0 ^b	0.6	< 0.050

^{a,b}Within a row, means with different superscripts differ ($P \leq 0.05$).

¹Steers fed ad libitum amounts of transvala hay (*Digitaria decumbens*) with no more than 60 d of regrowth, produced under irrigation and associated with 27% perennial peanut (*Arachis pintoi*) on a DM basis.

²Steers fed ad libitum amounts of tanner hay (*Brachiaria arrecta*) produced in dryland conditions.

³Steers fed ad libitum amounts of tanner hay (*Brachiaria arrecta*) produced in dryland conditions plus a daily supplement of 1 kg of molasses (as is) mixed with 46 g of urea diluted in 1 L of water and hand mixed with the hay, divided in two equal offerings.

⁴Pooled standard error of treatment means, n=9 steers/treatment.

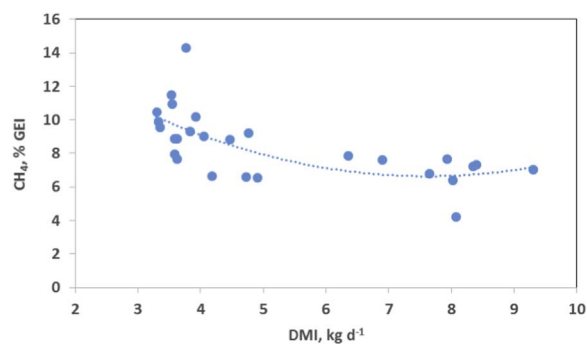


Fig. 1. Relationship between methane emitted as percent of gross energy intake (% GEI) and dry matter intake (DMI, kg/d) in Brahman steers consuming hay of varying qualities. CH₄ emission (% GEI) = 0.1893 × DMI² - 2.881 × DMI + 17.605 ($r^2 = 0.19$, $P < 0.001$).

for enteric CH₄ emitted by bovines in tropical countries, corresponding to 21.45 g of CH₄/kg of DMI. In this study, according to the aforementioned and considering the emission of CH₄ as percent of total GE consumed, the least ($P < 0.001$) estimated Y_m value was observed with GOOD (6.9%), when compared with POOR (9.3%) and POOR+MU (9.0%), which did not differ between them ($P > 0.05$; Table 3).

Across treatments, there was a linear and positive ($r = 0.62$, $P < 0.001$) relationship [CH₄ emission (g/d) = 15.096 × DMI (kg/d) + 59.917] between daily CH₄ emissions expressed as g/d, and DMI expressed as kg/d, (data not shown). Regarding enteric CH₄ emitted as percentage of GE intake (GEI), a polynomial relationship ($r^2 = 0.19$, $P < 0.001$) was detected with DMI expressed as kg/d, given by the following equation: CH₄ emission (% GEI) = 0.1893 × DMI² - 2.881 × DMI + 17.605 (Fig. 1).

4. Discussion

The results observed in terms of DMI in the present study, are in agreement with the findings on apparent total tract OM digestibility, in which steers consuming POOR hay had decreased OM digestibility when compared with POOR+MU and GOOD. This reduction in magnitude of OM consumed was of 39.9%, which is in agreement with the 36.1% reduction in OM digestibility observed between the same treatments (POOR vs. the average of the other treatments). Thus, the decrease in intake of poor quality hay in this study was directly proportional to the depression in digestibility, being most likely explained by the accumulation of undigested hay in the reticulo-rumen. When comparing the nutrient quality of the three forages offered in the study (Table 1) with the nutrient requirements of growing cattle, it becomes evident that POOR hay was likely limiting in both energy and protein. The addition of 1 kg/d of molasses and 46 g/d of urea was, apparently, not sufficient to overcome these limitations. When calculating the CP concentration in the POOR+MU diet (using the average DMI of POOR for all periods and analyzed CP values for urea and molasses), the dietary CP concentration only increases from 3.3% (POOR; Table 1) to 5.8% (POOR+MU; data not shown). The latter, is yet considered a limiting amount of CP for ruminal fermentation in most beef cattle production scenarios (Kunkle et al., 2000). Ciriaco et al. (2015) showed an increased digestion in apparent total tract digestibility of OM when bermudagrass (*Cynodon dactylon*) hay was supplemented with 1.36 kg/d of a 50:50 mixture of crude glycerol: molasses in beef heifers. However, no response in OM digestion was observed when 0.45 kg/d of the same liquid supplement was fed in addition to hay (Ciriaco et al., 2015). The amount of molasses supplemented in the POOR+MU diet in this study may have not been sufficient, based on previous findings (Moore et al., 1999; Kunkle et al., 2000; Ciriaco et al., 2015), to elicit a response in terms of increased OM digestibility.

Enteric CH₄ emissions in livestock systems can be reported in several ways, including g/d, g/kg of DMI, relative to product output (e.g., kg of BW gained or milk produced), or as energy loss as percentage of the gross energy consumed. Considering the challenges that lie ahead in terms of global food production, there is consensus in the scientific community that future strategies to mitigate emissions should focus on productivity of the systems. Thus, increasing animal productivity can be one of the more successful strategies to decrease emissions intensity (Hristov et al., 2013). In the present study, improving the quality of the diets offered, resulted in more efficient use of consumed energy and that is reflected in the Y_m values obtained (Table 3). In addition, the improvement in the quality of the type of forage offered (*D. decumbens* vs. *B. arrecta*), resulted in greater feed intakes and decreased CH₄ emissions (Tables 2, 3). The inclusion of urea and molasses supplementation in diets based on poor quality *B. arrecta* hay had no effect on methane emissions. However, nutrient digestibility was greatly improved, proving to be a useful management practice to increase the productivity of beef production systems that rely on hay feeding.

The greater amount of CH₄ produced per day in steers consuming GOOD hay when compared with the other two treatments, was directly related to the increased DMI (Table 2), likely as a result of the improved nutritional quality and digestibility of this diet. This is in agreement with what has been reported elsewhere under confinement or grazing conditions (McGeough et al., 2010). Digestibility in particular, has been positively related with feed intake (Kurihara et al., 1999; Boadi and Wittenberg, 2002; Pinares-Patiño et al., 2016), and methane emissions (Boadi and Wittenberg, 2002; Archimède et al., 2011; Cota et al., 2014). Typically a greater production of enteric methane is observed with diets of increased digestibility, because they provide greater quantities of fermentable substrates for methanogenic archaea. This well documented relationship between digestibility, intake and methane emissions was also evidenced in the present study.

The emissions of CH₄ observed in this study were similar to those reported in Latin America by Pedreira et al. (2009), Moysés do Nascimento (2007), and Primavesi et al. (2004), and also to results obtained in more temperate regions (McGinn et al., 2004; Chaves et al., 2006). The type of hay fed in the POOR treatment in the present study was chosen to represent the typical management of beef cattle production systems in the dry forest ecosystem of Costa Rica during the summer time (December to May). This is a time of the year in which forage availability is reduced because of dry climatic conditions, lasting approximately six months in Costa Rica, which represents a large proportion of the annual beef production cycle. Thus, if the methane emission measured when feeding POOR hay is considered as typical during the summer months for this animal category, the diets fed with GOOD and POOR+MU treatments would produce approximately 64% and 14% more enteric methane expressed as g/d, respectively, than feeding POOR hay. Thus, results expressed as g/d of CH₄ emitted can be misleading because animal productivity is not being fully considered, since GOOD and POOR+MU represent an increase in nutritional value over POOR hay. Therefore, to avoid the adoption of management practices that are detrimental to the productivity of the system, as discussed above, it is useful to express CH₄ emissions relative to a unit of productivity or, at the very least, relative to feed intake.

The efficiency in the use of diet energy, and the amount of CH₄ emitted per kg of feed consumed are ways to express emissions relative to animal performance. The values of these relationships observed in the current study are in agreement with what has been reported elsewhere (Chaves et al., 2006; McGeough et al., 2010; Clark et al., 2011). Therefore, while greater CH₄ emissions can be expected as a result of increased DMI because of improved digestibility, also an improvement in animal performance increases, which in turn decreases emissions intensity, this is, emissions per unit of product (Hristov et al., 2013; Pinares-Patiño et al., 2016).

A study conducted by Morales González (2007) in Costa Rica evaluated a similar hay to that offered in GOOD in the current

experiment. When, calculating the emissions intensity using animal performance data from Morales González (2007), feeding GOOD hay in this study would decrease emissions intensity by 34% (meaning less CH₄ per kg of BW gained) when compared to feeding POOR hay, which would be considered typical in Costa Rican production systems during the dry season.

When expressed as CH₄ yield (Y_m), emissions measured in our study are in agreement with those reported previously (Kurihara et al., 1999; Primavesi et al., 2004; Kennedy and Charmley, 2012). Furthermore, the Y_m value obtained for steers fed GOOD hay (Table 3) was similar to that suggested by IPCC (2006; 6.5 ± 1.0%) for CH₄ emitted by bovines in tropical countries. Methane yield (Y_m) declined with increasing level of intake. Thus, maximizing intake in growing cattle is expected to improve the efficiency of feed conversion because CH₄ losses, as a proportion of the energy consumed, are reduced with increased intake. It is important to take into consideration that this research resembles only the summer conditions in the Pacific North of Costa Rica. Thus, there is a need to adjust this value to the average annual climatic condition of this zone in order to obtain a more realistic estimation for the whole year.

Despite the fact that the conditions evaluated in this study represent only a fraction of the livestock systems in Costa Rica during an entire year, CH₄ emissions obtained in this study with GOOD hay extrapolated to a year (66.2 kg/year) would be 18% greater than the emission factor suggested by the Tier 1 IPCC methodology (56 kg/year). Conversely, steers fed for POOR hay and POOR+MU, had yearly calculated CH₄ emissions that were 28% and 18% lesser, respectively, than those from Tier 1 of IPCC. This demonstrates the importance of obtaining reliable measurements of CH₄ emissions obtained locally, under the conditions in which the livestock activity in each region is developed, reducing the uncertainty when conducting national level inventories of greenhouse gases by the livestock production sector.

5. Conclusion

Emissions of CH₄ obtained from Brahman steers under management conditions typical of the dry forest ecosystem of Costa Rica during the summer time, were closely related to the daily feed intake which is, in turn, intimately associated with forage quality and digestibility. While the addition of urea and molasses improved the digestibility of poor quality tanner hay (*Brachiaria arrecta*), this was not sufficient to offset the increased CH₄ emissions intensity (g/kg of DMI) when compared with good quality transvala hay (*Digitaria decumbens*). Thus, feeding good quality hay during the summer months of the dry forest ecosystem of Costa Rica can lead to reductions of 30% in CH₄ emissions intensity, representing an interesting management tool to decrease the carbon footprint of livestock systems in tropical regions.

Estimations of CH₄ yield (Y_m) by the IPCC Tier 1 methodology for tropical regions were similar to those observed with good quality hay in this study; however, the IPCC methodology underestimated CH₄ emissions when poor quality hay was fed. This study represent the first effort to successfully report in vivo CH₄ emissions from livestock systems in Costa Rica, and the data generated are of great importance for the development of national inventories of GHG emissions.

Conflict of interest

We confirm that there are no conflicts of interest associated with this publication.

References

- AOAC, 1995. Official Method of Analysis 16th ed.. Association of Official Analytical Chemists, Arlington, VA.
- Archimède, H., Eugène, M., Magdeleine, C., Boval, M., Martin, C., Morgavi, D.,

- Lecomte, P., Doreau, M., 2011. Comparison of methane production between C3 and C4 grasses and legumes. *Anim. Feed Sci. Technol.* 166–167, 59–64.
- Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A., 2008. Nutritional management for enteric methane abatement: a review. *Aust. J. Exp. Agric.* 48, 21–27.
- Boadi, D.A., Wittenberg, K.M., 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF₆) tracer gas technique. *Can. J. Anim. Sci.* 82, 201–206.
- Chacón, A., Jiménez, G., Montenegro, J., Sasa, J., Blanco, K., 2014. Inventario nacional de emisión de gases con efecto invernadero y de absorción de carbono en Costa Rica en el 2010. MINAE-IMN, p. 64.
- Chaves, A.V., Thompson, L.C., Iwaasa, A.D., Scott, S.L., Olson, M.E., Benchaar, C., Veira, D.M., McAllister, T.A., 2006. Effect of pasture type (alfalfa vs. grass) on methane and carbon dioxide production by yearling beef heifers. *Can. J. Anim. Sci.* 86, 409–418.
- Ciriaco, F.M., Henry, D.D., Mercadante, V.R.G., Schulmeister, T., Ruiz-Moreno, M., Lamb, G.C., DiLorenzo, N., 2015. Effects of different levels of supplementation of a 50:50 mixture of molasses: crude glycerol on performance, Bermuda grass hay intake, and nutrient digestibility of beef cattle. *J. Anim. Sci.* 93, 2428–2438.
- Clark, H., Kelliher, F., Pinares-Patiño, C., 2011. Reducing CH₄ emissions from grazing ruminants in New Zealand: challenges and opportunities. *Asian-Aust. J. Anim. Sci.* 24, 295–302.
- Cohen, R.D., Stevens, J.P., Moore, A.D., Donnelly, J.R., Freer, M., 2004. Predicted methane emissions and metabolizable energy intakes of steers grazing a grass/alfalfa pasture and finished in a feedlot or at pasture using the GrassGro decision support tool. *Can. J. Anim. Sci.* 84, 125–132.
- Cole, N.A., McCuiston, K., Greene, L.W., McCollum, F.T., 2011. Effects of concentration and source of wet distillers grains on digestibility of steam-flaked corn-based diets fed to finishing steers. *Prof. Anim. Sci.* 27, 302–311.
- Cota, O., de Figueiredo, D., Branco, R., Magnani, E., do Nascimento, C., de Oliveira, L., Mercadante, M., 2014. Methane emission by Nelore cattle subjected to different nutritional plans. *Trop. Anim. Health Prod.* 46, 229–234.
- DeRamus, H.A., Clement, T.C., Giampola, D.D., Dickison, P.C., 2003. Methane emissions of beef cattle on forages: efficiency of grazing management systems. *J. Environ. Qual.* 32, 269–277.
- Eckard, R.J., Grainger, C., de Klein, C.A.M., 2010. Options for the abatement of methane and nitrous oxide from ruminant production: a review. *Livest. Sci.* 130, 47–56.
- Eun, J.S., Fellner, V., Gumpertz, M.L., 2004. Methane production by mixed ruminal cultures incubated in dual-flow fermentors. *J. Dairy Sci.* 87, 112–121.
- FAO, 2006. *Livestock's Long Shadow. Environmental Issues and Options.* Food and Agriculture Organization of the United Nations, Rome, Italy.
- Grainger, C., Beauchemin, K.A., 2011. Can enteric methane emissions from ruminants be lowered without lowering their production? *Anim. Feed Sci. Technol.* 166–167, 308–320.
- Grainger, C., Clarke, T., McGinn, S.M., Auldist, M.J., Beauchemin, K.A., Hannah, M.C., Waghorn, G.C., Clark, H., Eckard, R.J., 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF₆) tracer and chamber techniques. *J. Dairy Sci.* 90, 2755–2766.
- Henry, D.D., Ruiz-Moreno, M., Ciriaco, F.M., Kohmann, M., Mercadante, V.R.G., Lamb, G.C., DiLorenzo, N., 2015. Effects of chitosan on nutrient digestibility, methane emissions, and in vitro fermentation in beef cattle. *J. Anim. Sci.* 93, 3539–3550.
- Herrero, M., Gerber, P., Vellinga, T., Garnett, T., Leip, A., Opio, C., Westhoek, H.J., Thornton, P.K., Olesen, J., Hutchings, N., Montgomery, H., Soussana, J.-F., Steinfeld, H., McAllister, T.A., 2011. Livestock and greenhouse gas emissions: the importance of getting the numbers right. *Anim. Feed Sci. Technol.* 166–167, 779–782.
- Hristov, A.N., Ott, T., Tricarico, J., Rotz, A., Waghorn, G., Adesogan, A., Dijkstra, J., Montes, F., Oh, J., Kebreab, E., Oosting, S.J., Gerber, P.J., Henderson, B., Makkar, H.P.S., Firkins, J., 2013. Mitigation of methane and nitrous oxide emissions from animal operations: III. A review of animal management mitigation options. *J. Anim. Sci.* 91, 5095–5113.
- Intergovernmental Panel on Climate Change (IPCC), 2006. *Climate Change 2006: The Scientific Basis.* Cambridge University Press, Cambridge, UK.
- Johnson, K.A., Huyler, M.T., Westberg, H.H., Lamb, B.K., Zimmerman, P., 1994. Measurement of methane emissions from ruminant livestock using a SF₆ tracer technique. *Environ. Sci. Technol.* 28, 359–362.
- Johnson, K.A., Westberg, H., Michal, J., Cossalman, M., 2007. The SF₆ tracer technique: methane measurement from ruminants. In: Makkar, H.P.S., Vercoe, P.E. (Eds.), *Measuring Methane Production from Ruminants*, 33–67.
- Kennedy, P.M., Charmley, E., 2012. Methane yields from Brahman cattle fed tropical grasses and legumes. *Anim. Prod. Sci.* 52, 225–239.
- Krizsan, S.J., Huhtanen, P., 2013. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. *J. Dairy Sci.* 96, 1715–1726.
- Kunkle, W.E., Johns, J.T., Poore, M.H., Herd, D.B., 2000. Designing supplementation programs for beef cattle fed forage-based diets. *J. Anim. Sci.* 77, 1–11.
- Kurihara, M., Magner, T., Hunter, R.A., McCrabb, G.J., 1999. Methane production and energy partition of cattle in the tropics. *Br. J. Nutr.* 81, 227–234.
- Lassey, K.R., 2007. Livestock methane emission: From the individual grazing animal through national inventories to the global methane cycle. *Agric. For. Meteorol.* 142, 120–132.
- Lassey, K.R., Pinares-Patiño, C.S., Ulyatt, M.J., 2002. Methane emission by grazing livestock: some findings on emission determinants. In: Ham, J., van; Baede, A.P., Guicherit, R., Williams-Jacobse, J.G. (Eds.), *Non-CO₂ Greenhouse Gases: Scientific Understanding, Control Options and Policy Aspects.* Millpress, Rotterdam, 95–100.
- McGeough, E.J., O'Kiely, P., Hart, K.J., Moloney, A.P., Boland, T.M., Kenny, D.A., 2010. Methane emissions, feed intake, performance, digestibility and rumen fermentation of finishing beef cattle offered whole-crop wheat silages differing in grain content. *J. Anim. Sci.* 88, 2703–2716.
- McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto, D., 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. *J. Anim. Sci.* 82, 3346–3356.
- Moore, J.E., Brant, M.H., Kunkle, W.E., Hopkins, D.I., 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. *J. Anim. Sci.* 77, 122–135.
- Morales González, J., 2007. Comparación de la calidad del heno de pasto Transvala (*Digitaria decumbens* cv. Transvala) producido bajo riego, heno de pasto Transvala comercial y pajas de paja de arroz mediante la ganancia de peso de toros establecidos. *Alcances Tecnológicos* 1, 27–36.
- Moyes do Nascimento, C.F., 2007. Emissão de metano por bovinos Nelore ingerindo *Brachiaria brizantha* em diferentes estádios de maturação (Emissions of methane from Nelore cattle consuming *Brachiaria brizantha* in different stages of maturity). Dissertation (MSc) Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Nutrição e Produção. Animal, 67.
- Muñoz, C., Yan, T., Wills, D.A., Murray, S., Gordon, A.W., 2012. Comparison of the sulfur hexafluoride tracer and respiration chamber techniques for estimating methane emissions and correction for rectum methane output from dairy cows. *J. Dairy Sci.* 95, 3139–3148.
- Navarro-Villa, A., O'Brien, M., Lopez, S., Boland, T.M., O'Kiely, P., 2011. Modifications of a gas production technique for assessing in vitro rumen methane production from feedstuffs. *Anim. Feed Sci. Technol.* 166–167, 163–174.
- Neto, G.B., Berndt, A., Nogueira, J.R., Demarchi, J.J., Nogueira, J.C., 2009. Monensin and protein supplements on methane production and rumen protozoa in bovine fed low quality forage. *South African J. Anim. Sci.* 39, 280–283.
- O'Mara, F.P., 2011. The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. *Anim. Feed Sci. Technol.* 166–167, 7–15.
- Pedreira, M., Primavesi, O., Aparecida Lima, M., Frighetto, R., de Oliveira, G.S., Berchielli, T.T., 2009. Ruminant methane emission by dairy cattle in Southeast Brazil. *Sci. Agric. (Piracicaba, Braz.)* 66, 742–750.
- Pinares-Patiño, C.S., Baumont, R., Martin, C., 2003. Methane emissions by Charolais cows grazing a monospecific pasture of timothy at four stages of maturity. *Can. J. Anim. Sci.* 83, 769–777.
- Pinares-Patiño, C.S., Machmüller, A., Molano, G., Smith, A., Vlaming, J.B., Clark, H., 2008. The SF₆ tracer technique for measurements of methane emission from cattle effect of tracer permeation rate. *Can. J. Anim. Sci.* 88, 309–320.
- Pinares-Patiño, C.S., Franco, F.R., Molano, G., Kjestrup, H., Sandoval, E., MacLean, S., Battistotti, M., Koolaard, J., Laubach, J., 2016. Feed intake and methane emissions from cattle grazing pasture sprayed with canola oil. *Livest. Sci.* 184, 7–12.
- Primavesi, O., Shiraiishi, R., dos Santos, M., de Lima, M., Berchielli, T., Barbosa, P., 2004. Metano entérico de bovinos leiteiros em condições tropicais brasileiras. *Pesq. agropec. Bras.* 39, 277–283.
- Scheehle, E.A., Kruger, D., 2006. Global anthropogenic methane and nitrous oxide emissions. *Energy J.* 3, 33–44, (special issue).
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597.