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Article

Enteric Methane Emissions from Holstein Cows Grazing Kikuyu (*Cenchrus clandestinus*) Grass in a Herd in Colombian High Tropic at Two Seasons of the Year

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Simple Summary

Enteric methane emissions from dairy cows represent an important source of greenhouse gases, yet they are poorly understood and hardly measured in tropical grazing systems. This study evaluated methane emissions in dairy cows grazing kikuyu grass in Colombian high-tropic using a sensor mask that measured exhaled gases directly in the field. Twelve cows with high or low milk production levels were monitored during low and high rainfall seasons. Although seasonal changes affected forage quality and composition, they did not significantly influence methane emissions, feed intake, or milk production. Instead, the cow's productivity level played a more important role. Due to their greater intake, high producing cows emitted more methane per day, but they were more efficient by emitting less methane per unit of milk and losing less energy as methane. Remarkably, methane emission per unit of dry matter intake was lower than previously reported values, suggesting that the employed system may overstate enteric emissions in tropical systems. The study also demonstrated that the sensor mask is a practical and affordable tool for measuring methane in grazing animals. These findings can help to improve the accuracy of greenhouse gases inventories and support more sustainable dairy production in tropical regions.

Abstract

Enteric methane emissions (EME) from grazing dairy systems in tropical regions remain poorly quantified, increasing uncertainty in national greenhouse gas inventories. This study aimed to quantify EME using electronic spirometry masks (ESM) in dairy cows in Colombian high tropics during two precipitation seasons. Six high milk yield (HMY; >30 L/d) and six low milk yield (LMY; <15 L/d) grazing kikuyu grass (*Cenchrus clandestinus*) and supplement with concentrate feed were monitored by EME, exhaled air volume, feed intake, milk yield and composition. Data was analyzed in a 2 × 2 factorial arrangement (season × production level). Season affected Kikuyu chemical composition ($P < 0.05$) but not dry matter intake (DMI), milk production, quality, nor EME ($P > 0.05$). Despite HMY cows having a greater DMI (kg DM/d; $P < 0.01$) and EME (g/d, L/d; $P < 0.05$) exhibited lower methane intensity (g / kg fat-corrected milk) and gross energy intake lost as methane ($P < 0.05$). Positive correlations were found between EME and total dry matter intake ($r = 0.638$) and milk production ($r = 0.726$). The observed methane yield was comparable to previous studies for tropical kikuyu-based systems but lower than reports from temperate regions, suggesting seasonal-driven kikuyu quality does not translate into EME changes in high tropic regions. Animal productivity level was a key driver of EME magnitude and efficiency, effectively measured by ESM which may represent a practical tool for narrowing EME estimates for tropical pasture-based dairy systems.

Keywords: sensors; spirometry mask; greenhouse gases; cattle

1. Introduction

Cattle population in Colombia ascends to 29.5 million head [1], occupying 77.2% of the total agricultural area [2]. This activity generates more than 1.4 million direct jobs and represents over 21.8% from national agricultural GDP [3] through beef and milk production, both of which are mainly pasture-based. Milk production in Colombia exceeded 8,405 million liters [4] from various systems, among which specialized dairy operations contribute nearly 45% [5,6]. These systems are characterized by using high-yield dairy breeds (mostly Holstein and Jersey), introduced pastures such as kikuyu grass (*Cenchrus clandestinus*), and supplementation with starch-rich supplements [7]. They are located mainly in high Andean regions and inter-Andean valleys from Colombian tropic [8].

Kikuyu grass is the main forage in dairy operations in the high tropics of the Department of Antioquia, representing more than 70% of the grazing area in this region [9]. As a C4 grass, it accumulates higher amounts of structural carbohydrates (cellulose and hemicellulose), which reduce ruminal fermentability, DMI, and milk production [10]. Kikuyu's chemical composition may vary throughout the year depending on seasonal variables such as ambient temperature, rain and solar radiation, affecting ruminal fermentability. Previous studies reported that kikuyu fermentability is higher during the high-precipitation season compared with the low-precipitation season [11]. To overcome these compositional limitations, producers have traditionally relied on supplementing commercial supplements rich in starch, which modify ruminal fermentation end products, including methane [12]. Methane (CH₄) is a greenhouse gas with a higher warming potential than carbon dioxide and has received increased attention in recent years to estimate emissions from different production systems and to identify strategies for their mitigation [13]. Ruminal fermentation has been identified as one of the most important anthropogenic sources of CH₄ [14].

The Intergovernmental panel on climate change (IPCC) has proposed several methodologies to estimate annual CH₄ emissions based on assumptions that often do not cover the wide diversity of production conditions, potentially leading to inaccurate estimates. For Colombia, the IDEAM et al. uses the IPCC Tier 2 methodology for estimating enteric methane emissions (EME) from cattle [15]. This involves the Agriculture, Forestry and Other Land Uses (AFOLU1 Colombia) model, based on models developed for animals under temperate production conditions, such as the NRC [16] beef cattle model, and the Australian model for domesticated ruminants [17]. As a result, it is possible that the estimates do not fully correspond to the conditions under which these models were developed, generating errors and uncertainty. In addition, AFOLU 1 estimates EME by animal groups, creating noise when a specific animal type must be assigned to one of these categories, further increasing errors and uncertainty in the estimation process.

The most widely accepted methodology in temperate regions for measuring EME from grazing cattle involves CH₄ quantification in continuous airflow collected during multiple visits to the feeding station of the GreenFeed System (C-lock Inc. Rapid City, SD), which captures individual daily variation in CH₄ emissions [18]. However, this system fail to account for EME released during periods of resting and rumination [19]. To the best of our knowledge, studies on EME quantification under grazing conditions in Colombia are limited and have primarily relied on laser-based sensors to measure CH₄ concentration and estimate total emissions [20]. Therefore, reducing the uncertainty and improving the precision of estimates from cattle in countries such as Colombia, is essential to properly quantify EME under grazing conditions. For this purpose, the electronic spirometry mask (ESM) has recently been proposed as a low-cost, easy-to-assemble device to which animals adapt quickly [21]. The ESM does not alter animal behavior, covers only the nostrils, and is capable of measuring EME at high temporal resolution during grazing activity. Therefore, the aim of this study was to quantify EME from dairy cows in the high tropical region of Antioquia during two seasons.

We hypothesize that seasonal differences may affect forage nutritional quality influencing ruminal fermentation and, thereby, CH₄ production.

2. Materials and Methods

2.1. Experimental Animals

This experiment was conducted at the Paysandú Agricultural Station of the Universidad Nacional de Colombia, located east of Medellín (Antioquia) at 2200 m above sea level, with a mean annual temperature of 16 °C and average relative humidity above 75%, characteristic of the bH–MB ecological zone [22]. All research procedures involving animals were reviewed and approved by the Institución Universitaria Vision de las Americas Ethic committee (CICUA), protocol number 62 from June 28th – 2025.

The herd is divided into two different areas: one assigned to high-producing cows, managed with a more intensive fertilization plan and shorter pasture rest periods (HMY), and another designated for medium- to low-producing cows (LMY). Each area has an independent milking parlor where cows are milked at 12 h intervals. A portable weather station (Watchdog 2700, Spectrum Technologies, Inc., Plainfield, IL, USA) was installed in the milking parlor for the high-producing cows to record precipitation, relative humidity, and ambient temperature.

2.2. Experimental Design

During a high-precipitation period (April 2025) and a medium-to-low precipitation period (July 2025), 12 adult lactating Holstein cows were selected: six with milk production above 30 L/day (high milk yield, HMY) and six with production below 15 L/day (low milk yield, LMY), grazing different paddocks. The animals grazed kikuyu (*Cenchrus clandestinus*) pastures with 30 to 33 days of regrowth and received supplementation (0.25 lb/L after 10 L of milk yield) with a supplement based on maize and soybean meal at each of the two daily milkings (Table 2). The experimental phase for each period (High and low precipitation) lasted 12 days.

At the beginning of each experimental period, milk yield was recorded, body weight (BW), and body condition score (BCS) were determined for each cow [23]. During the study, 15 g/cow of chromium oxide (Cr) was supplied at each milking as an external indicator to estimate fecal output. The indicator was mixed with supplement (1:1) and pelleted to ensure complete consumption. From day 10 to 12 of experimental period, milk yield and milk samples (morning and afternoon) were recorded daily, pasture and supplement supplement (1, 4, 7, 10, and 12d), and feces (daily at each milking) were collected. On the final day of the experimental period, cows were weighed again and BCS was reassessed.

Milk samples were analyzed for protein, fat, lactose, and total solids using infrared spectroscopy (ISO 9622:2013). Pasture and supplement samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, chromium (Cr), and gross energy (GE) following AOAC (2016) methods. In vitro dry matter digestibility was determined (IVDMD) using the pepsin–cellulase technique [24]. Indigestible dry matter in fecal samples was determined in vitro after 144 h of incubation and used as an internal marker [25]. Forage dry matter intake (DMI_f) was estimated using the following equation [26]:

$$\text{DMI}_f = ([\text{iDM}_{fc}] \times (\text{H}/0.8) - [\text{iDM}_s] \times \text{DMI}_s) / [\text{iDM}_f]$$

Where DMI_f is expressed in kg of DM/cow/d, iDM_{fc} is the indigestible dry matter determined in feces; H/0.8 indicates the external marker recovery percentage (80%) [25]; iDM_s is the indigestible dry matter determined in the supplement; DMI_s is the dry matter intake of supplement, and iDM_f is the indigestible dry matter determined in the pasture.

2.3. Enteric Methane Emissions (EME)

The adaptation to the electronic spirometry masks (ESM) involved fitting each cow with a polyester halter between days 3 and 6. Then, a dummy mask was attached to the halter between days 7 and 10 [27]. On days 10 and 12 of each experimental period, the cows wore the ESM from 08:00 to 18:00 h. To measure CH₄ emissions from each cow, the ESM units were equipped with an electrochemical CH₄ sensor (MQ4, Hanwei Electronics, Zhengzhou, China) and a flow sensor (DN-40, Louchen ZM, Hunan, China), connected to an Arduino UNO board programmed to record exhaled air volume and CH₄ concentration in exhaled air every 500 ms onto a MicroSD memory card. The sensors were calibrated according to the procedure described by Jaimes et al. [21] before the start of each experimental period. Raw data was recorded in Excel (Microsoft) for further analysis, and EME was calculated as follows:

$$\text{CH}_4 = \text{CH}_{4\text{ppm}} \times E / 1000000$$

Where CH₄ is expressed in L / cow / d; CH_{4 ppm} is the methane concentration in exhaled air (ppm) and E is the volume of exhaled air (L/min).

2.4. Statistical Analysis

The effects of season and pasture type (assigned to LMY or HMY) on pasture chemical composition were analyzed using a completely randomized design with a 2 × 2 factorial arrangement (season (S) × production level (L)) using the PROC GLM procedure of SAS 9.4 (SAS Institute Inc, Cary, NC). Statistical analyses of the performance variables measured in the animals were conducted under a completely randomized design with a 2 × 2 factorial arrangement (season × production level) using the PROC MIXED procedure of SAS (1998). by applying the following mixed model:

$$Y_{ijk} = \mu + L_i + S_j + LS_{ij} + C_j(L)_i + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the mean of all observations, L_i is the fixed effect of level of production of cows, S_j is the fixed effect of weather season, LS_{ij} is the interaction of level production of cows with the weather season, C_j(L)_i is the random effect of cow i within treatment j, and ε_{ijk} is the random residual error. Mean analysis was carried out with LSMEANS test using the SAS statistical package. Differences were considered significant at P ≤ 0.05.

3. Results

Accumulated precipitation and relative humidity at the Paysandú Agricultural Station during April (high-precipitation season) were higher (356 mm and 87%, respectively) than those observed in June (medium-low precipitation season) (222 mm and 83%, respectively). In contrast, average daily bright sunshine hours were higher during the medium-low precipitation season (8.2 h/day) than during the high-precipitation season (6.5 h/day), whereas mean daily temperature was similar in both seasons (16.9 °C).

While no significant S×L interactions were detected for the chemical composition of kikuyu grass pastures (Table 1), some chemical fractions were affected by season (p < 0.05), except CP, in vitro dry matter digestibility (IVDMD) and GE, with no differences between pastures assigned to HMY and LMY cows (P > 0.1). The supplement offered to HMY cows had higher CP, GE, and IVDMD, but lower NDF, ADF, and ash content compared to the supplement fed to LMY cows (Table 2).

Table 1. Chemical composition of kikuyu grass (*Cenchrus clandestinus*) pastures assigned to experimental cows in the high- and low-production groups during the two evaluation seasons.

Item ¹	Production Level ²				SEM ⁵	P – value ⁶		
	HMY		LMY			S	L	S × L
	Hig ³	Med ⁴	Hig ³	Med ⁴				
% of DM								
CP	20.0	17.8	18.1	17.8	0.96	0.053	0.132	0.121
NDF	67.1	62.2	67.9	63.1	3.57	0.002	0.467	0.953

ADF	37.8	35.0	37.5	34.8	3.77	0.040	0.807	0.966
Ash	8.58	7.11	8.98	7.75	0.37	0.005	0.179	0.733
IVDMD, %	49.8	46.7	48.3	47.9	2.71	0.214	0.513	0.380
GE, Mcal/kg	4.38	4.10	4.34	4.12	0.01	0.864	0.006	0.680

¹ CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; IVDMD: in vitro dry matter digestibility; GE: gross energy. ²HMY: high milk yield group; LMY: low milk yield group. ³High: season of high precipitation. ⁴Med: season of medium-low precipitation. ⁵Pooled standard error of treatment means, n = 6 cows. ⁶Observed significance level for the effect of season (S) level of milk yield (L), and their interaction (S×L).

Table 2. Chemical composition of the feed supplements offered to high- and low-production cows during the evaluated seasons.

Item ¹ % of DM	Production Level ²			
	HMY		LMY	
	Hig ³	Med ⁴	Hig ³	Med ⁴
CP	15.60	16.10	11.30	13.30
NDF	21.80	20.90	40.40	43.40
ADF	12.80	12.30	25.30	27.20
Ash	7.87	8.22	11.60	9.75
IVDMD, %	80.70	83.50	61.01	57.50
GE, Mcal/kg	4.26	4.24	4.07	4.17

¹ CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; IVDMD: in vitro dry matter digestibility; GE: gross energy. ²HMY: high milk yield group; LMY: low milk yield group. ³High: season of high precipitation. ⁴Med: season of medium-low precipitation. .

Season did not affect DMI_f ($P = 0.33$), DMI_s ($P = 0.87$), or DMI_t ($P = 0.40$) and, consequently, did not affect gross energy intake (GEI) (Table 3). However, level of production affected all the intake parameters, thus, HMY cows showed higher DMI_f, DMI_s, DMI_t, and GEI ($P < 0.01$). Similarly, season did not significantly affect variables associated with milk production and quality ($P > 0.1$; Table 4). In contrast, HMY cows exhibited a higher lactose content ($P = 0.01$) compared to LMY. A season × production level interaction was observed for milk fat content ($P < 0.02$), indicating that milk fat increased during the medium-low precipitation season in HMY cows, whereas the opposite occurred in LMY cows.

Table 3. Forage, supplement, and total intake of experimental cows in the high- and low-production cows during the two evaluation periods.

Item ¹ kg/cow/d	Production Level ²				SEM ⁵	P – value ⁶		
	HMY		LMY			S	L	S × L
	Hig ³	Med ⁴	Hig ³	Med ⁴				
DMI _f ,	11.8	12.9	10.6	10.5	0.52	0.339	0.010	0.319
DMI _s ,	8.3	7.5	2.4	3.3	0.37	0.864	0.001	0.058
DMI _t ,	20.0	20.4	13.0	13.9	0.69	0.403	0.001	0.696
DMD, %	72.3	71.3	65.8	66.5	1.27	0.898	0.002	0.513
GEI, Mcal/d	87.5	87.0	54.9	58.8	2.97	0.412	0.001	0.673

¹ DMI_f: dry matter intake of forage; DMI_s: dry matter intake of supplement; DMI_t: total dry matter intake; DMD: dry matter digestibility; GEI: gross energy intake. ²HMY: high milk yield group; LMY: low milk yield group. ³High: season of high precipitation. ⁴Med: season of medium-low precipitation. ⁵Pooled standard error of treatment means, n = 6 cows. ⁶Observed significance level for the effect of season (S) level of milk yield (L), and their interaction (S×L).

Table 4. Body weight, milk yield, and milk quality of experimental cows in the high- and low-production groups during the two evaluation periods.

Item ¹	Production Level ²				SEM ⁵	P – value ⁶		
	HMY		LMY			S	L	S × L
	Hig ³	Med ⁴	Hig ³	Med ⁴				
BW, kg	594	616	607	487	43.0	0.281	0.214	0.137
Yield, L/cow/d	37.5	35.6	13.0	17.8	1.88	0.463	0.001	0.110
Fat, %	2.53	2.93	4.07	3.59	0.14	0.786	0.001	0.015
CP, %	3.30	3.15	3.72	3.21	0.14	0.051	0.135	0.250
Lactose, %	5.20	4.92	4.27	4.57	0.19	0.954	0.011	0.179
Solids, %	12.0	11.9	12.9	12.3	0.42	0.408	0.171	0.518
FCM, L/cow/d	29.3	29.9	13.1	16.8	1.77	0.264	0.001	0.423

¹ BW: Bodyweight; Yield: Individual Milk yield; CP: milk crude protein acid; Solids: Total solids in milk; MFT: 4% Fat-Corrected milk. ²HMY: high milk yield group; LMY: low milk yield group. ³High: season of high precipitation. ⁴Med: season of medium-low precipitation. ⁵Pooled standard error of treatment means, n = 6 cows. ⁶Observed significance level for the effect of season (S) level of milk yield (L), and their interaction (S×L).

Similarly, season did not affect the parameters associated with methane emissions ($P > 0.1$; Table 5). However, HMY cows showed a greater volume of exhaled air and higher total CH₄ production, but lower CH₄ emission intensity and a lower percentage of GE as CH₄ ($P < 0.04$).

Table 5. Exhaled air volume and methane emissions of high- and low-production cows during the evaluation seasons, using the spirometry mask method.

Item ¹	Production Level ²				SEM ⁵	P – value ⁶		
	HMY		LMY			S	L	S × L
	Hig ³	Med ⁴	Hig ³	Med ⁴				
Exhal, L/min	149	135	111	127	9.10	0.904	0.036	0.142
<i>Methane</i>								
Concentration, ppm	1975	2084	193	2125	78.9	0.096	0.989	0.628
Volume, L/d	423	406	303	388	19.9	0.128	0.009	0.032
Volume, g/d	303	290	217	278	14.2	0.128	0.001	0.032
Yield, L/kg DMI _s	21.2	24.6	23.5	24.6	2.05	0.166	0.451	0.451
Yield, g/kg DMI _s	15.2	17.6	16.8	17.6	1.05	0.174	0.467	0.467
Intensity, L/L milk	11.3	11.6	23.5	22.1	1.70	0.739	0.001	0.627
Intensity, L/FCM	14.5	13.8	23.2	23.6	1.73	0.926	0.001	0.766
Ym, %	4.62	4.36	5.18	6.16	0.36	0.346	0.012	0.124

¹ Exhal: Exhalations. Ym = percentage of gross energy intake lost as CH₄ (% of gross energy intake) ²HMY: high milk yield group; LMY: low milk yield group. ³High: season of high precipitation. ⁴Med: season of medium-low precipitation. ⁵Pooled standard error of treatment means, n = 6 cows. ⁶Observed significance level for the effect of season (S) level of milk yield (L), and their interaction (S×L).

4. Discussion

4.1. Forage Chemical Composition

Kikuyu grass is a species with a rhizomatous and stoloniferous growth habit [28], which makes it more resilient to environmental changes. This is because both structures (rhizome, stolons) perform different but complementary functions in plants. Stolons act as supporting stems for photosynthesis,

whereas rhizomes accumulate reserve carbohydrates and meristematic tissue, conferring a high degree of plasticity under varying environmental conditions [29]. These structures also ensure plant reproduction and expansion more efficiently than seeds [30], contributing to greater persistence over time. Despite these properties, in this experiment the nutritional quality of kikuyu grass was affected by season, showing higher contents of CP, NDF, ADF, ash, and GE during the high-precipitation season than during the medium-low precipitation season (Table 1). This may be explained by the fact that rainfall during the high-precipitation season was 60.4% greater and sunshine hours were 26.1% lower than in the medium-precipitation season, which could have affected soil nutrient uptake and the photosynthetic capacity of the pasture and, consequently, its chemical composition. Similarly, da Silva et al. [31] found that both precipitation and solar radiation influence CP and NDF contents in several tropical grasses, including *Cynodon* spp. cultivar Tifton 85, another rhizomatous and stoloniferous species.

The chemical composition of the feed supplements offered to HMY and LMY cows during the two evaluation seasons falls within the values previously reported for this type of feed in northern Antioquia [10,31]. These supplements are formulated according to the animals' milk yield and the average quality of the pastures offered, with the aim of improving the supply of energy and key nutritional fractions that are often limiting in pastures, such as rumen-undegradable protein and non-structural carbohydrates [10].

4.2. Animal Performance

Differences in pasture chemical composition due to season did not affect pasture dry matter intake (DMI_p; Table 3). Similar results have been reported by other authors [33,34], which may be associated with the fact that DMI_p in animals grazing high-NDF pastures, as observed in this experiment, is limited by rumen fill capacity [34,36]. Although some studies have reported a strong inverse relationship between NDF content and DMI_p [37], others have shown high variability and low correlation between these two variables [37,38]. Thus, although in this experiment the difference in average pasture NDF content between seasons was 4.8%, it was apparently insufficient to promote a significant effect on rumen fill and, consequently, on DMI_p.

Mertens [39] estimated that maximum NDF intake in lactating cows corresponded to 1.20% of body weight (BW). Later studies showed that this value was underestimated and could reach up to 1.83% of BW [40]. Data reported by Correa et al. [10] indicate that NDF intake from kikuyu grass may represent between 1.0 and 1.62% of the animals' BW, suggesting considerable plasticity in animals' capacity to respond to the effects of NDF on rumen fill. Likewise, Jaimes et al. [41] reported a similar maximum kikuyu NDF intake (1.57% of BW) with wide variability.

Another factor that may have contributed to the lack of a seasonal effect on forage dry matter intake (DMI_f) is the substitution effect exerted by feed supplements, which also shows high variability, ranging from 0.11 to 0.50 kg supplement / kg forage (Mayne and Wright, 1988). In the current experiment, the average substitution rate (SR) was estimated at 0.336 ± 0.278 kg/kg. To determine this, the regression between supplement DMI (independent variable) and forage DMI (dependent variable) was first calculated (DMI_f, kg/cow/d = $9.708 + 0.132$ DMI_s, $r^2 = 0.94$). Then, the intercept (DMI_f when cows are not supplemented) was incorporated into the formula proposed by Bargo et al. [42]:

$$SR (kg/kg) = (\text{pasture DMI in the unsupplemented treatment} - \text{pasture DMI in the supplemented treatment}) / \text{supplement DMI}.$$

As shown, the SR found in this study falls within expected values but also shows high variability (CV = 82.9%), which may have contributed to the lack of seasonal effect on DMI_p. Furthermore, because milk production level was similar between the two assessed seasons and concentrate supplementation was adjusted according to milk production, no differences in total DMI were observed between precipitation seasons (Table 3).

Pasture DMI (DMI_p) was lower in LMY cows, likely due to their lower energy and nutrient requirements. The NRC (2001), based on the theory of energy intake regulation, states that cows

adjust intake according to their level of milk production. In this experiment, the correlation between kikuyu grass DMI_p and milk yield was 0.748 ($P < 0.01$), slightly lower than the value reported by Correa et al., [10], which was 0.86 in Holstein cows grazing kikuyu pastures in Antioquia. Yet, because in dairy production systems in Antioquia, the amount of supplement offered to lactating cows is adjusted to the milk production level, as was also done in this experiment, the correlation between supplement DMI (DMI_s) and milk yield was very high (0.993, $p < 0.001$). Overall, the correlation between total DMI (DMI_t) and milk yield in this experiment was 0.983 ($p < 0.001$), notably greater than those reported by Bilal et al. [43] ($r = 0.59$), Britt et al. [44] ($r = 0.44$), and Madilindi et al. [45] ($r = 0.32$) and Kebede et al. (2010) ($r = 0.40$).

The lack of seasonal effect on milk yield and quality (Table 4) is consistent with the absence of seasonal effects on both DMI_p and DMI_t. Although production level did not affect body weight, milk from HMY, as expected, had lower fat and total solids contents but higher lactose and milk urea nitrogen (Table 4). These results agree with previous studies reporting, who observed a negative correlation between milk yield and milk fat, [46,47] and between yield and total solids [47]. In contrast, Antanaitis et al. [48] and Barbosa et al. [47] reported a positive correlation between milk yield and lactose content.

4.3. Enteric Methane Emissions (EME)

The exhaled air volume of the experimental cows found in this study (Table 5) fell within the expected range for cows with the body weights reported here. According to Gallivan et al. [49], pulmonary ventilation in adult cattle averages 0.218 ± 0.044 L/kg/min, a range that includes the values observed in this study (0.232 ± 0.029 L/kg/min). Gallivan et al. [50] later reported that pulmonary ventilation in adult cows can reach 0.244 ± 0.035 L/kg/min, while Zhou et al. [51] indicated that Holstein cows under a 16 °C environment may exhibit a pulmonary ventilation rate of 145 L/min for cows weighing approximately 680 kg, equivalent to 0.216 L/kg/min. In this experiment, HMY cows showed a greater exhaled air volume (L/min), likely associated with increased metabolic activity and oxygen demand for milk production [52,53]. Similarly, Pinto et al. [54] reported an increased respiratory rate in higher-producing dairy cows, attributed to greater metabolic demands.

Methane concentrations in exhaled air observed in this study (Table 5) were greater than those reported by Antanaitis et al. [55], who measured EME in housed Holstein cows using a laser detector and found concentrations ranging from 187 to 348 ppm. Likewise, Difford et al. [56] reported an average CH₄ concentration of 410 ppm in Holstein and Jersey cows measured in respiration chambers, a value slightly higher than that reported by van Breukelen et al. [57] in the Netherlands using the GreenFeed system (average 325 ppm in cows with more than 75% Holstein genetics), and by Sahraei et al. [58], who reported an average of 235 ppm in lactating Holstein cows using the Sniffer system. In contrast, the CH₄ concentrations reported in Table 5 fall within the range reported by Washburn and Brody [59] (1010 to 4190 ppm), who used spirometry masks that completely covered the muzzle of lactating cows.

Methane production (L/cow/day) (MP) is a raw metric influenced by various factors, particularly, feed intake level and diet digestibility [60,61]. MP values found in this study for HMY cows are higher than those reported by Noguera and Posada [62] (402 ± 40 L/cow/day), who measured MP using respiration chamber in lactating Holstein cows consuming kikuyu grass. This difference may be explained by the lower milk production level of HMY cows (26.9 ± 1.9 L/cow/day) in the experimental cows of Noguera and Posada [62]. MP values found here for LMY cows are similar to those reported by Molina et al. [63] in Holstein × Jersey crossbred cows grazing kikuyu grass, where EME was measured using the polytunnel method. However, these values are lower than those reported by Ulyatt et al. [64] (363 g/cow/day), who used the SF₆ technique with lighter cows (438 kg body weight) grazing kikuyu grass in New Zealand, where pasture NDF content was nearly 20% lower than in the present study. Likewise, values for HMY cows found here are lower than those reported by Montenegro et al. [65] in Holstein cows in Costa Rica (390 g/cow/day) grazing a mixture

of African star grass (*Cynodon nlemfuensis*) and kikuyu but receiving more than 14.0 kg/cow/day of feed supplements, likely with higher digestibility than that observed here (Tables 1 and 2).

Because no differences were found in forage intake, supplement intake, or total DMI between evaluation periods (Table 3), no effect of season on CH₄ production was observed (Table 5). Nevertheless, MP was higher in HMY cows ($P < 0.016$), reflecting their greater pasture, supplement, and total feed intake. Accordingly, the correlation between milk yield level and MP was high and positive ($r = 0.726$, $P < 0.007$), as was the correlation between total DMI and MP ($r = 0.638$, $P < 0.027$). Similar relationships have been reported by other authors [66–68] and have been used to develop regression equations for estimating CH₄ production [69,70].

Methane yield (g/kg DMI) (MY) is a more refined EME metric that reflects the efficiency with which consumed feed is converted into CH₄. In this experiment, MY was similar to that reported by Noguera and Posada [62] (16.1 ± 4.0 g/kg DMI) in Holstein cows consuming kikuyu grass, but higher than that reported by Molina et al. [63] in Holstein × Jersey crossbred cows grazing kikuyu grass, likely due to their lower reported DMI. In contrast, Montenegro et al. [65], reported higher MY values in Holstein cows grazing African star grass (*Cynodon nlemfuensis*) and kikuyu pastures in Costa Rica (20.5 ± 1.3 g/kg DMI), despite similar milk yield and DMI levels to those exhibited by HMY cows in this study; this difference may be explained by their higher MP values. The MY factors currently used in other regions of the world are generally higher than those observed here. For example, average value of 18.2 ± 3.71 and 21.4 ± 3.39 g CH₄/kg DMI have been reported in the United States and Europe respectively [71], while in New Zealand the value is 22.1 g/kg DMI [61]. Methane intensity (L CH₄/FCM or g CH₄/kg milk yield) (MI) is perhaps the most relevant efficiency metric for evaluating the environmental impact of dairy systems [72] and has been long suggested to be included in dairy genetic selection programs to improve efficiency traits [73].

Measurements of methane emitted by ruminants have been published since the 19th century [74,75], when this gas was simply called “hydrocarbon” (Kohlenwasserstoff) or “swamp gas” (sumpgas), and its chemical formula was considered to be CH₂. Although the caloric values of methane were known from 1838 [76], the idea that methane excreted by animals represents an energy loss was not introduced until the beginning of the 20th century [77]. Then, was incorporate into energy system in the calculus of metabolizable energy by Armsby [78]. The percentage of gross energy intake lost as CH₄ Ym; [79] has been referred to by other authors as the “methane conversion factor” [80], “methane emission factor” [81], or “cattle methane yield” [82]. This parameter was particularly important in energy studies in ruminants until the 1970s [60]. However, when CH₄ was classified as a greenhouse gas in the mid-1970s [83], its importance in studies on ruminant nutrition, physiology, and metabolism increased significantly, and today, its relevance as a source of energy loss in ruminants is reinforced by its importance as greenhouse gas. The Ym showed in Table 5 are between the range reported by Moe and Tyrrell [60] (1.6 to 9.9%) in their analysis of energy balance trials with Holstein cows and in the range reported by Niu et al. [84] in an intercontinental database (2.7 to 9.8%), but are lower of Ym proposed by IPCC (2019) to dairy cows (5.7 to 6.5%) indicating that the values of IPCC [85] may overestimate methane emission as previously indicated by Noguera and Posada [62]. These authors reported a mean of Ym of $4.9 \pm 1.2\%$ in Holstein cows under a respirometry chamber in Northern of Antioquia with a mean of milk yield of 26.9 ± 1.9 L/d.

In this experiment, while Ym was positively correlated with MY ($r=0.606$, $p<0.036$) and with MI ($r=0.786$, $p<0.003$), do not was correlated with MP ($r=0.047$, $p>0.885$) suggesting that the last metric may not be an appropriated indicator to analysis of methane emission in ruminants under the evaluated experimental conditions. However, Ym is affected by several factors including dry matter intake, feed nutrient composition [71,86], genetic background [87], and milk yield [88]. In this experiment, Ym was correlated negatively with total DMI ($r = -0.728$, $p<0.0074$) and fat corrected milk yield ($r=0.860$, $p<0.001$) indicating that with higher intake and higher milk fat-corrected yield, a lower gross energy intake is lost in the form of methane as was observed by others [69,87].

The use of a spirometry mask represents a significant methodological advantage for quantifying EME because it enables direct and continuous measurement of exhaled gases during behavioral states

(i.e., grazing, rumination, resting) that are typically underrepresented or not captured by other techniques. Technologies such as GreenFeed (C-Lock, Inc. Grand Rapids, SD) rely on voluntary (or guided) animal visits to the system and therefore provide discontinuous measurements that may bias estimates towards feeding periods [89,90]. Similarly, SF₆ technique [91] integrates emissions over time but lacks the temporal resolution to associate EME with specific activities during grazing [64,91]. Respiration chamber, although considered the gold standard for EME determination, restrict animal movement and behavior, limiting their applicability under grazing conditions and potentially altering intake and emission patterns [92,93]. In contrast, the spirometry mask allows for high-frequency, real-time measurements of CH₄ concentration and airflow, enabling the characterization of emissions across the full range of daily activities under grazing conditions, including rumination and idling periods when CH₄ production remains substantial but is often unmeasured by other systems. This capacity to capture temporal variability improves the accuracy of daily EME estimate and may provide a more mechanistic understanding of emission dynamics in grazing cattle systems.

4. Conclusions

Under the conditions of this study, DMI and EME were primarily driven by production level rather than rainy season, as no seasonal effects were observed on intake, milk yield or CH₄ production. High-producing cows in the evaluated environment, exhibited greater pasture, supplement and total DMI, which translated into greater absolute EME, while CH₄ yield remained within reported ranges for grazing systems based on kikuyu grass. Strong positive correlations between milk yield, total DMI and CH₄ production confirm that intake is a major determinant of EME output. Despite differences in milk composition associated with production level, overall animal performance and CH₄ intensity reflected biological relationships, supporting the robustness of intake-driven prediction of EME in grazing systems. The observed exhaled air volumes and CH₄ concentrations were consistent with literature value, indicating that the spirometry mask methodology provides reliable estimates of respiratory exchange and CH₄ output under grazing conditions. Values of Y_m found in this experiment agreed with other reports but were lower than the values proposed by the IPCC.

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Abbreviations

The following abbreviations are used in this manuscript:

EME Enteric methane emissions

ESM	Electronic spirometry mask
HMY	High milk yield
LMY	Low milk yield
DMI	Dry matter intake
CH ₄	Methane
BW	Body weight
BCS	Body condition score
CP	Crude protein
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
Cr	Chromium
GE	Gross energy
iDM	Indigestible dry matter
IVDMD	In vitro dry matter digestibility
FCM	4% Fat-Corrected milk
Ym	Percentage of gross energy intake lost as CH ₄

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