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Concentrate supplementation on milk yield, methane and CO_2 production in crossbred dairy cows grazing in tropical climate regions

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Abstract The objective of this study was to evaluate the level of concentrate supplementation on the production and chemical composition of milk from 12 crossbred F1 dual-purpose cows (½ *Bos taurus* – ½ *Bos indicus*) and estimate the emission of CH_4, N_2O, and CO_2 gases. The study included 12 crossbred F1 dual-purpose cows over 60 days of lactation. The cows grazed on 28% tropical native grassland and 72% *Brachiaria spp.* and *Cynodon neumfluensis,* supplemented with 0, 150, 300, and 450g of concentrate per kg daily milk production, during three experimental periods of 15 days each in a crossover design. Pasture and concentrate samples were collected and were analyzed for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber. Milk production (kg d^{-1}) was recorded daily, nitrous oxide (N_2O), and emissions from excreta and daily CH_4 production were calculated. Results were analyzed with the SAS MIXED procedure. Concentrate supplementation in tropical crossbred dairy cows did not improve milk yield but increased CH_4 and N_2O production ($P < 0.0001$) per cow as the concentrate increased in the diet; the Ym factor from the tropical region yielded less CH_4 than the IPCC Ym model ($P < 0.0001$). In conclusion, the calculation of CH_4 using specific emission factors for the tropical climate region is better than the IPCC default emission factors in order not to overestimate the CH_4 emissions.

Keywords crossbreed, N_2O, supplementation, tropical pastures

1. Introduction

Livestock and agriculture are intensifying and expanding to meet the growing global demand for food (Foresight 2011); cow's milk production systems are responsible for producing 4–19% of global food protein (FAO 2019); however, they also emit 14% of anthropogenic greenhouse gas (GHG) emissions (Smith et al 2007).

The main sources of GHG emitted from dairy farms are methane (CH_4) from enteric fermentation and nitrous oxide (N_2O), which is eliminated through feces and urine (Selbie et al 2015). CH_4 and N_2O have 28 and 298 times the global warming potential of carbon dioxide (CO_2), respectively (IPCC 2006).

In this sense, animal feed plays an important role in the sustainability of livestock production systems by affecting GHG emissions, since quality and excesses increase this emission (Makkar 2013). Therefore, it is a challenge to develop methodologies to estimate and monitor GHG emissions and then develop mitigation strategies (Norse 2012).

Currently, IPCC (2006) provides methodologies based on default values to estimate GHG emissions. However, the IPCC mentions the importance of using country-specific emission factors, since using default emission factors may lead to an overestimation of the inventories of these gases (Niu et al 2018; Ledgard et al 2020).

The Food and Agriculture Organization of the United Nations (FAO), in consultation with an expert group, developed Sustainable Animal Diets (STAnD), which aim to reduce the adverse impacts of animal diets and to provide a framework for introducing changes in livestock systems practices (Makkar and Ankers 2014), including minimizing GHG emissions, the use of local native resources, and diminishing the number of grains in animal feeds.

In tropical and sub-tropical climate regions milk production is based on crossbred dual-purpose cows (about 22.1% crossbred), which produce 48% of the world's milk

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(CVS 2016). Thus 40% of milk production in Mexico is based on crossbred dual-purpose cows from the tropics and is usually supplemented with grain cereal-based concentrates (SAGARPA 2016). However, the effect of milk production with concentrate supplementation and the environmental impact caused must first be evaluated, to suggest mitigation strategies appropriate to production systems in this region. The objective of this study was to evaluate the level of concentrate in the production and chemical composition of milk from 12 crossbred F1 dairy cows (1/2 Bos taurus – 1/2 Bos indicus) and estimate the emissions of CH_4, N_2O, and CO_2 using the Tier II methodology (IPCC 2006), in addition to using a calculated Ym factor in the tropical climate region (Montoya-Flores et al 2020) and comparing the emission values.

2. Materials and Methods

2.1. Experimental site

The study was conducted at the Center for Teaching, Research and Extension in Tropical Livestock Production (CEIEGT), of the Faculty of Veterinary Medicine and Animal Science, Universidad Nacional Autonoma de Mexico, located in the municipality of Tlapacoyan, Veracruz (20 ° 04' north latitude and 97 ° 03 west longitude), at an altitude ranging from 99 to 123 m above sea level, an average annual temperature of 24.5°C and average annual rainfall 1991±352 mm (Castillo et al 2005).

2.2. Animals and diets

Twelve crossbred F1 dairy cows (*Bos taurus – Bos indicus*), with 60 ± 6 days in milk and average milk production of 8.414 kg/day were used. All animals were grazing on swards of mixed grasses, composed of 72% native species (*Paspalum notatum, P. conjugatum, Axonopus affinis, Desmodium triflorum*) and 28% introduced species (*Brachiaria Humidicola, Cynodon niemfluensis, Brachiaria brizantha var. Toledo, Brachiaria decumbens, Digitaria decumbens, Brachiari brizanta var. Marandu*). Besides, the cows were randomly grouped and their diets supplemented (0, 150, 300, and 450 g DM/kg milk production) with commercial dairy concentrate (ABATEZ®, 18.3% CP, 7.11 MJ ME/kg DM). The concentrate was offered during milking time (10:00 hours), in three experimental periods of 15 days each in a crossover design. Table 1 shows the chemical composition of the pastures and the concentrate provided in the diets.

Table 1 Chemical composition (g/kg DM) of the meadow pasture and concentrate, supplemented in crossbreed F1 dairy cows in tropical areas.

	DMœ	OM	CP	NDF	ADF	ADL	GE MJ/kg
Meadow pasture	200	910	109	724	385	56	16.44
Concentrate*	888	889	183	374	262	90	18.74

œDM g/kg, expressed as fresh matter, Commercial Dairy Concentrate (ABATEZ®): 0, 150, 300 and 450g DM per cow, per day per liter produced of milk, supplied during milking time (10:00 h); DM, dry matter; OM, Organic matter; CP, Crude Protein NDF, Neutral detergent fiber analyzes; ADF, acid detergent fiber; ADL, Acid detergent lignin; GE, gross energy (MJ d^{-1})

2.3. Sampling and analysis

Fifteen grass samples (1 kg on a wet basis) were obtained per period, through the manual mimicry technique of sharecropping (Wallis De Vries, 1995), and 15 samples of concentrate (0.5 kg) per period, of which pools of 5 samples representing one sward (paddock) or concentrate batch per treatment and period were made, obtaining nine grass samples and nine concentrate samples. The samples were analyzed (AOAC 1990) for dry matter (DM, method 934.01), ash (method 942.05), nitrogen (N, method 954.01), and ether extract (EE, method 920.39). Analysis of neutral detergent fiber (NDF) (Van Soest et al 1991), acid detergent fiber (ADF), and lignin (ADL) (AOAC 1990), method 973.18) were performed in an ANKOM200 Fiber Analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA), following the method 973.18 (AOAC 1990). However, for NDF the analysis was without the use of an alpha-amylase but with sodium sulfite in the NDF.

In the last five days of each experimental period, the daily milk production per animal (kg d^{-1}) was recorded, and a 200 mL sample was taken to determine its chemical composition, utilizing an automatic lactose analyzer SL60 (Bulgaria). The contents (g/100 g) of protein, fat, non-fat solids, lactose, density (kg/m^3), and salts (minerals) were determined.

2.4. Estimations

Dry matter intake (DMI) was estimated through the equation (NRC 2001):

$$\mathrm{DMI}\left(\frac{\mathrm{kg}}{\mathrm{d}}\right) = 0.372 * \mathrm{FCM} + 0.0968 * \mathrm{LW}^{\wedge 0.75} * \left(1 - \mathrm{e}^{(-0.192*(\mathrm{WL}+3.67))}\right) \quad (1)$$

where FCM (kg d^{-1}) is the fat corrected milk 3.5% of fat content, LW is the live weight of the animal (kg), WL is the week of lactation.

$$3.5\ \%\ \mathrm{FCM}(\mathrm{kg\ d^{-1}}) = (\mathrm{kg\ milk} * 0.432) + [(\mathrm{kg\ fat\ d^{-1}}) * 16.23] \quad (2)$$

Total digestible nutrients (TND) were calculated from the acid detergent fiber content of diet (ADF, g/kg DM) (Van Soest et al 1991):

$$\mathrm{TND} = 88.9 - (\mathrm{ADF} * 0.779) \quad (3)$$

The net energy for maintenance (NE_m, Mcal/kg DM), weight gain (NE_g), and lactation (NE_l) were determined using the NRC (2001) equations using the TND:

$$\mathrm{NE_m} = \left((\mathrm{TND} * 0.01318) - 0.132\right) * 2.2 \quad (4)$$

$$NE_g = ((TND * 0.01318) - 0.046) * 2.2 \quad (5)$$

$$NE_l = ((TND * 0.01114) - 0.054) * 2.2 \quad (6)$$

The nitrogen balance was calculated according to the following formula:

Nitrogen balance (g d^{-1}) = N intake (g d^{-1}) - [N feces (g d^{-1}) + N urine (g d^{-1})] (7)

The following equations were used to estimate the nitrogen excreted in feces (N feces), urine (N urine), and milk (N milk) (Jones 1931; Ramin and Huhtanen 2013):

$$N_{feces} = \text{Crude Protein excreted in feces}(g\ d^{-1})/6.25 \quad (8)$$

$$N_{urine} = \text{Crude Protein excreted in urine}(g\ d^{-1})/6.25 \quad (9)$$

$$N_{milk} = \text{crude Protein excreted in milk } (g\ d^{-1}) / 6.25 \quad (10)$$

Crude protein excreted in urine was estimated using the urinary energy (UE) (Jones 1931; Ramin and Huhtanen 2013):

$$[UE(MJ\ d^{-1})/9 * 1000] \quad (11)$$

The excretion of urinary energy (UE) was calculated from:

$$UE\ (MJ\ d^{-1}) = [-2.71 + (0.028 * (\text{Total CP intake it / DMI, kg } d^{-1}) + (0.589 * DMI, Kg\ d^{-1})] \quad (12)$$

$$NBalance = Nintake - (Nfeces + Nurine) \quad (13)$$

$$N\ intake = \text{Total Crude Protein intake} / 6.25 \quad (14)$$

Enteric methane (CH_4) production was calculated from the gross energy (GE) intake (MJ/ head d^{-1}) from concentrate and grass intake. Daily methane production was calculated based on the IPCC (2006) equation:

$$CH_4 = \frac{GE*\left(\frac{Ym}{100}\right)}{55.65} \quad (15)$$

where CH_4: methane emission (g /head d^{-1}), GEI: gross energy intake (MJ/head d^{-1}) and Ym is the percentage of GEI converted to methane. Two values of Ym were used in this study: Ym of 6.5% proposed by IPCC (2006) and Ym of 5.54% reported by Montoya-Flores et al (2020) in tropical regions:

Ym = methane conversion factor

$Ym = (6.5\%\ \text{of GEI})$ (IPCC 2006) (16)

$Ym = (5.54\%\ \text{of GEI})$ (Montoya-Flores et al 2020) (17)

The constant 55.65 (MJ/ kg CH_4 d^{-1}) is the energy content of methane:

DE: digestible energy(% of GE) (18)

where DE = GEI x digestibility energy.

Kilograms of methane per day were expressed as kg CH_4 divided by kg of milk produced (FCM 3.5%).

Kg CH_4/ kg milk (FCM 3.5%)

Carbon dioxide (CO_2) was estimated from the grams of CH_4 (IPCC 2006), as follows:

$$CO2 = g\ CH_4\ d^{-1} * 28 \quad (19)$$

The IPCC (2006) equation was used to estimate Nitrous oxide (N_2O) emissions from excreta. The amount of excreted nitrogen (Nex) was based on CP intake and dry matter digestibility (DMD), calculated with the following model:

$$Nex = (PCintake/6.25) * (1 - DMD/100) \quad (20)$$

Enteric CH_4 and nitrous oxide (N_2O) emissions were converted to CO_2 equivalents (CO_2-eq) using the global warming potential of 28 and 298, respectively IPCC (2007).

2.5. Statistical analysis

For the chemical and energy composition of the diets, milk production, N_2O, and CO_2 were analyzed using the General Lineal Models, using the SAS program (1990), according to the following model:

$Y_{ijk} = \mu + Tx_i + P_j + C_k + \varepsilon_{ijk}$

where Y_{ijk} = is each treatment observation; μ is the overall average; Tx_i is the treatment effect (i = 4), P_j is the effect due to the period (j=3), C_k is the effect due to the animal (k=12) and εijk is experimental error.

The CH_4 production data were analyzed by the GLM procedure, with the SAS program (SAS 1990) according to the model:

$Y_{ij} = \mu + M_i + Tx_j + (M_i \times Tx_j) + \varepsilon_{ij}$

where: Yijk=CH_4 output, μ= is the overall average, M (i=2) is the effect of the model used, Tx (j =4) is the treatment effect, M (i=2) x Tx (j=4) is the effect of the interaction between the model and the treatment used; and εij is experimental error. The averages of each variable ($P < 0.05$) were compared with Tukey's test.

Milk production, CH_4 N_2O, CO_2 means were subjected to trend analysis using orthogonal polynomials (Cochran and Cox 1992). Effects were considered significant if they were less than $P < 0.05$, using the Tukey's test for comparison of means.

3. Results

3.1. Diets

Table 2 shows the estimated DM and nutrient intakes for each treatment. The metabolic live weight ($LW^{0.75}$) of the cows was similar ($P > 0.05$) between treatments. The dietary content of CP, ADL, and energy intake increased ($P < 0.05$) with concentrate supplementation levels increased, and NDF and ADF intake decreased ($P < 0.05$), respectively.

3.2. Production and chemical composition of milk

Milk production and quality are presented in Table 3. No differences ($P > 0.05$) were observed between treatments in terms of milk production and chemical composition and FCM 3.5%. Similarly, feed intake (kg DM d^{-1}) was no different ($P > 0.05$).

3.3. Nitrogen digestibility, balance and metabolism

Nitrogen intake, N excretion, as well as the N balance, showed linear increases ($P < 0.05$) with concentrate supplementation (Table 4). Supplementing cows with 450g concentrate/kg increased ($P < 0.05$) N excretion in feces by 25.6% and N excretion in urine by 12.6%, compared with other treatments. Expressed as a percentage of N intake, fecal N showed a linear increase with concentrate supplementation, whereas urinary N presented a linear decrease ($P < 0.0001$).

3.4. Production of CH_4, N_2O and CO_2

The production of CH_4 L / cow d^{-1}, CH_4 g d^{-1}, methane emission intensity (CH_4 g / Kg Milk FCM 3.5%), methane yield (CH_4 g /Kg DM intake), and GEI intake were higher ($P < 0.001$) as the concentrate increased in the diet, with the group receiving 450g DM concentrate/ kg milk produced being the one with the highest amounts (Table 5). Similarly, when comparing by methods (IPCC 2006; Montoya-Flores et al 2020) differences were found ($P < 0.001$), where more CH_4 was produced with the IPCC (2006) model, using Ym 6.5%.

Emissions of CH_4 and N_2O expressed as CO_2-eq, were both largest in treatment 450g among all treatments (Table 6). However, there was no difference between treatments when CO_2-eq emissions were adjusted to the FCM3.5% production ($P > 0.05$).

4. Discussion

4.1. Diets

The diets with the highest amount of ingested forage are those with the highest concentration of NDF and ADF, as well as the lowest amounts of energy and CP, as is the case of the control diet. However, the weight of the cows was not affected ($P > 0.05$) between the different treatments. These results show that cows fed grass and low amounts of concentrate can produce good amounts of milk without affecting their weight (Whelan et al 2017) when their nutritional requirements are met from pasture.

Table 2 Effect of concentrate supplementation level (kg d^{-1}) in the diet of grazing crossbreed F1 dairy cows in tropical areas.

Variable	Treatments†				SEM	*P- value*
	0.00	0.15	0.30	0.45		Treatments
$LW^{0.75}$	108.53	107.60	109.09	109.99	0.399	0.7403
DMI, g/kg $LW^{0.75}$	117.92	117.09	113.52	122.34	2.150	0.3509
Concentrate, kg DM d^{-1}	0.00^{c}	1.24^{b}	2.11^{b}	4.36^{a}	0.024	0.0001
Meadow pasture, kg DM d^{-1}	12.78^{a}	11.33^{b}	10.25^{c}	9.08^{d}	0.019	0.0001
Organic matter	11.6	11.58	11.54	11.50	0.003	0.1637
Crude Protein	1.37^{c}	1.48^{b}	1.52^{b}	1.67^{a}	0.001	0.0001
NDF	9.19^{a}	8.77^{b}	8.44^{b}	7.8^{c}	0.005	0.0001
ADF	4.77^{a}	4.64^{ab}	4.54^{b}	4.33^{c}	0.001	0.0001
ADL	0.68^{c}	0.72^{bc}	0.77^{b}	0.83^{a}	0.001	0.0001

*Significant linear effect ($P < 0.05$) of the treatment on the indicated variable. †kg of concentrate as DM/kg daily milk production; NDF, neutral detergent fiber; ADF, Acid detergent fiber; ADL, Acid detergent lignin, $LW^{0.75}$, Metabolic live weight $^{0.75}$, DMI, dry matter intake; NDF, Neutral detergent fiber analyzes; ADF, acid detergent fiber; ADL, Acid detergent lignin.

Table 3 Effect of the level of concentrate (kg/kg of milk produced) on milk production (kg/cow d^{-1}) and chemical composition (g/100g).

Variable	Treatment†				SEM	*P- value*		
	0.00	0.15	0.30	0.45		Treatment	Lineal	Quadratic
DM intake, kg d^{-1}	12.78	12.57	12.35	13.43	0.1186	0.2113	0.5523	0.0911
Milk yield, kg d^{-1}	8.71	8.26	7.00	9.68	0.4019	0.1797	0.8601	0.0736
Milk / DM intake	0.68	0.65	0.56	0.71	0.0153	0.2812	0.5596	0.1187
FCM★, 3.5%	8.58	8.22	7.16	10.30	0.9353	0.1575	0.7316	0.0702
Fat, g/100g	3.50	3.57	3.70	3.79	0.284	0.8893	0.4633	0.9691
Protein, g/100g	2.38	2.41	2.44	2.51	0.0851	0.7599	0.3679	0.7958
Non-Fatty solids, g/100g [2]	6.54	6.65	6.60	6.94	0.291	0.7730	0.5589	0.6502
Lactose g/100g	3.50	3.54	3.67	3.79	0.1439	0.4949	0.1631	0.7888
Fat g d^{-1}	297.00	287.00	254.77	377.33	38.84	0.3561	0.5423	0.0971
Protein g d^{-1}	206.73	199.93	169.27	243.43	21.68	0.1380	0.8900	0.0711
Non-Fatty solids, g d^{-1}	572.00	545.88	452.00	665.89	57.91	0.0952	1.0000	0.0464
Lactose, g d^{-1}	303.00	292.67	252.33	367.33	32.89	0.1200	0.7176	0.0658
Density, kg/m^3	1.02	1.02	1.23	1.02	0.01	0.9896	0.7926	0.9650
Minerals %	0.61	0.62	0.67	0.64	0.028	0.3555	0.1194	0.4296

Significant linear effect ($P < 0.05$) and Quadratic effect ($P < 0.05$) of treatment on the indicated variable. ★FCM, fat-corrected milk 3.5 % = (kg milk* 0.432) + [(kg fat d^{-1}) * 16.23 milk. †Treatments, kg of concentrate as DM / kg daily milk production. DMI, dry matter intake.

Table 4 N balance (g d^{-1}) in lactating crossbred dairy cows, fed diets with increased concentrate supplementation (kg) per kg of milk yield produced in grazing tropical areas.

Variable	Treatment†				SEM	*P- Value*		
	0.00	0.15	0.3	0.45		Treatment	Lineal	Quadratic
N Intake, g d^{-1}	219.16^{c}	230.32^{bc}	237.03^{b}	282.38^{a}	4.20	0.0001	0.0004	0.0628
N excreted, g d^{-1}								
Faeces	60.56^{b}	64.22b^{b}	66.48^{b}	80.08^{a}	1.25	0.0001	0.0002	0.0617
Urine	138.98^{b}	140.40^{b}	140.85^{b}	157.68^{a}	1.56	0.0106	0.0298	0.0769
Milk	32.40	31.34	26.53	38.16	3.39	0.1380	0.8900	0.0711
N excreted, g d^{-1}	199.54^{b}	204.62^{b}	207.34^{b}	237.75^{a}	6.74	0.0014	0.0047	0.0693
N Balance, g d^{-1}	-12.78^{b}	-5.64^{b}	3.16^{a}	6.47^{a}	1.54	0.0001	0.0001	0.7343
Fecal N / N Intake (%)	27.60^{d}	27.90^{c}	28.07^{b}	28.33^{a}	0.003	0.0001	0.0001	0.5781
Urinary N / N intake (%)	63.42^{a}	61.02^{b}	59.47^{b}	56.12^{c}	0.010	0.0001	0.0001	0.1548
Milk N/N intake (%)	14.85	13.46	11.09	13.17	1.08	0.1288	0.0721	0.1205
Excreted N/ N Intake (%)	27.60^{d}	27.90^{c}	28.07^{b}	28.33^{a}	0.02	0.0001	0.0001	0.4966

*Significant linear effect ($P < 0.05$) and Quadratic effect ($P < 0.05$) of treatment on the indicated variable; N, nitrogen; N balance (g /d) =N intake – (N feces + N urine); †Treatment, kg of concentrate as DM / kg daily milk production.

4.2. Production and chemical composition of milk

Dietary supplementation with concentrate did not modify milk production and quality (Table 3) in the present study ($P > 0.05$). These results are similar in grazing cows (Lawrence et al 2015; Dale et al 2015), mentioning that the intake of different levels of concentrate does not affect milk production and chemical composition. Garcia et al (2007) found that milk production increases in grazing cows when they are provided with the required amount of forage and concentrate based on the requirements of each cow, which is possible in the present study as the cows produced 8 L/d, and the pastures provided enough energy and protein (109 g CP /kg, 16.44 GE MJ /kg) for their nutrient requirements for milk production. Hills et al (2015) mention that when the forage assigned to each cow and provided with the required amount of forage, the potential milk production tends to increase, in pasture-based systems, DMI is recognized as the factor limiting milk production to the greatest degree, so we can confirm that not only the concentrate influences milk production in grazing cows but also the amount of forage provided.

Most studies on dairy cows mention that increasing the proportion of concentrate in the diet increases milk production since feed digestibility is improved; however, these studies are conducted with cows that produce more than 20 kg of milk per day in temperate climates (Sanh et al 2002; T. Yan et al 2010; Olijhoek et al 2018). In tropical regions the cows are mixed (50% meat genotype - 50% milk genotype), mainly crossbreeding of *Bos indicus* with *Bos taurus*, so their milk production is between 10 to 15 kg per day approximately (Hatungumukama et al 2009; Ku-Vera et al 2018; Valencia et al 2018) since they are not selected for their milk production, but their resistance to diseases and hostile climates. Thus, the milk production of the cows in this study is within the established parameters (Sanh et al 2002). If neither production nor quality can be improved due to genetic issues, a reduction in feed costs could be considered since feeding represents 70% of the cost of milk production; the use of the region’s pastures provides a good alternative, as observed in this study.

4.3. Nitrogen digestibility, balance and metabolism

N excretion was higher ($P < 0.05$) for diets containing higher amounts of concentrate (Table 4). It has been reported that only 5-30% of the total N consumed is used, which means that N losses are around 70-95%, and the greatest loss is through urine (Selbie et al 2015), which coincides with the present study.

Some studies (Pacheco and Waghorn 2008) report that in dairy cow’s nitrogen excretion is up to 72%, which is mainly eliminated through urine and feces. The results obtained in the present study showed that more N was eliminated through urine, Castillo et al (2001) mention that when there is an intake above 400 g N d^{-1}, excretion increases exponentially in urine, while through milk and feces it decreases linearly. Olmos and Broderick, (Olmos and Broderick 2006) mention that any increase over 16.5% of CP in the diet, the loss of nitrogen is generated through urine, increasing the volume of urine from 17.3 to 21.7 L d^{-1} in response to increased N supplementation.

Excess urinary N can be reduced by diets with lower N and higher energy forages (de Klein et al 2010). As mentioned above, diets with more than 16.5% CP increase N excretion in urine, however, a high N in the diet is not a reflection of increased urine excretion, as these diets also increase water consumption which helps dilute the amounts of N excreted (Selbie et al 2015).

In the present study, negative balances were obtained for cows that consumed more forage, probably due to the lower N intake, which is reflecting higher mobility of body reserves (Santos et al 2011), however, no differences ($P > 0.1$) were observed in live weight and milk production.

Table 5 Gross energy intake (MJ d^{-1}) and CH_4 emissions from F1 crossbred dairy cows grazing tropical pastures supplemented with different levels of concentrate (per kg of milk yield production) in humid tropics.

Variable	Model		Treatment †				SEM	*P- value*		
	Ym§ 6.5%	Ym§§ 5.54%	0.00	0.15	0.3	0.45		Model	Treatment	M x T
GEI (MJ d^{-1})	225.27	225.27	212.70^b	217.19^b	219.26^b	251.94^a	5.27	0.988	0.0001	0.989
CH_4 (L d^{-1})	578.97^a	493.47^b	506.29^b	516.99^b	521.91^b	599.69^a	12.60	0.0001	0.0001	0.9741
CH_4 (g d^{-1})	413.56^a	352.47^b	361.63^b	369.27^b	372.79^b	428.35^a	9.00	0.0001	0.0001	0.9741
Methane emission intensity	51.96^a	44.29^b	43.23^b	47.91^{ab}	55.00^a	46.38^{ab}	2.68	0.0058	0.022	0.9956
Methane yield, g /Kg DM intake	32.30^a	27.53^b	28.30^d	29.39^c	30.26^b	31.81^a	0.125	0.0001	0.0001	0.4566

*Significant linear effect ($P < 0.05$) and Quadratic effect ($P < 0.05$) of treatment on the indicated variable; Ym§ (methane conversion factor) = 6.5% IPCC, (2006); Ym§§ (methane conversion factor) = 5.54% Montoya et al. (2020); CH_4, methane; GEI, gross energy intake; Methane emission intensity (CH_4 g / Kg Milk FCM 3.5%), Methane yield (CH_4 g /Kg DM intake); †Treatment, kg of concentrate as DM / kg daily milk production

4.4. Production of CH_4, N_2O and CO_2

Many strategies are currently being used for CH_4 mitigation, one of which is increasing concentrate in diets (Sauvant and Giger-Reverdin 2009); however, exceptions may occur, where CH_4 emissions increase as in the present study. The diet with 450g DM of concentrate/kg of milk was the one that presented the highest production of CH_4 (Table 5). Some studies have shown that the use of rations high in concentrate can increase the total production of CH_4 per animal per year by up to 23%. This higher production is associated with a higher feed intake (Lovett et al 2005), although this emission can be reduced by up to 40% if milk production per animal is increased (Boadi et al 2004). O'Neill et al (2012) mention that in grazing cows supplemented with a mixed ration, higher CH_4 yields were found than those that were not supplemented; moreover, no higher milk production or feed intake occurred.

In the present study, no differences were found ($P > 0.05$) in milk production. However, when comparing the CH_4 production divided by the FCM 3.5%, the CH_4 production is affected, and it can be seen that the CH_4 production in the treatment with a higher amount of concentrate is only 6% higher compared to the control group; when this correction is not done, CH_4 emitted is up to 16% in the treatment with higher amount of concentrate. An increase in milk production per head is a mechanism for decreasing GHG, reducing CH_4 production per kg milk yield (Muñoz et al 2015). However, currently, not only the kg of milk produced per cow is considered, but also the amount of fat and protein in that milk (FPCM), to compare results on a common basis (IDF 2015).

Table 6 Estimation of greenhouses gases emissions (expressed as CO_2-eq kg d^{-1}) from in F1 crossbred dairy cows in pasture supplemented with different levels of concentrate in humid tropics.

Variable	Treatment †				SEM	*P- value*		
	0.00	0.15	0.3	0.45		Treatment	Lineal	Quadratic
CH_4 (CO_2-eq kg d^{-1}) Ym§6.5%	10.93^b	11.16^b	11.27^b	12.95^a	0.38	0.0028	0.0093	0.0682
CH_4 (CO_2-eq kg d^{-1}) Ym§§5.54%	9.31^b	9.51^b	9.61^b	11.03^a	0.32	0.0028	0.0093	0.0682
N_2O (CO_2-eq kg d^{-1})	1.728^b	1.805^b	1.858^b	2.214^a	0.069	0.0011	0.0004	0.0628
CO_2-eq kg d^{-1}/kg FCM 3.5%	1.307	1.448	1.663	1.402	0.114	0.0895	0.0548	0.0887

*Significant linear effect ($P < 0.05$) and Quadratic effect ($P < 0.05$) of treatment on the indicated variable; Ym§ (methane conversion factor) = 6.5% IPCC, (2006); Ym§§ (methane conversion factor) = 5.54% Montoya et al. (2020); CH_4 methane; N_2O nitrous oxide; CO_2-eq carbon dioxide equivalent. †Treatment, kg of concentrate as DM / kg daily milk production; FCM, fat-corrected milk 3.5 %.

GHG emissions by cattle are of global concern (IPCC 2013), which is why the use of methodologies for calculating emissions is so important at present. However, as noted in this study, the values used to calculate emissions are critical points, as they may overestimate GHG emissions (Ledgard et al 2020). While the IPCC (2006) provides default values for emission calculations (i.e, YM 6.5%), which are used in most publications, it also specifies that emission factors need to be specific and validated in each country (Van Lingen et al 2019). In the present study, the IPCC energy partition factor of 6.5% was used, as well as the region-specific Ym factor (Montoya-Flores et al 2020), Ym 5.54% of energy intake. This factor was the result of an exhaustive series of research trials in Mexico, (crossbred F1dairy cows from the tropics, with diets, is similar to those in the present study), where direct CH_4 emissions were measured in open-circuit respiration chambers (Ku-Vera et al 2018; Valencia Salazar et al 2018). Observing the results of CH_4 emissions calculated with the IPCC Ym factor resulted in a 15% higher amount compared to the Ym factor of Montoya et al (2020).

Grazing milk production systems are also responsible for 22% of the N_2O emissions worldwide, so it is important to try to calculate their emissions and reduce them (van der Weerden et al 2017). Table 6 show that as the concentrate in the diet increases, the N_2O excreted into the environment increases. Several articles mention that the most important factor for increased N_2O excretion in urine and feces is the increase in dietary N intake (Olmos Colmenero et al 2006; Selbie et al 2015; van der Weerden et al 2017). However, not all the N consumed and excreted is converted into N_2O; e.g. urine is the main route of elimination, with only 2% of the excretions being in the form of N_2O (Selbie et al 2015). Although the amount of N_2O excreted into the environment is very low, its global warming potential is 298 times higher than CO_2 (IPCC 2006); hence the importance of reducing emissions as urine deposited by grazing animals is one of the main sources of N_2O production.

The use of concentrates in animal diets should be evaluated in detail because the costs are high, as well as increasing N_2O emissions, so the use of local feed resources (i.e, fodder) with well-balanced diets is an alternative for animal feed, in addition to not competing with human food (Makkar and Ankers 2014; Gard et al 2016).

5. Conclusions

The use of a country-specific emission factor and Ym factor for the calculation of CH_4 is the best alternative in order not to overestimate CH_4 emissions. The supplementation of concentrate per liter produced of milk in dual-purpose cows grazing in tropical areas did not have an effect on the increase in milk production and its chemical composition. However, higher productions of CH_4 and N_2O were registered with the diets that presented greater amounts of concentrate, for which it is convenient to analyze the use of concentrate in grazing cows on small-scale systems in tropical areas since the use of large amounts of concentrate does not increase milk production, but increases milk production costs and results in higher CH_4 and N_2O emissions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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