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In vitro Fermentation Profile and Methane Production of Kikuyu Grass Harvested at Different Sward Heights

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Highly digestible forages are associated with an *in vitro* low-methane (CH_4) rumen fermentation profile and thus the possibility of reducing CH_4 emissions from forage-based systems. We aimed to assess the *in vitro* ruminal fermentation profile, including CH_4 production, of the top stratum of Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) harvested at different sward heights (10, 15, 20, 25, and 30 cm). Herbage samples (incubating substrate) were analyzed for their chemical composition, *in vitro* organic matter digestibility (IVOMD), and morphological components. *In vitro* incubations were performed under a randomized complete block design with four independent runs of each treatment. Gas production (GP), *in vitro* dry matter digestibility (IVDMD), CH_4 production, total volatile fatty acid (VFA) concentration, and their acetate, propionate, and butyrate proportions were measured following 24 and 48 h of incubation. Herbage samples had similar contents of organic matter, neutral detergent fiber, and crude protein for all treatments. However, a higher acid detergent fiber (ADF) content in taller sward heights than in smaller sward heights and a tendency for metabolizable energy (ME) and IVOMD to decrease as sward height increased were found. Similarly, the stem + sheath mass tended to increase with increasing sward height. Amongst the nutrients, ME ($r = -0.65$) and IVDMD ($r = -0.64$) were negatively correlated with sward height ($p < 0.001$) and ADF was positively correlated with sward height ($r = 0.73$, $p < 0.001$). Both the GP and IVDMD were negatively related to the sward height at both incubation times. Sward heights of Kikuyu grass below 30 cm display an *in vitro* profile of VFAs high in propionate and low in acetate, with a trend toward lower methane production of CH_4 per unit of IVDMD. These findings are important to aid decision-making on the optimal sward height of Kikuyu grass and manage animal grazing with the opportunity to reduce CH_4 production.

Keywords: methane mitigation strategy, methanogenic potential, sward structure, tropical grass, forage nutritive value, grazing management

INTRODUCTION

Livestock is under fire of critics for its major share in the environmental impact of the agricultural sector. Total global greenhouse gas (GHG) emissions from livestock (animals, manure, feed production, and land-use change) are estimated to account for 14.5% of total anthropogenic emissions (Gerber et al., 2013). Among livestock production systems, grassland-based ruminants are the most controversial in the present-day literature (Teague et al., 2016; Gerssen-Gondelach et al., 2017). On the one hand, ruminants produce methane (CH_4) as a natural byproduct of microbial fermentation of feed in the rumen, contributing approximately 6% of the global anthropogenic GHG emissions (40% of all livestock emissions; Gerber et al., 2013; Beauchemin et al., 2020). On the other hand, grazed pastures which are the basis of those systems, when properly managed, potentially improve the sustainability of livestock production (Lobato et al., 2014; Elgersma, 2015; French et al., 2015), provide many social and environmental services (Werling et al., 2014; Mottet et al., 2017; Horrocks et al., 2019; Zubieta et al., 2020), and improve soil health indicators in tropical systems (Teutscherová et al., 2021). Hence, current grazing systems are being redesigned to link animal production with environmental management (Boval and Dixon, 2012; Carvalho, 2013) in light of current demands for sustainable agricultural production around the world (Herrero et al., 2010; Mottet et al., 2017).

The profitability and sustainability of forage-based dairy systems depend on efficient management (Herrero et al., 2000). In this regard, grazing management is of particular importance since when properly managed, it can improve the quantity and quality of herbage consumed by the animals and ultimately reduce CH_4 emissions (Congio et al., 2018; Savian et al., 2018, 2021). Previous studies have shown that the sward height is a useful and reliable tool to optimize pasture management (Carvalho et al., 2011; Kunrath et al., 2020). The literature suggests that under moderate- to low-intensity grazing management, animals ingest a diet with high nutritive value composed primarily of leaf lamina from the top stratum of the sward (Savian et al., 2018, 2020; Zubieta et al., 2021). Likewise, it is well known that diet digestibility declines from the top to the bottom of the sward, showing a vertical quality gradient of forages (Delagarde et al., 2000; Benvenutti et al., 2016, 2020). Moreover, as pasture matures, the sward height increases and the nutritive value decreases (Benvenutti et al., 2020). High forage digestibility is associated with a fermentation profile in the rumen that is unfavorable to CH_4 production (Hristov et al., 2013; Muñoz et al., 2016). Therefore, if grazed herbage is the main source of nutrients for animals, it is pivotal to offer a highly digestible forage that may have a high potential for mitigating enteric CH_4 emissions.

Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*), widely known as *Pennisetum clandestinum Hochst*, is a highly productive subtropical grass of African origin that is well adapted to the forage-based dairy systems of some countries of Latin and Central America (e.g., Colombia, Brazil, and Mexico) and Oceania [e.g., Australia and New Zealand; (García et al., 2014; Sbrissia et al., 2018; Marín-Santana et al., 2020)]. When managed correctly, Kikuyu grass is recognized for its moderate to good quality and high yield potential, especially in high-fertility soils (Reeves et al., 1996; Fulkerson et al., 2006; García et al., 2014). Commonly, grazing management goals of Kikuyu grass are based on plant characteristics associated with the regrowth age, phenological state, leaf stage, critical leaf area index, among others (Reeves et al., 1996; Fulkerson and Donaghy, 2001; Schmitt et al., 2019b). Currently, and for several forage species, including Kikuyu grass, the sward height is proposed as an easy-to-use grazing management criterion and a key performance predictor (Marin et al., 2017; de Souza Filho et al., 2019; Kunrath et al., 2020), as there is a strong relationship with the quantity and quality of the herbage that animals ingest. On the other hand, *in vitro* studies may predict enteric CH_4 production with reasonable accuracy and precision (Danielsson et al., 2017) and can help to identify promising strategies for *in vivo* studies oriented to reduce the environmental impact of livestock (Danielsson et al., 2017; Valencia Echavarria et al., 2019; Molina-Botero et al., 2020). Previous studies examined the effects of stage of regrowth on the nutritive value of whole plants of Kikuyu pastures and on the *in vitro* fermentation parameters (Ramírez et al., 2015; Vargas et al., 2018). Basic and key information regarding the sward height relationship with the nutritive attributes of Kikuyu grass and the main ruminal fermentation parameters, including CH_4 production, has not yet been established.

We hypothesized that the top stratum of the Kikuyu grass harvested at intermediate sward heights (15, 20, and 25 cm) has highly digestible leaves and displays an *in vitro* low-CH_4 rumen fermentation profile with similar chemical and sward structural characteristics. Thus, this study aimed to assess the effect of the sward height of Kikuyu grass from herbage samples of the top stratum (incubating substrate that reflects the potentially grazed stratum) on the *in vitro* ruminal fermentation profile. We also evaluated the *in vitro* CH_4 production and identified the sward heights that may offer the largest opportunity to mitigate enteric CH_4 production from grazing cattle fed with Kikuyu grass.

MATERIALS AND METHODS

Origin of Herbage Material

Herbage samples for the *in vitro* incubations were produced within a grazing trial with dairy heifers at the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), municipality of Lages, S.C., Brazil (27°47′10.5″S, 50°18′20.5″W, 937 m a.s.l.). According to Köppen's climate classification, the region is humid subtropical under oceanic influences. It has an annual average temperature of 17°C and annual average precipitation of 1460 mm (Alvares et al., 2013). The soil was classified as Humudept (with an umbric epipedon) according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). The soil is developed from sedimentary rocks (sandstone and siltstone) and has an acidic pH, high aluminum content and low sum and base saturation (Rauber et al., 2021).

The grazing trial was carried out in a 5000-m^2 permanent pasture of Kikuyu grass (*Cenchrus clandestinus - Hochst. ex Chiov*) established in the early 1990s and grazed by dairy and beef cattle since then. The whole area was mowed homogeneously until 5 cm of height and divided into ten paddocks of 500 ± 5 m^2. Fertilizers were split into two applications depending on rainfall occurrence and considering a two-period evaluation. The pasture received one application of 250 kg/ha of fertilizer (N-P-K, 9–33–12) and 135 kg/ha of urea on 26 January 2017 (first evaluation period). On 22 March 2017, 67.5 kg/ha of urea was applied (second evaluation period). Due to the frost event and low temperatures in winter and sometimes in spring, the Kikuyu growth season is from the final period of spring and early autumn (Sbrissia et al., 2018); therefore, the herbage collection in both periods lasted from 28 Feb to 15 Apr 2017.

Treatments and Experimental Design

Treatments consisted of herbage samples from the top stratum of Kikuyu grass harvested at five sward heights (10, 15, 20, 25, and 30 cm). The grazing trial was conducted in a randomized complete block design with two spatial (paddocks) and two temporal (morning or afternoon) replicates. The blocking criterion was the time of day due to differences that may exist in the herbage chemical composition and dry matter yield within a day (Delagarde et al., 2000; Gregorini, 2012). Each sward height of the Kikuyu grass was randomly assigned in two paddocks, each one evaluated once in the morning and once in the afternoon (two periods of evaluation), in an alternated scheme with random start. Once target sward height was achieved after the initial mowing and before to start a grazing assessment, herbage sampling was performed (i.e., in the morning, period one). After that, the sward was mowed again to half of the treatment sward height (residuals were retired), and when it reached the set sward height again, a second herbage sampling was conducted (i.e., in the afternoon, period two). A total of four herbage samples from the top stratum per treatment were collected for *in vitro* incubations.

The *in vitro* incubation experimental design was carried out through four independent runs of each treatment, two ruminal liquids from steers (unmixed), and two independent sets corresponding to 24 and 48 h of incubation. In addition, four blanks (no substrate) for each incubation time were included.

Sward Measurement and Herbage Sampling

The sward height was measured at 150 random points per paddock using a sward stick (Barthram, 1985). When the treatment sward height of individual paddocks was confirmed, metallic quadrants (0.25 m^2) were placed at three random sites; average sward heights were calculated from five readings taken inside the quadrants with the sward stick to perform herbage clipping at half of the canopy height (samples representing the grazing stratum). Half of the herbage samples were separated into morphological components (leaf lamina, stem + sheath, and dead material) and dried in a forced-air oven at 55°C for 72 h. The dry weights of morphological components were used to calculate total herbage mass (kg DM/ha) as the sum of each component's mass. The other half was also dried and then pooled per paddock and time of the day for chemical analysis and *in vitro* incubations.

Chemical Composition and *in vitro* Organic Matter Digestibility

The herbage samples were analyzed in duplicate for dry matter (DM, method 930.04; AOAC, 2016), ash (method 930.05; AOAC, 2016), and for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991) by using an Ankom 200 fiber analyzer without heat-stable alpha-amylase. ADF and NDF procedures are not ash-free. Samples were also characterized for N content by the Kjeldahl digestion. The crude protein amount was calculated as N × 6.25 (N, method 984.13; AOAC, 2016). The two-stage Tilley and Terry (1963) technique (incubation with rumen fluid followed by acid-pepsin digestion) was used to estimate the *in vitro* organic matter digestibility (IVOMD). The total digestible nutrient (TDN) concentration of the simulated grazing samples was estimated as a percentage of IVOMD (Moore et al., 1999). The metabolizable energy (ME) were estimated using the following equations of NRC (NRC, 2001): DE (Mcal/kg) $= 0.04409 \times$ TDN (%), and ME (Mcal/kg) $= 1.01 \times$ DE (Mcal/kg) -0.45.

In vitro Ruminal Fermentation

Procedures involving animals were carried out in accordance with the relevant guidelines, regulations, and requirements of Colombian law No 84/1989 and the following protocol, approved by the Ethics Committee of the International Center for Tropical Agriculture (CIAT).

The *in vitro* incubations were conducted according to Theodorou et al. (1994) in the Forage Quality and Animal Nutrition Laboratory (certified by the FAO-IAG proficiency test of feed constituents 2017 including *in vitro* gas production) at CIAT located in the Valle del Cauca department, Colombia (3°29′34″N, 76°21′37″W, 965 m a.s.l.). Rumen fluid was collected at 7:30 am from two rumen-fistulated *Bos indicus* Brahman steers with an average body weight of 720 ± 42 kg, which were grazed on *Cynodon plectostachyus* (star grass) pasture, with free access to water and mineral salts.

The rumen fluid was filtered using a 250 μm nylon pore size cloth, dispensed into two thermal flasks prewarmed to 39 ± 0.5°C, and immediately transferred to the laboratory. The time between rumen fluid collection and inoculation did not exceed 30 min. Five-hundred milligrams of each herbage sample (DM basis) was incubated in 160 mL glass bottles, prewarmed in an incubator at 39°C, with 20 mL filtered rumen fluid mixed with 80 mL rumen medium in a 1:4 ratio (Menke and Steingass, 1988), and dispensed with continuous flushing of CO_2. The bottles were slightly stirred, sealed with rubber stoppers and aluminum caps, and incubated in a water bath at 39°C in two different sets corresponding to incubation times of 24 and 48 h. Four blanks of rumen medium (bottles without substrate that contained only inoculum and medium) per each set were also incubated. The gas production was measured at 3, 6, 9, 12, 24, and 48 h using a pressure transducer (Lutron Electronic Enterprise Co. Ltd., Taipei, Taiwan) connected to a digital wide-range manometer (Sper Scientific, Arizona, USA) and a 60 mL syringe through a three-way valve (Theodorou et al., 1994). After each measurement, the gas of the bottles was released to avoid partial dissolution of CO_2 (Tagliapietra et al., 2010) and possible disturbance of microbial activity (Theodorou et al., 1994). Cumulative pressure values were converted into volume (GP, mL) from measured pressure changes at incubation times and after correction for blank pressure values using the ideal gas law and expressed per unit of dry matter incubated (DMi) and *in vitro* dry matter degraded (IVDMD) (López et al., 2007).

In vitro Methane Production and Calculations

Methane (CH_4) analyses were carried out in the Greenhouse Gas Laboratory CIAT. A gas sample in the headspace was collected into a 5 mL vacuum vial (Labco Ltd., High Wycombe, England) at 24 and 48 h. The CH_4 concentration was determined using a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan) equipped with a Hayesep N packed column (0.5 m × 1/8" × 2 mm ID) and flame ionization detector (FID). The operating temperatures of the column, detector, methanizer, and valves were 80, 250, 380, and 80°C respectively. Ultrahigh purity 5.0-grade N was used as the carrier gas with a linear velocity of 35 mL/min. The CH_4 concentration was calculated using a standard of 10% CH_4 balanced in N (Scott-Marrin Inc., Riverside, CA) and corrected for the CH_4 blank values. The volume of CH_4 (mL) produced at the end of each incubation time (24 and 48 h) was calculated as a product of the total gas produced (mL) multiplied by the concentration of CH_4 (%) in the analyzed sample, as described by Lopez and Newbold (2007).

Volatile Fatty Acids and *in vitro* dry Matter Digestibility

Following 24 and 48 h of incubation, the fermentation was stopped by dipping the bottles in cold water with ice and then processing to determine volatile fatty acids (VFAs) and the *in vitro* digestibility of dry matter (IVDMD). Ruminal fluid samples (10 mL) were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (1.6 mL) was transferred into a 2 mL Eppendorf tube, and 0.4 mL of metaphosphoric acid (25% w/v) was added for VFA analysis. Samples were then stored frozen at −20°C and later analyzed for acetate, propionate, and butyrate concentrations by high-performance liquid chromatography (HPLC) with an SPD-20AV UV-VIS detector (SHIMADZU, Prominence UFLC System) fitted with a BIO-RAD Aminex HPX-87H, 300 × 7.8 mm Ion Exclusion Column. The total VFA concentration was calculated as the sum of the individual VFA concentrations in the ruminal fluid and was corrected for the blank values. Based on the obtained results, the proportion of each VFA in the total VFA amount was calculated. The acetic: propionic ratio was also calculated. All contents remaining in the bottle were finally filtered through preweighed sintered glass crucible pore number 1 (Pyrex®) and dried in a forced-air oven at 105°C for 24 h to determine the IVDMD.

Statistical Analysis

All statistical analyses were performed using R 3.5.3 (R Core Team, 2018). Herbage chemical composition and sward characteristics were analyzed with ANOVA in a randomized block design: $Yijk = \mu + \alpha i + \beta j + \varepsilon ijk$, where: $Yijk$ is the response variable, μ is the overall mean, αi treatments (herbage samples from the top stratum), βj is the effect of the block (time of the day), and ϵijk is the residual error. HSD Tukey's test was used to compare means among treatments; significance was declared at $p \leq 0.05$ and tendencies at $0.05 < p \leq 0.10$. The nutritive value (NDF, ADF, CP, ME, IVDMD) and *in vitro* fermentation parameter (GP, acetate, propionate, and butyrate) results were submitted to Pearson's correlations and visualized using the R package corrplot (Wei et al., 2017).

The *in vitro* fermentation data were analyzed as linear ($Y = \beta 0 + \beta 1SH + \varepsilon$), quadratic ($Y = \beta 0 + \beta 1SH + \beta 2SH^2 + \varepsilon$), and a double linear function of sward height ($Y = f\{p + a1 \times (SH - v), p + a2 \times (SH - v)\}$), where Y is IVDMD, GP, *in vitro* CH_4, VFA (acetate, propionate, and butyrate), f is the min or max function, v and p are the coordinates of the crossing point of sward height, SH are the observed values of sward height, and a1 and a2 are the slopes of the component lines adapted from Mezzalira et al. (2017). Linear and quadratic regression models were fitted by using R lm{stats} function and double linear models were fitted by deviance minimization with the optim{stats} function. After fitting a regression model, the residual plots were checked and the Shapiro–Wilk test was carried out using the R function shapiro.teststats. The best model was selected by the smaller value of Akaike's information criterion (AIC). The objective of the regression analysis was to understand how the nutritive value of the top stratum of Kikuyu grass, harvested at different sward heights, influences the *in vitro* ruminal fermentation profile.

RESULTS

Sward Characteristics and Chemical Composition of the Herbage Incubated

The sward heights obtained were close to the nominal treatment heights and different between treatments ($p < 0.001$, **Table 1**). Herbage mass in 10 cm swards was less than in the 30 cm swards but did not differ among the other sward heights. The 25 and 30 cm sward heights resulted in a higher green leaf mass than the 10 cm sward height ($p < 0.01$) but did not differ between 15 and 20 cm ($p > 0.05$, **Table 1**). The stem + sheath mass tended to increase with increasing sward height ($p = 0.09$, **Table 1**).

No differences were found for OM, NDF, and CP contents ($p > 0.05$, **Table 2**), however, the ADF concentration was greater at 30 cm sward heights than at 10 cm sward heights, but not different from other sward heights ($p = 0.02$, **Table 2**). The IVOMD and ME tended to decrease with increasing sward height ($p = 0.16$ and $p = 0.10$, respectively; **Table 2**).

Relationship Between Sward Height, Chemical Composition, and *in vitro* Fermentation Parameters

The correlation values among the sward height, nutritive value and *in vitro* fermentation parameters at 48 h are presented in **Figure 1**. The sward height showed a moderate negative correlation with IVDMD ($r = -0.64$), GP ($r = -0.46$), CP ($r = -0.45$), and ME ($r = -0.65$). Conversely, a high and positive correlation ($r = 0.73$) between the ADF (g/kg) and

TABLE 1 | Sward characteristics of herbage samples from the top stratum of five Kikuyu sward height.

Item	Sward heights (cm)					*p*-value	SEM
	10	15	20	25	30		
Sward height (cm)	9.8^e	15.1^d	20.1^c	24.3^b	31.3^a	<0.0001	0.51
Herbage mass (kg DM/ha)	426.0^b	502.0^{ab}	796.0^{ab}	870.3^{ab}	950.3^a	0.01	107.2
Green leaf mass (kg DM/ha)	363.9^b	463.1^{ab}	737.4^{ab}	791.3^a	842.8^a	0.01	93.6
Stem + sheath mass (kg DM/ha)	31.9	24.25	52.94	73.88	91.1	0.09	18.0

Common superscript letters among the same row denote non-significant difference at 0.05 level, as determined by HSD Tukey's test. DM, dry matter; S.E.M, standard error of the mean.

TABLE 2 | Chemical composition and *in vitro* organic matter digestibility (IVOMD) of herbage samples from the top stratum of five Kikuyu sward heights.

Item	Sward heights (cm)					*p*-value	SEM
	10	15	20	25	30		
DM (g/kg of DM)	923.7	913.0	918.0	923.9	919.8	0.10	2.8
OM (g/kg of OM)	907.1	911.6	905.3	905.8	902.1	0.22	2.7
NDF (g/kg of DM)	535.9	541.9	543.1	541.1	545.6	0.98	11.0
ADF (g/kg of DM)	194.1^b	198.7^{ab}	210.9^{ab}	213.1^{ab}	218.8^a	0.02	3.8
CP (g/kg of DM)	316.8	301.8	305.0	302.9	281.3	0.22	8.0
IVOMD (g/kg of OM)	686.6	657.5	635.1	610.7	592.3	0.16	31.0
ME (Mcal/kg of DM)	2.3	2.2	2.1	2.0	1.9	0.10	0.1

Common superscript letters among the same row denote non-significant difference at 0.05 level, as determined by HSD Tukey's test. SEM, standard error of the mean; DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy.

sward height was observed (**Figure 1**). The GP exhibited a high positive correlation with IVDMD ($r = 0.74$) and ME ($r = 0.62$), and at the same time, IVDMD was highly and positively related to ME ($r = 0.84$) (**Figure 1**). The total CH_4 had a moderate and positive correlation with GP ($r = 0.39$); however, it was poorly related to the other variables evaluated. In addition, acetic acid had a strong negative correlation with propionic acid ($r = -0.79$, **Figure 1**). Pearson's correlation of dataset at 24 h (**Supplementary Figure 1**) and the correlation matrix at 24 and 48 h (**Supplementary Tables 1**, **2**, respectively).

The *in vitro* Fermentation Parameters

The GP, expressed as milliliters per unit of dry matter incubated (mL/g DMi), and IVDMD (g) linearly decreased with sward height at both incubation times (24 and 48 h are shown in **Figures 2A,B**, respectively). However, when the GP was expressed as milliliters per unit of *in vitro* digestible dry matter (mL/g IVDMD), it was not related to the sward height either at any incubation time (data not shown). There was no relationship between the total *in vitro* CH_4 production, expressed in terms of milliliters per dry matter incubated (mL/g DMi), and the sward heights studied at any incubation time (data not shown). However, after 24 h of fermentation, the *in vitro* CH_4 production expressed as milliliters per unit of *in vitro* digestible dry matter (mL/g IVDMD) fitted a double linear trend model ($p = 0.060$). The minimum value of CH_4 production at 24 h (15.4 mL/g IVDMD) occurred at 21.3 cm (**Figure 3A**). CH_4 production, first described a straight line slightly inclined but not different between 10 and 20 cm (a1 = −0.22 g mL/IVDMD/cm, $p = 0.32$), and then increased with the sward height (a2 = 0.61 g mL/IVDMD/cm, $p = 0.02$) (**Figure 3A**). Likewise, CH_4 production (mL/g IVDMD) at 48 h tended to increase linearly as a function of sward height (**Figure 3B**).

Meanwhile, the total VFA (mM/L) concentration did not differ between treatments for any incubation time, but it was close to double at 48 h relative to 24 h (data not shown). The main VFA proportions, acetate, propionate, and butyrate (mol/100 mol), were unrelated to sward height at 24 h (data not shown) but significant changes were found after 48 h of incubation. Overall, the acetate, propionate, and acetate: propionate ratio following 48 h of fermentation showed that the minimum methanogenic profile occurred below 30 cm (**Figures 4A,B,D**). The acetate and propionate molar proportions and the acetate: propionate ratio were well described by a double linear model (**Figures 4A,B,D**, respectively). The relationship between acetate (mol/100 mol) and sward height first described a straight line slightly inclined (a1 = −0.09 mol/100 mol/cm, $p = 0.06$) and after 28.4 cm tall, it showed a steeper line with a higher and more significant slope (a2 = 1.55 mol/100 mol/cm, $p < 0.0001$). Conversely, the propionate (mol/100 mol) first increased (increasing slope, a1 = 0.20 mol/100 mol/cm, $p = 0.002$) until 28.42 cm and then decreased (decreasing slope, a2 = −1.34 mol/100 mol/cm, $p < 0.0001$) with sward height. The butyrate showed a negative and linear fit as the sward heights increased ($p < 0.0001$, **Figure 4C**). The acetate: propionate ratio subtly decreased with sward height between 10 and 28.8 cm (decreasing slope, a1 = −0.013 units/cm,

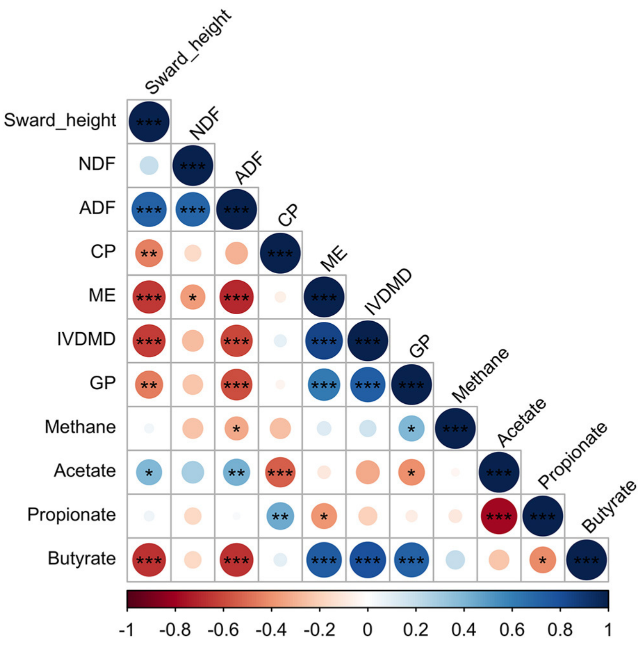

FIGURE 1 | Correlation plot between the sward height, nutritive value, and *in vitro* fermentation parameters at 48 h of Kikuyu grass harvested at different sward heights ($n = 36$). Positive and negative correlation coefficients are displayed in blue and brown scale, respectively. Sward_height, (cm); NDF, neutral detergent fiber (g/kg of DM); ADF, acid detergent (g/kg of DM), CP, crude protein (g/kg of DM); ME, metabolizable energy Mcal/kg of DM; IVDMD, *in vitro* dry matter digestibility (g); GP, Gas production (mL/ g DMi). DMi, dry matter incubated. Methane (total in vitro CH_4 production, ml), acetate, propionate, and butyrate (mol/100 mol). Significance level (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$).

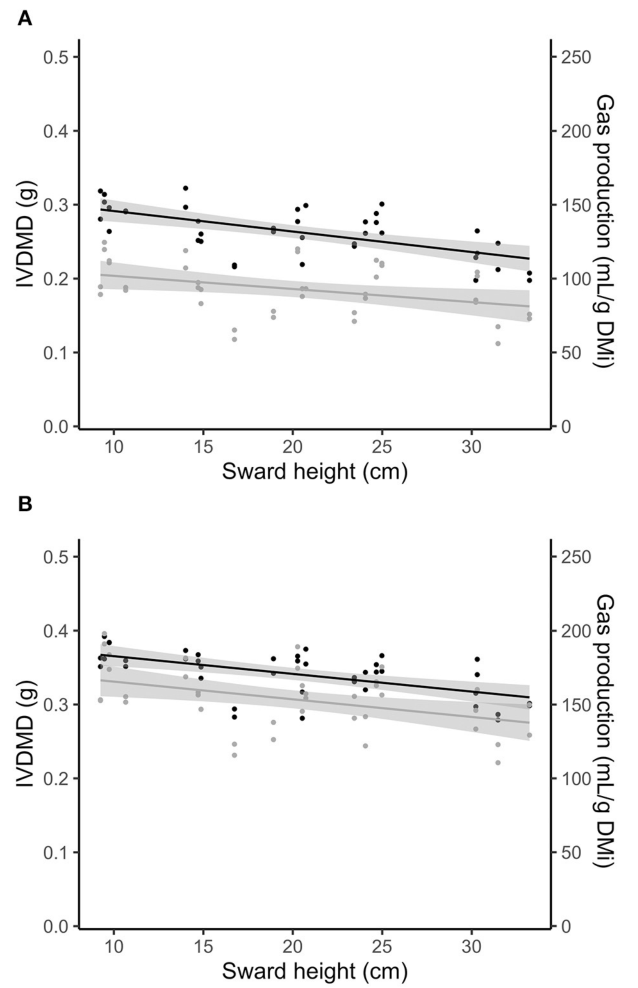

FIGURE 2 | Relationship between gas production (GP, mL/g DMi; gray dots) and *in vitro* dry matter digestibility (IVDMD, g; black dots) and sward height (SH, cm) of kikuyu grass. **(A)** include all data of GP and IVDMD at 24 h of fermentation ($n = 40$); equation for: GP = 110.74–0.90SH, $p < 0.01$), $R^2 = 0.12$. IVDMD = 0.32–0.002SH, $p < 0.0001$, $R^2 = 0.40$. **(B)** include all data of GP and IVDMD at 48 h of fermentation ($n = 40$); equation for: GP = 177.42–1.19SH, $p < 0.05$, $R^2 = 0.16$; IVDMD = 0.32–0.002SH, $p < 0.0001$, $R^2 = 0.32$. DMi, dry matter incubated.

$p = 0.004$) and then increased at sward heights taller than 28.8 cm (increasing slope, a2 = 0.14 units/cm, $p = 0.0001$, **Figure 4D**).

DISCUSSION

Moderate to low-intensity grazing management strategies favor animals to select bites of the top stratum of plants, whose diet is mainly composed of highly digestible leaves with high CP and low fiber content (Savian et al., 2018; Zubieta et al., 2021). This study assessed the effect of the sward height of Kikuyu grass from herbage samples of the top stratum on the *in vitro* ruminal fermentation profile and its relationship with the chemical composition and IVDMD. The key finding was that the sward heights of Kikuyu grass below 30 cm display a profile of VFAs high in propionate and low in acetate, with a trend toward lower CH_4 production per unit of IVDMD. Although the chemical composition between the treatments was similar, the tendency for stem and sheath mass to increase led to an increase in ADF contents and a tendency to decrease the IVOMD with sward height, shifting the fermentation profile toward an *in vitro* rumen environment more favorable to CH_4 production at sward heights above 28 cm.

Sward Characteristics and Chemical Composition

The chemical composition of herbage from the top stratum of the Kikuyu grass showed many similarities between the sward heights. The overall tendency to decrease IVOMD and increase ADF contents with sward height is consistent with the changes in the relative proportions of the leaves and stems + sheath within the top stratum as the sward height increases. In swards of *Cenchrus clandestinus*, Schmitt et al. (2019a) observed that NDF and ADF contents of herbage samples from the upper stratum did not change between 10 and 25 cm heights. Previous studies on the vertical distribution of chemical composition and digestibility of a perennial ryegrass sward showed little variation

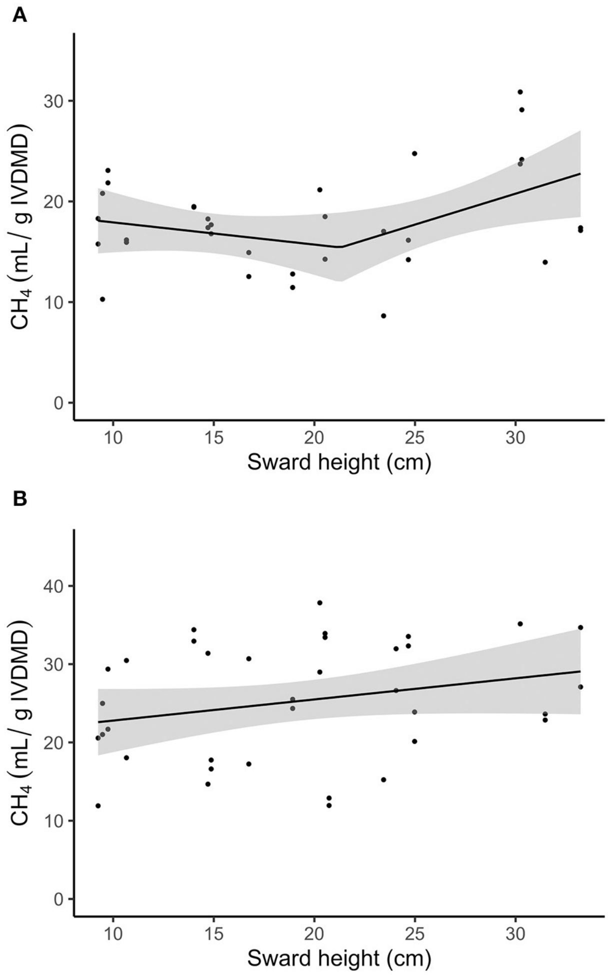

FIGURE 3 | Relationship between *in vitro* CH_4 (mL/g IVDM) and sward height (SH, cm) of kikuyu grass. Equation for: CH_4 = min [15.4–0.22 (SH−21.3)], [(15.4+ 0.61 (SH−21.3)], $p < 0.06$, $R^2 = 0.11$, following 24 **(A)**; and CH_4 = 20.1–0.26SH, $p < 0.12$, $R^2 = 0.04$, following 48 **(B)**.

in NDF and organic matter digestibility at different regrowth ages and at different times of the day (Delagarde et al., 2000). Regardless of the regrowth age, leaves were located mainly in the top stratum, while steams were present mainly in the bottom stratum of Kikuyu pastures; consequently, CP decreased, and NDF and ADF increased with age of regrowth and from top to bottom of the swards (Benvenutti et al., 2020). For a given stratum of the sward, the differences between regrowth age are commonly more marked between vegetative and reproductive stages (Schmitt et al., 2019a; Benvenutti et al., 2020). In the vegetative stage, the nutritive value differs little among plant parts (Laca et al., 2001; Benvenutti et al., 2020).

The results concerning the NDF, ADF, CP, ME, and IVOMD are consistent with those values found from the upper stratum of the Kikuyu sward (Benvenutti et al., 2020). However, CP exhibited higher values than usually reported for the whole plant (Correa et al., 2008; García et al., 2014) or the upper stratum of this species (Schmitt et al., 2019a). Nonetheless, when the nutritional value was evaluated by strata through the vertical distribution, the observed CP values were consistent with the CP content of the upper layer of the plant (Benvenutti et al., 2020). Previous studies have shown that the CP contents of leaves change significantly with anatomical characteristics along the length of leaf blades (Garcia et al., 2021). In addition to the high CP content of the upper stratum due to green leaves, the higher N levels due to fertilization could have influenced the results. According to Correa et al. (2008), the higher CP content (true protein and nonprotein nitrogen (NPN)) in highly fertilized Kikuyu swards is closely related to the higher amounts of ruminal ammonia ($N\text{-}NH_3$) and lower N use efficiency. Even though high N fertilizer rates are common for Kikuyu ryegrass pasture systems, animal excreta on pasture can negatively affect the Nitrogen efficiency of the cows (Marais, 2001; Viljoen et al., 2020) and contribute to nitrous oxide (N_2O) emissions (Maire et al., 2020).

Relationship Between Chemical Constituents and *in vitro* Fermentation Parameters

The strong and positive correlation between GP and the IVDMD at 48 h and the high and positive correlation between ME and GP and IVDMD were expected once GP was directly related to the amount of OM fermented by rumen bacteria, which is consistent with the principles of the *in vitro* gas production technique (Theodorou et al., 1994; Mauricio et al., 1999). It is widely known that GP can be a good index of forage ME content and provides an effective method for assessing the nutritive value of the feeds (Menke and Steingass, 1988). On the other hand, the negative correlation between sward height and GP and chemical components such as ME, IVDMD, CP and at the same time the positive correlation between sward height with the ADF is an interesting result; since the sward height has a consistent correlation with herbage mass and it is a practical and reliable indicator to optimize grazing management (Carvalho et al., 2011; Kunrath et al., 2020).

The chemical composition of forages is influenced by several factors, including sward structure, stage of maturity, season of harvest, and stratum harvested (Benvenutti et al., 2020; Marín-Santana et al., 2020). In general, the correlations between pasture chemical components and *in vitro* fermentation parameters in this study are consistent with previous studies with tropical grasses (Bezabih et al., 2014; Kulivand and Kafilzadeh, 2015), and with other studies using different types of feeds and forages (Getachew et al., 2004). However, unlike expected, CH_4 production had a poor and negative relationship with NDF and ADF content. This discrepancy is probably due to the high variability of CH_4 data at both incubation times. The highly significant correlation between ME and butyrate and the negative relationship between ADF and butyrate indicate the contribution of these components to VFA production (Ungerfeld, 2015).

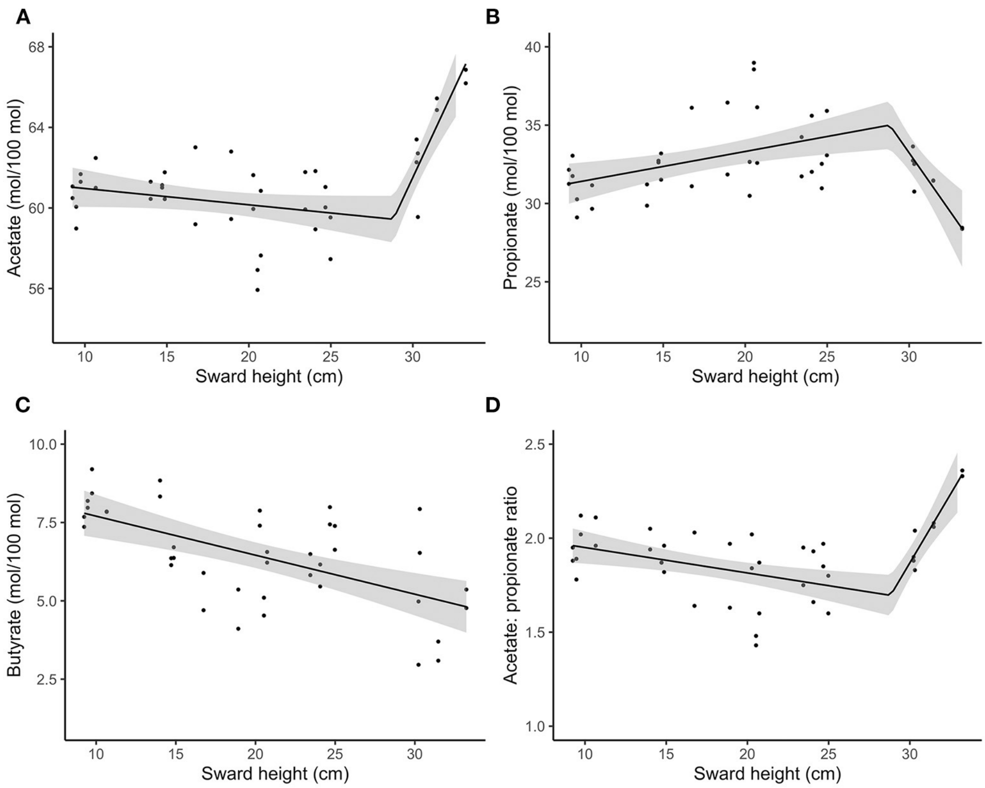

FIGURE 4 | Relationship between VFAs (mol/100 mol) and acetate to propionate ratio with sward height (SH, cm) of kikuyu grass following 48 h of fermentation. Equation for: Acetate = min [59.33–0.09 (SH−28.4)], [59.33 + 1.55 (SH−28.4)], $p < 0.0001$, $R^2 = 0.54$, **(A)**; Propionate = min [35.04 + 0.20 (SH−28.42)], [35.04–1.34 (SH − 28.42)], $p < 0.001$, $R^2 = 0.30$, **(B)**; Butyrate = 8.94–0.12SH, $p < 0.0001$, $R^2 = 0.34$, **(C)**; and Acetate: propionate ratio = min [1.70–0.013 (SH−28.8)], [1.70 + 0.14 (SH−28.8)], $p < 0.0001$, $R^2 = 0.44$, **(D)**.

In vitro Fermentation Parameters

The sward height of Kikuyu grass influenced its nutritive value and *in vitro* rumen fermentation profile. Since the stems + sheath mass tended to increase and IVOMD tended to decrease as a function of sward height, the GP and IVDMD also decreased. As stated above, *in vitro* gas production is a suitable indicator to predict the carbohydrate degradation of forages (Menke et al., 1979; Theodorou et al., 1994; Danielsson et al., 2017). It is widely accepted that the higher the IVDMD is, the higher the GP (Durmic et al., 2010; Meale et al., 2011). Consistently, taller sward heights (>28 cm) displayed a higher methanogenic profile than shorter (10 cm) and intermediate (15, 20, and 25 cm) sward heights due to the changes in morphological components and chemical composition, which resulted in a higher acetate: propionate ratio at 48 h of fermentation. The highest methanogenic profile of sward heights of Kikuyu grass above 28 cm, is due to the tendency of more stems + sheath with the sward height, and the tendency of the ME and IVDMD diminished with the sward height. CH_4 production in an *in vitro* gas system is strongly associated with the fermentation of structural carbohydrates. It has been previously reported that decreasing the digestibility of herbage and increasing the fiber content with advancing plant maturity influences not only total VFA production but also the molar proportions, with greater acetate and lower propionate, and therefore a higher acetate: proportionate ratio and higher CH_4 production per unit of degraded dry matter (Boadi et al., 2002; Beauchemin et al., 2008; Navarro-Villa et al., 2011; Purcell et al., 2011). In our study, the GP reduction as a function of sward height may reflect a higher structural carbohydrate content at taller heights than at shorter heights. Likewise, the trend toward lower *in vitro* CH_4 production with sward height is explained by the lower IVDMD as a function of sward height. Assessing the *in vitro* CH_4 output from different maturity stages of Kikuyu grass, other studies have shown a lower CH_4 production per unit of degraded organic matter (Vargas et al., 2018) and per gram of digestible dry matter (Ramírez et al., 2015), in the youngest forages than in the most mature forages.

The end products of *in vitro* ruminal fermentation, such as the acetate, propionate, and butyrate proportions, are consistent with the data published by other authors (Burke et al., 2006; Marín et al., 2014; Ramírez et al., 2015; Vargas et al., 2018) who also evaluated the *in vitro* fermentation of Kikuyu grass. The lack of differences found in the total VFA concentration and the molar proportions of the main VFAs measured at 24 h may be associated with subtle changes in the fermentation pathways during the first h of fermentation. In agreement with (Meale et al., 2011), batch culture *in vitro* fermentation has a low sensitivity to elucidate small differences between the same type of substrate (e.g., herbage) in the early fermentation. However, prolonged incubation in a closed system potentially favors VFA production changes and their proportions (Ungerfeld and Kohn, 2006), as observed at 48 h. The high molar proportion of acetate and the low of propionate in Kikuyu pastures harvested above 28 cm of sward height matched with a tendency toward more *in vitro* CH_4 output (mL/g IVDMD) and suggested a low *in vitro* rumen fermentation efficiency at tall sward heights. It is also widely known that forages that increase propionate and decrease acetate are often associated with reducing ruminal CH_4 production (Moss et al., 2000; Beauchemin et al., 2009; Meale et al., 2011). Nevertheless, the lower proportion of propionate at smaller heights was unexpected due to the similarities of the chemical composition and IVDMD at sward heights below 25 cm. A possible explanation of this finding could be related to the increase in butyrate concentration at the expense of propionate, as the sward height increases. In this study, the butyrate seems to have acted as an alternative H_2 sink (Moss et al., 2000; Ungerfeld, 2015), which is also in agreement with the trend toward lower CH_4 production per unit of IVDMD (mL/g IVDMD) at sward heights below 28 cm. Changes in the fermentation pathways could be associated with superior CP concentrations and, probably, with the higher nitrate concentration in the evaluated Kikuyu structures as a product of the high N fertilization of the Kikuyu, as suggested by Lovett et al. (2004). Nitrate is an alternative H_2 sink and an effective inhibitor of methanogenesis (McAllister and Newbold, 2008; Van Zijderveld et al., 2010; Yang et al., 2016; Patra et al., 2017). Other studies have suggested that the inclusion of nitrate in *in vitro* ruminal fermentation could increase the molar proportion of acetic acid and reduce the molar proportion of propionic acid (Navarro-Villa et al., 2011).

The similar chemical composition of herbage samples from swards heights of 10, 15, 20, and 25 cm in this study suggests an *in vitro* rumen environment less favorable to CH_4 production, therefore the possibility of flexible grazing management. However, Kikuyu swards managed with the 10 cm sward height target could result in low herbage and green leaf mass, which may affect herbage intake and animal performance (Marin et al., 2017; Schmitt et al., 2019b). Therefore, grazing managers must make strategic decisions considering a holistic management framework.

Another important consideration is that *in vitro* CH_4 production may not reflect the *in vivo* conditions and should be interpreted with care (McAllister et al., 2011; Klop et al., 2017). Therefore, it is recommended to carry out long-term grazing studies that include *in vivo* CH_4 and dry matter intake measurements (Yáñez-Ruiz et al., 2016).

CONCLUSIONS

We conclude that Kikuyu grass harvested below 30 cm displays an *in vitro* profile of VFAs high in propionate and low in acetate, with a performance less favorable to CH_4 production per unit of IVDMD. Our findings suggest that grazing management sward height targets of Kikuyu grass at intermediate sward heights (15 to 25 cm) may be a promising strategy to reduce CH_4 emissions. Further studies based on *in vivo* measurements may be necessary before practical application.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Procedures involving animals were carried out in accordance with the relevant guidelines, regulations, and requirements of Colombian Law No 84/1989 and following protocol, approved by the Ethics Committee of the International Center for Tropical Agriculture (CIAT).

AUTHOR CONTRIBUTIONS

AM, JB, AZ, and PF: conceptualization and methodology. AM and GC: performed the statistical analysis. AM: writing – original draft preparation. AM, JB, AZ, PF, GC, JA, and NC: writing – review & editing. JB, PF, and JA: supervision. JA: project administration. PF, NC, and JA: funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.682653/full#supplementary-material

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