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Research article

Influence of carbohydrate fractions on degradability, rumen fermentation, and methane emission in selected tropical forages using an *in vitro* study

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Abstract

Importance of the work: Understanding of how forage composition affects energy production, nutrient utilization, and methane emissions in ruminants has been enhanced, supporting the development of feeding strategies that improve animal productivity while reducing environmental impact.

Objectives: To examine the contribution of carbohydrates to *in vitro* degradability, rumen fermentation, methane emission, and their interrelationships.

Materials and Methods: Six forage types were evaluated: the Pakchong and Taiwan cultivars of Napier grass (*Pennisetum purpureum*), the Samurai 1 and Samurai 2 varieties of sorghum (*Sorghum bicolor*), sweet corn (*Zea mays saccharata*), and branches of *Indigofera zollingeriana*. The study specifically examined the contribution of carbohydrates to *in vitro* dry matter and organic matter degradability, ruminal fermentation characteristics (pH, ammonia concentration, short-chain fatty acids (SCFAs), and total gas production), methane emissions, and their interrelationships. A completely randomized design was used with the six forage types as treatments, each replicated five times. A randomized group design was used for the *in vitro* fermentation study. Data were analyzed using analysis of variance, and treatment means were compared using Tukey's *post hoc* test.

Results: There were significant ($p < 0.05$) differences in the nutrient contents among the various selected forages tested based on dry matter, crude ash, crude protein, ether extract, crude fiber, and non-fibrous carbohydrates, as well as structural carbohydrates (neutral detergent fiber and acid detergent fiber) and nonstructural carbohydrates (total sugars and starch). Structural carbohydrates were negatively ($p < 0.05$) correlated with forage degradability, total SCFAs, and gas production, while nonstructural carbohydrates were positively ($p < 0.05$) correlated with these factors. There was an inverse correlation with methane emission, indicating the potential of carbohydrates as indicators for ruminal nutrient fermentation.

Main finding: Methane mitigation could be achieved by selecting forage types low in structural and high in nonstructural carbohydrates.

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Introduction

Ruminant animals derive most of their energy from forage, which undergoes microbial fermentation in the rumen to produce short-chain fatty acids (SCFAs), methane (CH_4) and carbon dioxide (CO_2), with SCFAs, such as acetate, propionate, and butyrate, being absorbed through the rumen wall and serving as the primary energy source for ruminants, substantially influencing their health and productivity (Xiao et al., 2020). However, typically, forage contains low protein and high fiber. Fiber can be classified into two main groups: structural carbohydrates (comprising hemicellulose and cellulose) and non-structural carbohydrates (comprising sugars and starches) (Villalba et al., 2021). Structural carbohydrates, which form the cell walls in plants, are more resistant to microbial degradation, requiring cellulolytic microorganisms, such as bacteria, fungi, and protozoa, to break them down. In contrast, non-structural carbohydrates, such as sugars and starches, are stored within plant cells and are more readily fermented by amylolytic bacteria, contributing to rapid SCFA production (Gonzalez-Ronquillo et al., 2023). Various factors, including species, varieties, cultivars, and age, can lead to differences in nutrient contents among different types of forage, ultimately affecting rumen degradability, fermentation characteristics, gas and methane production and nutrient utility in the animal (Yalew et al., 2020; Ridla et al., 2023; Rodrigues et al., 2023).

Tropical grasses (C4 plants), including species such as Napier grass, accumulate energy reserves primarily in the form of starch, whereas temperate grasses (C3 plants) store energy as fructose (McDonald et al., 1991; Heckman et al., 2024). The distinction between C3 and C4 plants extends beyond carbohydrate storage; C4 plants are more photosynthetically efficient under high temperatures and intense light conditions, while C3 plants are better suited to cooler environments (Miyake, 2016). Regardless of their photosynthetic pathways, carbohydrates synthesized via photosynthesis play a crucial role in determining forage quality, degradability, and energy availability for ruminants (Villalba et al., 2021).

Non-structural carbohydrates undergo rapid fermentation in the rumen, resulting in efficient SCFA production, which is essential for the animal's energy metabolism (Waters et al., 2025). These carbohydrates are more easily degraded than structural carbohydrates that require longer microbial fermentation periods (Zhao et al., 2023). Furthermore, the fermentation of non-structural carbohydrates increases the production of propionate, a hydrogen-utilizing SCFA, which mitigates methane emissions by reducing the amount of hydrogen available for methanogenesis (Li et al., 2021). Since methane is a potent greenhouse gas, reducing methane emissions through strategic forage selection is a key focus in sustainable livestock production systems (Ridla et al., 2023).

SCFA production is a critical indicator of the efficiency of carbohydrate fermentation in the rumen, directly influencing energy intake and overall animal performance (Liu et al., 2022). However, an overabundance of non-structural carbohydrates can lead to ruminal acidosis, a condition in which rapid fermentation lowers rumen pH, which subsequently disrupts microbial populations and impairs fiber degradation (Zain et al., 2024). Therefore, achieving an optimal balance between structural and non-structural carbohydrates in forage is essential for maintaining rumen health and maximizing fermentation efficiency.

The composition of structural and non-structural carbohydrates in forage provides valuable insights into its fermentation characteristics in the rumen (Ridla et al., 2023). Despite the widespread use of different types of tropical forage in Indonesia, research has remained limited on the contribution of these carbohydrate fractions to ruminal fermentation, digestibility, and methane mitigation (Jayanegara et al., 2018a; Makmur et al., 2019). Therefore, further investigation is needed to elucidate the roles of structural and non-structural carbohydrates in tropical forages cultivated in Indonesia, with a particular focus on *in vitro* fermentation characteristics, degradability, and methane production.

Techniques using in vitro fermentation are widely used to assess the fermentation potential of forage, providing preliminary insights into gas production and nutrient degradability before *in vivo* trials, which offer more precise measurements of digestibility, feed efficiency, and methane emissions (Scicutella et al., A. 2025). Integrating both approaches is essential for developing effective methane mitigation strategies without compromising ruminant productivity. Therefore, the current study aimed to investigate the contribution of carbohydrates to *in vitro* degradability, ruminal fermentation, methane emissions and their interrelationships, with the overarching goal of developing sustainable feeding strategies that optimize nutrient utilization while mitigating greenhouse gas emissions.

Materials and Methods

Forage selection and sample preparation

The experiment used various types of forage: Napier grass (*Pennisetum purpureum*), cultivars Pakchong (Pc) and Taiwan (Tw), sorghum (*Sorghum bicolor*) varieties Samurai 1 (Sm1) and Samurai 2 (Sm2), sweet corn (*Zea mays saccharata*) (Sc), and *Indigofera zollingeriana* branches (Izb).

The different types of forage were cultivated at the IPB University Research Station (6°28'24.7"S, 107°00'46.8"E) on Ultisol soil under natural rainfall conditions. Precipitation averaged 325 mm/mth during the cultivation period (November–December 2023), with no supplemental irrigation.

Fertilization was performed using both organic and inorganic fertilizers. Chicken manure was applied at planting at a rate of 4 t/ha. Additionally, inorganic fertilizers were applied two weeks post-planting at rates of 100 kg P_2O_5/ha, 100 kg K_2O/ha and 200 kg N/ha.

Forage cultivation was conducted in experimental plots measuring 5 m × 5 m, with five replicates per treatment. Pc and Tw, along with *I. zollingeriana*, were planted at a spacing of 1 m × 1 m, whereas sorghum (Sm1 and Sm2) and sweet corn were planted at a spacing of 20 cm × 75 cm.

Pest and disease management was performed manually by removing pests from the plants. In cases of severe infestations, the pesticide Sagri-beat 7/30 WP (provided by Satya Agro Indonesia) was applied at a concentration of 1 g/L and sprayed onto the plants.

Harvesting was performed based on the optimal growth stage of each forage species. PC and Tw, along with *Indigofera*, were harvested at the peak vegetative stage at 60 days after planting (DAP), while the sorghum and sweet corn were harvested at 80 DAP during the milk stage. Forage harvesting was conducted above the first node, approximately 10 cm above ground level, while the *Indigofera* plants were harvested at 1 m above ground level.

Each forage sample (3 kg) was chopped into 5 cm pieces and sun-dried, followed by oven-drying at 60°C for 48 hr to achieve a uniform moisture content. Then, each dried sample was ground using a laboratory-scale mill and sieved through a 1 mm mesh. Chemical composition analysis and fermentation assessments were conducted in a laboratory at IPB University, Indonesia.

Chemical analysis

The different forage types underwent a comprehensive chemical composition analysis following established protocols. The dry matter (DM) content was determined based on oven-drying at 105°C for 24 hr, the crude ash (CA) content based on combustion in a muffle furnace at 550°C for 6 hr, the crude protein (CP) content using the Kjeldahl method, the ether extract (EE) content based on ether extraction, and the crude fiber (CF) content using sequential digestion with hot acid and alkaline solutions (AOAC, 1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analyzed using the sequential detergent fiber analysis method (AOAC, 2011) with an ANKOM 2000 Fiber Analyzer (provided by ANKOM Technology). All fiber fractions were expressed, excluding residual ash. Non-fibrous carbohydrates (NFC) were estimated using Equation 1:

$$NFC = 100 - (CP + NDF + EE + CA) \quad (1)$$

The total sugar content was quantified using the anthrone method, in which carbohydrates react with anthrone reagent in sulfuric acid to form a yellow-green complex, with absorbance measured at 625 nm (Deriaz, 1961). The starch content was determined using the acid hydrolysis method, where starch is hydrolyzed with perchloric acid and subsequently measured colorimetrically at 540 nm (Pirt and Whelan, 1951).

In vitro fermentation characteristics assessment

Rumen fluid was collected from three Ongole Crossbred cattle at the IPB University slaughterhouse at 0300 hours. The cattle were maintained on a diet comprising 40% forage and 60% concentrate. The rumen fluid was collected in pre-warmed (40°C) insulated thermos bottles to maintain anaerobic conditions and ensure microbial viability (Ridla and Nahrowi, 2025). The handling, slaughtering, and sampling procedures adhered to Indonesian Animal Welfare and Health Regulations (Indonesian Government, 2012; 2021).

An *in vitro* fermentation technique was used to assess forage degradability and ruminal fermentation characteristics. The degradability assay followed the two-stage Tilley and Terry (1963) method, where 0.5 g of each dried and ground forage sample was incubated with 50 mL of a rumen fluid-to-buffer ratio of 1:4 at 39°C for 48 hr. This was followed by secondary digestion for 48 hr in an acid-pepsin solution (pH ~2) to simulate abomasal digestion.

After incubation, the residues were dried at 105°C to determine *in vitro* dry matter degradability (IVDMD), then ashed at 550°C to assess *in vitro* organic matter degradability (IVOMD).

Rumen fermentation characteristics were assessed based on separate *in vitro* incubations, in which each 0.5 g forage sample was anaerobically fermented with 50 mL of a rumen fluid-to-buffer ratio of 1:4 at 39°C for 4 hr. After incubation, the supernatant was analyzed for rumen pH using a digital pH meter (Horiba-18), Ammonia nitrogen ($N\text{-}NH_3$) using the micro-diffusion technique (Conway and O'Malley, 1942), and total short-chain fatty acids (TSCFAs) using the steam distillation method (Kromann et al., 1967).

In vitro gas and methane production were measured following the method of Theodorou et al. (1994). Feed samples (each 0.75 g) were incubated in gas-tight fermentation vessels containing rumen fluid and buffer at 39°C. Total gas production was recorded after 24 hr using a 50 mL syringe, while the methane concentration was analyzed using a Shimadzu 14B gas chromatograph equipped with a Propak column and a flame ionization detector.

All incubations were conducted in triplicate, with five independent fermentation runs, each including a blank control (rumen fluid with buffer only) to correct for endogenous gas and metabolite production.

Experimental design

A completely randomized design (CRD) was employed to assess the effects of varying nutrient levels in the different types of forage on the contents of CA, CP, EE, CF, NFC, NDF, ADF, total sugars, and starch on the results. The experiment included six distinct forage types (treatments), each tested with five replicates.

For the *in vitro* fermentation study, a randomized group design (RGD) was utilized, which is a slight modification of the CRD with the same six forage types but incorporating five groups designated as replicates. These groups were differentiated based on the replicates incubated with rumen fluid.

Data analysis

The data were analyzed using one-way analysis of variance (ANOVA) to determine the effects of different treatments. All statistical analyses were performed using the SPSS software, version 26 (IBM Corporation, 2019). When the ANOVA indicated significant differences among treatment means ($p < 0.05$), post-hoc comparisons were carried out using Tukey's honestly significant difference test to identify specific pairwise differences.

Results

Nutrient content of selected forage types

Based on the nutrient analysis (Table 1), there were significant differences among the forage types. Izb had the lowest ash content (2.45% DM) but the highest organic matter (97.55% DM). Sc had the highest crude protein (11.52% DM), followed by Izb (10.74% DM), Pc (10.52% DM), and Tw (10.07% DM), while Sm2 had the lowest (7.10% DM). The CF content was highest in Izb (38.83% DM), followed by Sm2 (26.52% DM) and Tw (25.75% DM), while Sm1 had the lowest (24.77% DM).

Table 1 Nutrient content of selected forages

Forage type	Dry matter	Crude ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
	--(%)--	(% DM)				
Pc	91.73 ± 1.03	16.07 ± 0.09^{a}	10.52 ± 0.23^{ab}	1.72 ± 0.05^{b}	24.18 ± 1.02^{bc}	47.51 ± 2.21^{b}
Tw	91.29 ± 1.11	15.99 ± 0.15^{a}	10.07 ± 0.31^{b}	2.11 ± 0.04^{a}	25.75 ± 1.11^{b}	46.07 ± 1.96^{b}
Sm1	91.36 ± 0.98	11.63 ± 0.10^{b}	7.83 ± 0.24^{c}	1.63 ± 0.05^{b}	24.77 ± 0.96^{bc}	54.14 ± 2.32^{a}
Sm2	91.00 ± 1.23	8.68 ± 0.11^{c}	7.10 ± 0.19^{c}	2.12 ± 0.03^{a}	26.52 ± 0.89^{b}	55.58 ± 2.44^{a}
Sc	90.77 ± 1.19	9.28 ± 0.12^{c}	11.52 ± 0.21^{a}	1.24 ± 0.01^{b}	23.91 ± 1.01^{c}	54.06 ± 1.99^{a}
Izb	91.13 ± 0.99	2.45 ± 0.06^{d}	10.74 ± 0.22^{a}	0.61 ± 0.01^{c}	38.83 ± 1.23^{a}	47.37 ± 1.89^{b}

DM = dry matter; Pc = Pakchong grass; Tw = Taiwan grass; Sm1 = sorghum Samurai1; Sm2 = sorghum Samurai2; Sc = sweet corn; Izb = *Indigofera zollingeriana* branch.

Values (mean ± SD) in the same column with different lowercase superscripts are significantly different at $p < 0.05$.

Structural and non-structural carbohydrates

As shown in Table 2, Izb had the highest ($p < 0.05$) neutral detergent fiber (NDF) (73.14% DM) and acid detergent fiber (ADF) (49.35% DM), followed by Sm2 and Sm1, suggesting lower degradability. In contrast, Sc had the lowest NDF (62.31% DM) and ADF (40.51% DM), followed by Tw and Pc, indicating better degradability.

For the non-structural carbohydrates, Sc had the highest ($p < 0.05$) total sugar (6.89% DM) and starch (7.06% DM), making it a superior energy source. Tw and Pc had intermediate values, while Izb (3.97% DM; 5.09% DM) and Sm2 (3.80% DM; 5.15% DM) had the lowest, indicating lower energy availability.

Degradability and fermentation characteristics

Table 3 presents the degradability, pH, ammonia (NH_3), and TSCFA concentrations of the different types, essential for evaluating nutritional value and its impact on rumen fermentation.

Degradability: Sc had the highest ($p < 0.05$) *in vitro* IVDMD (65.74%) and *in vitro* IVOMD (63.34%), suggesting superior degradability. Pc and Tw followed closely, while Sm1 and Sm2 had intermediate values. Izb had the lowest ($p < 0.05$) degradability (IVDMD, 61.89%; IVOMD, 60.91%).

Fermentation: There were no significant differences in pH; however, NH_3 concentrations varied ($p < 0.05$) in the range 5.97–8.25 mM. Sc had the highest NH_3 concentration, indicating efficient protein degradation, while Sm2 had the lowest (5.97 mM), suggesting lower protein degradation efficiency. Pc, Tw, and Izb had moderate NH_3 concentrations (6.13–6.59 mM).

TSCFA production: The TSCFA levels, a key indicator of carbohydrate fermentation, varied significantly. Sc and Tw had the highest concentrations (86.32 mmol/L), followed by Pc (78.79 mmol/L), while Sm2 had the lowest (72.65 mmol/L). These differences highlighted the variation in fermentability and energy availability among the different types of forage.

Gas and methane production

Total gas and methane emissions are critical indicators of rumen fermentation efficiency. Table 4 shows that Sc had the highest ($p < 0.05$) gas production (153.16 mL/g DM), followed closely by Tw (152.76 mL/g DM) and Pc. In contrast, Sm1, Sm2,F and Izb had significantly ($p < 0.05$) lower gas production levels. However, these lower-gas-producing forage types emitted more methane than Sc, suggesting the need for more digestible ingredients to improve fermentation efficiency and reduce methane emissions.

Table 2 Structural and nonstructural carbohydrate contents of different types of forage

Forage type	Structural carbohydrate		Nonstructural carbohydrate	
	NDF	ADF	Total Sugar	Starch
	(%DM)			
Pc	65.74 ± 2.12^{c}	42.90 ± 1.78^{c}	4.23 ± 0.23^{bc}	5.83 ± 0.32^{bc}
Tw	65.94 ± 2.08^{c}	42.55 ± 1.82^{c}	5.24 ± 0.18^{b}	6.48 ± 0.24^{b}
Ss1	67.22 ± 1.78^{b}	43.83 ± 1.67^{c}	4.25 ± 0.22^{bc}	5.96 ± 0.31^{bc}
Ss2	67.69 ± 1.99^{b}	47.51 ± 1.99^{b}	3.80 ± 0.20^{c}	5.15 ± 0.28^{c}
Sc	62.31 ± 2.11^{d}	40.51 ± 1.92^{d}	6.89 ± 0.19^{a}	7.06 ± 0.30^{a}
Izb	73.14 ± 2.32^{a}	49.35 ± 1.65^{a}	3.97 ± 0.17^{c}	5.09 ± 0.27^{c}

Dm = dry matter; Pc = Pakchong grass; Tw = Taiwan grass; Sm1 = sorghum Samurai1; Sm2 = sorghum Samurai2; Sc = sweet corn; Izb = *Indigofera zollingeriana* branch; NDF = Neutral detergent fiber; ADF = acid detergent fiber.
Values (mean ± SD) in the same column with different lowercase superscripts are significantly different at $p < 0.05$.

Table 3 Degradability and fermentation characteristics of different types of forage

Forage type	IVDMD (%)	IVOMD (%)	pH	NH_3 (mM)	TSCFA (mmol/L)
Pc	63.53 ± 0.93^{b}	62.15 ± 1.23^{b}	6.90 ± 0.02	6.27 ± 0.43^{bc}	78.79 ± 2.25^{b}
Tw	64.30 ± 1.23^{ab}	62.05 ± 1.11^{b}	6.85 ± 0.01	6.59 ± 0.38^{b}	79.13 ± 2.14^{b}
Sm1	62.48 ± 1.18^{bc}	60.40 ± 1.18^{c}	6.85 ± 0.03	6.15 ± 0.33^{bc}	74.81 ± 2.24^{bc}
Sm2	62.38 ± 1.33^{bc}	60.21 ± 1.22^{c}	6.88 ± 0.02	5.97 ± 0.42^{c}	72.65 ± 1.99^{c}
Sc	65.74 ± 1.04^{a}	63.34 ± 1.06^{a}	6.83 ± 0.04	8.25 ± 0.31^{a}	86.32 ± 2.32^{a}
Izb	61.89 ± 1.21^{c}	60.91 ± 1.19^{c}	6.78 ± 0.06	6.13 ± 0.39^{bc}	76.23 ± 1.89^{bc}

Pc = Pakchong grass; Tw = Taiwan grass; Sm1 = sorghum Samurai1; Sm2 = sorghum Samurai2; Sc = sweet corn; Izb = *Indigofera zollingeria*na branch; IVDMD = *in vitro* dry matter degradability; IVOMD = *in vitro* organic matter degradability; NH_3 = ammonia; TSCFA = total short-chain fatty acid.
Values (means ± SD) in the same column with different lowercase superscripts are significantly different at $p < 0.05$.

Table 4 *In vitro* total gas and methane production of different types of forage

Forage type	Total gas (mL/g DM)	Methane (mL/g DM)	Methane (% total gas)
Pc	135.57 ± 5.34[b]	16.52 ± 1.21[a]	12.19 ± 0.89[a]
Tw	152.76 ± 4.26[a]	15.19 ± 1.19[b]	10.05 ± 1.13[b]
Sm1	128.42 ± 5.31[c]	15.58 ± 2.18[ab]	11.31 ± 1.19[ab]
Sm2	124.05 ± 4.34[c]	16.28 ± 1.11[a]	12.32 ± 0.99[a]
Sc	153.16 ± 3.78[a]	14.39 ± 1.01[c]	9.05 ± 0.89[c]
Izb	135.27 ± 4.22[b]	16.29 ± 0.97[a]	12.35 ± 1.01[a]

DM = dry matter; Pc = Pakchong grass; Tw = Taiwan grass; Sm1 = sorghum Samurai1; Sm2 = sorghum Samurai2; Sc = sweet corn; Izb = *Indigofera zollingeriana* branch.
Values (mean ± SD) in the same column with different lowercase superscripts are significantly different at $p < 0.05$.

Methane production varied significantly ($p < 0.05$) among the different types of forage. Izb (16.29 mL/g DM), Pc (16.52 mL/g DM), and Sm2 (16.28 mL/g DM) produced the most methane, while Tw (15.19 mL/g DM) and Sm1 (15.58 mL/g DM) had moderate outputs. Sc produced the lowest methane emission (14.39 mL/g DM). Methane as a percentage of total gas followed a similar trend, with Izb (12.35%), Sm2 (12.32%), and Pc (12.19%) having the highest values, while Tw (10.05%) and Sc (9.05%) had the lowest.

Correlation between carbohydrates and fermentation characteristics

The correlation matrix (Table 5) illustrates the relationship between forage carbohydrate content and fermentation parameters (degradability, TSCFA and gas and methane production levels).

Structural carbohydrates (NDF and ADF) had strong negative correlations ($p < 0.05$) with TSCFA (correlation coefficient, r = -0.76 and -0.77, respectively) and gas production (r = -0.61 and -0.69, respectively), indicating reduced fermentability. Conversely, nonstructural carbohydrates were positively correlated with TSCFA, with total sugar (r = 0.79) and starch (r = 0.71) enhancing fermentation. Similarly, total sugar (r = 0.78) and starch (r = 0.72) were positively associated with gas production.

Forage degradability was negatively correlated ($p < 0.05$) with structural carbohydrates, as NDF (r = -0.83) and ADF (r = -0.84) reduced IVDMD and IVOMD. Total sugar (r = 0.79) and starch (r = 0.77) positively influenced degradability, highlighting the superior degradability of forage rich in nonstructural carbohydrates.

Methane production was positively correlated ($p < 0.05$) with structural carbohydrates, with methane concentration linked to NDF (r = 0.67) and ADF (r = 0.71). In contrast, nonstructural carbohydrates were negatively correlated with methane emissions, with total sugar (-0.82) and starch (-0.88) reducing methane. These findings suggested that increasing nonstructural carbohydrates might mitigate methane emissions, while structural carbohydrates contributed to greater methane production.

Correlation between methane emission and fermentation characteristics

Table 6 shows significant negative correlations between methane production and key fermentation parameters. Methane was negatively correlated with IVDMD (r = -0.81), IVOMD (r = -0.63), ammonia (r = -0.86), TSCFA (r = -0.75) and total gas production (r = -0.76). Similarly, methane was negatively correlated with IVDMD (-0.89), IVOMD (-0.74), ammonia (-0.88),

Table 5 Correlation coefficient between carbohydrates content and fermentation characteristics

Parameter	Structural carbohydrates		Nonstructural carbohydrates	
	NDF (% DM)	ADF (% DM)	Total sugar (% DM)	Starch (% DM)
IVDMD (%)	-0.83*	-0.81*	0.79*	0.79*
IVOMD (%)	-0.84*	-0.82*	0.78*	0.77*
TSCFA (mmol/L)	-0.76*	-0.77*	0.79*	0.71*
Total gas (mL/g DM)	-0.61*	-0.69*	0.78*	0.72*
Methane (mL/g DM)	0.67*	0.71*	-0.82*	-0.88*
Methane (% total gas)	0.73*	0.79*	-0.85*	-0.84*

DM = dry matter; IVDMD = *in vitro* dry matter degradability; IVOMD = *in vitro* organic matter degradability; TSCFA = total short-chain fatty acids; NDF = neutral detergent fiber; ADF = acid detergent fiber.
* = significant at $p < 0.05$

Table 6 Correlation coefficient between methane emission and fermentation characteristics

Parameter	IVDMD (%)	IVOMD (%)	pH	NH_3 (mM)	TSCFA (mmol/L)	Total gas (mL/g DM)
Methane (mL/g DM)	-0.81*	-0.63*	0.25	-0.86*	-0.75*	-0.76*
Methane (% total gas)	-0.89*	-0.74*	0.16	-0.88*	-0.82*	-0.85*

DM = dry matter; IDMD = *in vitro* dry matter degradability; IOMD = *in vitro* organic matter degradability; NH_3 = ammonia; TSCFA = total short-chain fatty acid.

* = significant at $p < 0.05$.

TSCFA (-0.82) and gas production (-0.85). These results indicated that improving degradability and fermentation efficiency could reduce methane emissions, enhancing both environmental sustainability and livestock nutrition.

Discussion

Nutrient content of different types of forage

The variations in nutrient content among the different types of forage emphasized the necessity of considering specific nutrient requirements when selecting forage options for livestock or other end-uses. Differences in nutrient composition may arise due to species, variety, cultivar, fertilization practices, harvesting age, and climatic conditions (Yalew et al., 2020; Ridla et al., 2023; Rodrigues et al., 2023). The nutritional suitability of a particular forage depends on its composition and intended application.

CP is a critical nutrient in animal feed, essential for growth, development, and tissue repair in ruminants by providing amino acids necessary for synthesizing tissues such as muscle, bone, hair, and organs (McDonald et al., 2022). Conversely, adequate fiber intake is crucial for maintaining rumen health and preventing disorders like acidosis, which can arise when dietary fiber is insufficient (McDonald et al., 2022). Among the different types of forage tested, Sc and Pc had the highest CP levels, making them valuable protein sources. On the other hand, Sm2 had the highest crude fiber content, which might be suitable for specific agricultural or dietary applications. These findings aligned with studies on the nutrient composition in different types of forage such as Pakchong grass (Mohamad et al., 2022), Taiwan grass (Malahubban et al., 2021), sorghum (Puteri et al., 2015), sweet corn crop residues (Sharvan et al., 2020) and *I. zollingeriana* (Antari et al., 2022).

Differences in the structural and nonstructural carbohydrate contents across the different types of forage tested highlighted the need for selecting forage types that aligned with specific nutritional needs. Forage with a higher structural carbohydrate content, such as Izb and Sm2, may require additional processing or supplementation to enhance ruminal degradability. On the other hand, forage rich in nonstructural carbohydrates, such as Sc, Tw, and Pc, provide greater energy availability, making them more suitable for energy-dense diets or specific livestock feeding strategies (Ahvenjärvi et al., 2024). Hamid et al. (2007) and Ridla et al. (2023) documented variations in the carbohydrate composition among different forage species. Additionally, Ismartoyoa et al. (2022) noted that NDF and acid ADF levels often indicated reduced forage degradability, while a higher starch content enhanced nutrient utilization and feed digestibility (Luo et al., 2017).

Degradability and fermentation characteristics

The pH value across the different types of forage remained relatively consistent, suggesting that these forage types supported a stable ruminal environment. The observed pH range (6.78–6.90) was within the optimal range for a healthy rumen (Permana et al., 2022), facilitating effective microbial digestion and fiber fermentation. The absence of significant differences in pH indicated that the different types of forage tested were unlikely to cause substantial shifts in ruminal acidity, thereby reducing the risk of rumen acidosis or alkalosis (Baek et al., 2021).

Ammonia (NH_3) concentration in rumen fluid plays a critical role in microbial protein synthesis and nitrogen utilization. For example, McDonald et al. (2022) reported that typical optimal NH_3 levels for microbial growth typically were in the range 6–21 mM. The NH_3 levels in the current study were within this range, supporting microbial activity in the rumen. Forage such as Sc, with a higher NH_3 concentration, might enhance microbial growth and nutrient utilization, whereas lower NH_3 levels, such as in Sm2, would suggest the need for strategies to improve protein degradability and nitrogen availability. Factors influencing the NH_3 concentration include nutrient profile and protein quality (Jayanegara et al., 2016) and the balance between degradable and undegradable protein (Permana et al., 2022).

The TSCFA concentrations in the rumen (72.65–86.32 mM) were within the typical range of 70–150 mmol/l (Baek et al., 2021). TSCFA serves as a primary energy source for ruminants and indicates forage fermentability (Ridla and Nahrowi, 2025). The production of TSCFA is influenced by microbial populations and the availability of digestible carbohydrates (Harris et al., 2021). Generally, higher TSCFA concentrations correlate with increased digestion efficiency and higher NH_3 levels (Kara et al., 2022).

The different types of forage tested in the current study posed challenges related to ruminal degradability, necessitating additional processing or supplementation to optimize their nutritional profiles. Izb, Sm1, and Sm2 exhibited reduced ruminal fermentation efficiency, which might contribute to increased greenhouse gas emissions in ruminant production systems. These findings aligned with Parnian-Khajehdizaj et al. (2023), who reported that species and variety influenced forage degradability. Additional factors affecting degradability include dietary composition (Ismartoyoa et al., 2022), harvesting time post-planting (Ronga et al., 2020), and fertilizer type (Bourih, 2017).

Nutrient content and methane production of different types of forage

The differences in gas and methane production among the different types of forage tested were driven primarily by variations in their structural and non-structural carbohydrate contents, which influenced microbial fermentation pathways and subsequent gas production (Sun et al., 2022).

Forage with high levels of NDF and ADF, such as Izb, Sm1, and Sm2, tended to promote acetate-dominant fermentation. The degradation of structural carbohydrates (cellulose and hemicellulose) by cellulolytic bacteria results in greater acetate production, which generates more hydrogen (H_2), a key substrate for methanogenesis (Jayanegara et al., 2020; Dong et al., 2022). Consequently, the higher fiber contents in different types of forage contributes to elevated methane emissions (Loza et al., 2021).

In contrast, forage rich in non-structural carbohydrates (soluble sugars and starch), such as Sc and Tw, favor propionate production. Propionate formation competes with methanogenesis for hydrogen, thereby reducing methane emissions (Ridla et al., 2023). The significant negative correlation between methane production and IVOMD/IVDMD further supported the notion that enhanced forage degradability shifted fermentation toward propionate and butyrate production, ultimately reducing methanogenesis (Parnian-Khajehdizaj et al., 2023).

Differences in the CP content among forage types also contributes to variations in fermentation end-products. For example, in the current study, the negative correlation between ruminal ammonia (NH_3) concentration and methane emission suggested that efficient nitrogen utilization might enhance microbial growth, leading to a shift in fermentation pathways away from acetate-driven methanogenesis (Astudillo-Neira et al., 2023). Forage with a higher CP content might promote the growth of ammonia-utilizing bacteria, which could contribute to lower methane production.

In the current study, the negative correlation between methane and TSCFA further supported the role of fermentation efficiency in methane mitigation. Forage yielding higher TSCFA concentrations typically facilitates rapid fermentation and increased propionate production, thereby reducing hydrogen availability for methanogenesis (Dhakal et al., 2024). This could explain why sweet corn forage and Taiwan grass, which had higher TSCFA concentrations, were associated with lower methane emissions compared to the more-fibrous forage types.

Thus, the variation in methane emissions among the different types of forage tested was a direct result of their chemical composition, particularly the balance between structural (NDF, ADF) and non-structural (sugars, starch) carbohydrates, as well as the crude protein content. The observed differences in IVOMD, IVDMD, NH_3, and TSCFA aligned with known fermentation pathways, where high-fiber forages promote acetate-driven fermentation and higher methane production, while non-fibrous, starch-rich forages favor propionate-driven pathways with reduced methane output.

Strategies to mitigate methane emissions in ruminants include dietary modifications (Ridla and Nahrowi, 2025) and the incorporation of plant-based additives such as tannins (Jayanegara et al., 2018b; Min et al., 2020) and saponins (Ridla et al., 2021). In addition, synthetic compounds, such as 3-nitrooxypropanol, have been shown to effectively reduce enteric methane production (Jayanegara et al., 2018c), while chitin and chitosan have been explored for their potential to alter ruminal fermentation pathways and suppress methanogenesis (Haryati et al., 2019). Selecting forage with an optimal balance of structural and nonstructural carbohydrates is another crucial strategy, as a lower structural carbohydrate content and higher nonstructural carbohydrate levels enhance ruminal fermentability, thereby reducing methane emissions while maintaining efficient rumen function and livestock productivity (Ridla et al., 2023). Ku-Vera et al. (2020) provided a comprehensive review of methane mitigation strategies in ruminants fed tropical forage, highlighting the role of forage composition in influencing fermentation dynamics. Furthermore, Ikhwanti et al. (2020) demonstrated strong correlations between the sugar, acid-soluble polysaccharide and total phenolic contents in tropical legumes with *in vitro* nutrient fermentability, suggesting that specific forage characteristics could be leveraged to optimize ruminal efficiency and minimize methane output.

Conclusion

Of the different types tested, the sweet corn (*Zea mays saccharata*) forage was identified as the most suitable option for reducing methane emissions, having the lowest methane production both in absolute concentration and as a proportion of total gas. The second most favorable forage was the Taiwan grass (*Pennisetum purpureum*), which also had comparatively lower methane emissions.

The reduced methane production observed in the sweet corn forage and Taiwan grass could be attributed to their higher nonstructural carbohydrate content and lower structural fiber fractions. These compositional characteristics promote a shift in ruminal fermentation toward propionate-dominant pathways, thereby reducing hydrogen availability for methanogenesis and mitigating methane emissions while maintaining fermentation efficiency.

Incorporating sweet corn forage and Taiwan grass into ruminant diets should enhance the sustainability of livestock production by reducing methane emissions relative to other forage options, contributing to improved environmental outcomes without compromising rumen fermentation dynamics.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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