Research Article

Impact of Sage, Ivy Gourd and Lemongrass on Rumen Fermentation and Methane Production: An *In Vitro* Study

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Abstract | This *in vitro* study investigated the effects of three phytogenic supplements (PSs)—sage (*Salvia officinalis*), ivy gourd (*Coccinia grandis*), and lemongrass (*Cymbopogon citratus*)—on rumen fermentation kinetics and methane (CH_4) production. The basal diet (60% roughage and 40% concentrate) without PSs served as the control diet, while phytogenic diets (PDs) included 1% dry matter (DM) of each PS and designed as sage, ivy gourd, or lemongrass diets, respectively. Data obtained from the *in vitro* trial were analyzed via one-way analysis of variance. Results indicated that PDs showed the tendency of better rumen fermentation kinetics, i.e., improved organic matter degradability, microbial protein synthesis, energy yield, short-chain fatty acids, and net gas production, compared to the control (P=0.063), while there was no significant differences among all PDs. Sage and lemongrass diets significantly reduced CH_4 production (by 18% and 5%, respectively), while ivy gourd had a minimal impact. Additionally, the CH_4 percentage of net gas and per mg neutral detergent fibre reduced by 16–30% in all PDs, with sage demonstrating the most pronounced reduction (P<0.01). Therefore, supplementation of individual PSs like sage, ivy gourd, or lemongrass at 1% DM of basal diet enhanced rumen fermentation kinetics, whereas both sage and lemongrass effectively reduced CH_4 production.

Keywords | Sage, Lemongrass, Ivy gourd, *In vitro*, Fermentation kinetics, Methane mitigation

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INTRODUCTION

Globally, ruminants generally consume grasses; however, in the Indian subcontinent, especially in Bangladesh, they are commonly given a combination of grasses, inferior rice straw, and concentrates (Rahman *et al.*, 2024a). The inclusion of rice straw or grass as primary components in diets often leads to poor nutrient utilization and contribute to greater environmental pollution (Khonkhaeng *et al.*, 2021). This pollution is primarily caused by methane (CH_4), which is the second most abundant greenhouse gas produced in the rumen by methanogenic archaea (Vargas-Ortiz *et al.*, 2022). A cow daily emits around 132–414 g of enteric CH_4, with a potential for global warming which is 28 times greater than CO_2 over a century (Lee *et al.*, 2017). Additionally, CH_4 production leads to a 10% energy loss from feed, reducing nutrient efficiency and causing a loss of 16–26 g/kg of feed consumed (Hristov *et al.*, 2013).

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To mitigate CH_4 emissions and improve nutrient utilization, researchers are investigating phytogenic supplements (PSs) as natural and sustainable solutions (Kholif *et al*., 2024). Sage (*Salvia officinalis*), a PS, exhibits varying effects on methanogenesis, ruminal fermentation, and nutrient degradation, depending on various doses like 0.5, 1.0, 1.5, and 2.0% dry matter (DM) of diet (Kholif *et al*., 2024). Among these doses, supplementation at 1% DM with basal diet enhance ruminal fermentation, nutrient degradability, and reduces CH_4 emissions, likely due to its phytochemicals, such as tannins, camphor, saponins, rosmarinic acid, and carnosol (Kholif *et al*., 2024).

Similarly, ivy gourd (*Coccinia grandis*) and lemongrass (*Cymbopogon citratus*), as promising PSs, have been used in egg production in laying hens (Rahman *et al*., 2021) and functional milk production in cows (Rahman *et al*., 2024b), respectively, due to their rich content of flavonoids, phenolics, tannins, and saponins. Tannins and saponins present in ivy gourd and lemongrass may reduce protozoa, methanogens, and fungal species by forming sterol complexes in their cell membranes, potentially decreasing enteric CH_4 emissions (Bodas *et al*., 2012; Goel and Makkar, 2012). However, to our knowledge, the anti-methanogenic potential of ivy gourd as a PS remains unexplored. On the contrary, it was reported that lemongrass, when included at 1% along with mangosteen peel (1%) or separately at 2% of the substrate level, significantly improves total gas production, nutrient degradability, and propionic acid production, while reducing CH_4 concentration and methanogens (Prachumchai *et al*., 2024; Totakul *et al*., 2024). Additionally, daily supplementation of lemongrass as a PS (100 g/cattle) has been reported to improve nutrient digestibility, milk yield, antioxidant status, and concentrations of desirable fatty acids (Rahman *et al*., 2024b). It also stimulates microbial protein synthesis and enhances the populations of cellulolytic and amylolytic bacteria in beef cattle (Wanapat *et al*., 2008).

In addition, the composition of basal diets along with PSs namely sage, ivy gourd, and lemongrass—particularly their crude protein levels and fibre fractions—are closely associated with CH_4 emissions (Bashar *et al*., 2024). When these PSs, particularly sage and lemongrass, are included at low levels (typically 1% of DM), their effectiveness is mainly attributed to secondary metabolites rather than primary nutrients (Kholif *et al*., 2024; Prachumchai *et al*., 2024). Moreover, the chemical composition of these PSs is influenced by various factors such as plant variety, region, soil type, and cultivation practices, which may influence rumen fermentation patterns and CH_4 production (Yalew *et al*., 2020). Among various varieties, lemongrass (*Cymbopogon flexuosus*) exhibited variable fibre fractions, with cellulose, hemicellulose, and lignin contents approximately 39.5%, 22.6%, and 28.5%, respectively (Madhu *et al*., 2017). However, to the best of our knowledge, the nutrient composition—particularly the fibre fractions and the anti-methanogenic properties of these PSs have not been studied in this subcontinent. Consequently, this study (*in vitro*) was designed to evaluate the composition of shrub (sage) and herbs (ivy gourd and lemongrass) and their effects on rumen organic matter degradability, microbial protein synthesis, digestible energy, metabolizable energy, short-chain fatty acids, net gas, and CH_4 production.

MATERIALS AND METHODS

Phytogenic Supplements Preparation

The experiment was under taken in the Department of Animal Nutrition at Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Phytogenic supplements (PSs), including sage (*Salvia officinalis*), ivy gourd (*Coccinia grandis*), and lemongrass (*Cymbopogon citratus* DC. staf.), were cultivated and harvested at 65 days post-planting (Rahman *et al*., 2021; Rahman *et al*., 2024b) from the Shahjalal Animal Nutrition Field Laboratory (SANFL) at BAU. The harvested PSs were oven-dried at 60°C and subsequently ground using a locally manufactured grinder equipped with a 1 mm sieve. Then, each PS was placed in a zipper-sealed bag and stored in a desiccator with silica gel at room temperature to maintain its stability and prevent moisture absorption.

Experimental Diets and Design For *In Vitro* Study

Green roughage namely Napier grass (*Pennisetum purpureum*) and dried rice straw were collected from the SANFL, BAU. Concentrate items, including corn, mustard oil cake, soybean meal, and wheat bran, were procured from the local market. These ingredients were oven-dried at 60°C and ground to a particle size of 1 mm using a locally made grinder. Then, the concentrate mixture was prepared on a dry matter (DM) basis by combining corn, mustard oil cake, soybean meal, wheat bran, di-calcium phosphate, and salt at 15.72, 9.60, 4.96, 8.00, 0.50, and 1.00%, respectively. The basal diet was formulated considering the roughages and concentrate mixture ratio at 60: 40 and designed as the control diet without PS (Table 1). The phytogenic diets (PDs) included the basal diet supplemented with 1% DM of sage, ivy gourd, or lemongrass and considered as sage, ivy gourd, or lemongrass diet, respectively. The control diet and three PDs were arranged in a completely randomized design.

Chemical Analysis

The proximate components namely DM, ash, crude protein (CP), crude fibre (CF), and ether extract (EE) of the basal diet, Napier grass, rice straw, concentrate mixture, and PSs including sage, ivy gourd, and lemongrass powder were determined in accordance with the AOAC, (2005). The fibre components of these samples, including neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL), were determined following the procedures described by Goering and Van Soest, (1970). Then, hemicellulose was calculated by subtracting the ADF from the NDF; whereas cellulose was quantified by subtracting the ADL from the ADF. Besides, non-structural carbohydrate (NSC) were calculated using the following formula, NSC (%) = 100 – % of (CP + ash + NDF + EE). The chemical composition of the basal diet, Napier grass, rice straw, and concentrate mixture are presented in Table 1.

Table 1: Ingredients and nutrient composition of the basal diet and nutrient composition of grass, straw, and concentrate mixture.

Ingredients	**g/kg dry matter**			
Napier grass	498.3			
Rice straw	103.9			
Corn	157.2			
Mustard oil cake	96.0			
Soybean meal	49.6			
Wheat bran	80.0			
Di-calcium phosphate	5.0			
Salt	10.0			
Chemical constituents	**% on dry matter**			
	Basal diet	**Napier grass**	**Rice straw**	**Concentrate mixture**
Organic matter	89.18	88.87	83.02	92.67
Crude protein	13.45	7.91	5.31	21.30
Crude fibre	25.07	37.44	40.00	5.48
Ether extract	3.32	4.97	3.34	4.82
Ash	10.82	12.13	16.98	7.85
Nitrogen free extract	47.33	38.55	34.36	61.07
Neutral detergent fibre	54.01	79.19	79.52	21.20
Acid detergent fibre	34.66	46.37	47.82	8.00
Nonstructural carbohydrate	18.40			
Acid detergent lignin	6.12	-	-	-
Cellulose	28.54	-	-	-
Hemicellulose	19.35	32.82	31.70	13.20

Ethical Approval and Rumen Liquor Collection

The rearing of cannulated beef cattle and the rumen liquor collection system were approved by the Animal Welfare and Experimentation Ethics Committee (AWEEC/BAU/2025 (2) /15(b)). The *in vitro* study was conducted in two phases. In the first phase, rumen liquor was collected from two Holstein crossbred beef cattle (390 ± 30 kg) at a local slaughterhouse using a pre-warmed conical flask filled with CO_2. The flask was placed in a thermo flask with hot water (39°C) to maintain temperature during collection and transportation. The rumen liquor was then homogenized and filtered through a two-layer cheesecloth into a CO_2-filled warm flask. In the second phase, rumen liquor was collected from one Holstein crossbred cattle (350 kg) and one cannulated Indigenous Zebu beef cattle (198.5 kg) following the same procedure. The cannulated animal, housed at SANFL, BAU, was maintained on a 52: 48 roughage-to-concentrate diet. Cattle was supplied a daily ration of around 12 kg German grass and 2.20 kg of a concentrate mixture (66.4% corn, 16.3% wheat bran, 15.4% mustard oil cake, 0.9% di-calcium phosphate, and 1.0% salt), with *ad libitum* water access. To accurately represent diet digestibility across both genetic types and avoiding variability from slaughterhouse sampling, rumen liquor was collected from a cannulated Indigenous Zebu cattle under controlled conditions and from slaughterhouse-sourced Holstein crossbred cattle.

In vitro Rumen Fermentation Kinetics

For both phases, *in vitro* gas production was measured described by Menke *et al.* (1979). For each phase, after measuring the basal diet (200 ± 10 mg) without PSs (control diet) or with PSs at 1.0% of DM (PDs: sage, ivy gourd, or lemongrass), fourteen piston syringes (2 per diet, 2 blanks, 2 hay and 2 concentrate standards) fitted with piston and long needle attached to silicon rubber tube was incubated at 39°C from the afternoon. The following day, one part of filtered liquor was mixed with two parts of an incubation medium saturated with CO_2 and stirred using a magnetic stirrer in a 39°C water bath to prepare the liquor-incubation medium. Subsequently, 30 mL of the rumen liquor-incubation medium was dispensed into each pre-warmed (39°C) piston syringe using an automatic pipette. After eliminating gas bubbles, the plastic clip on the silicon tube was secured, and the initial piston position was recorded. The syringes were then placed in an incubation apparatus set to rotate at a rate of one rotation per minute for 24 hours. The first reading was taken after 8 hours, followed by gas elimination, and the second reading was recorded at 24 hours.

Considering both hours reading of the gas for each diet, blank, hay, and concentrate standards, the net gas production (mL per 200 mg of DM) was determined for each diet (Menke *et al.*, 1979). Then, using net gas production, CP, and EE of each diet, digestible energy (DE), metabolizable energy (ME), and organic matter (OM) degradability were calculated according to Menke *et al.* (1979). Short-chain fatty acids (SCFAs) were estimated using the formula of Njidda (2010), while microbial protein (MP) synthesis was calculated as 19.3 g microbial nitrogen per kg OM degradability, following Khattab *et al.* (2016).

Methane and pH Measurement

After 24 hours reading, the silicon tube was inserted into the nozzle of a syringe containing 2 mL of 10 M NaOH solution. After securing the setup, the tube's clip was loos-

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ened, allowing gas to flow from the piston syringe into the NaOH solution. The displacement of NaOH in syringe indicated CH_4 volume, as CO_2 dissolves in the solution (Fievez *et al*., 2005). CH_4 generation per mL of NaOH was calculated based on the total gas volume injected and the corresponding NaOH displacement. The CH_4 percentage in the total gas was determined from the CH_4-to-CO_2 ratio, while CH_4 production per mg of NDF per hour was calculated using the NDF content of the diets. Immediately after gas collection, rumen fluid pH was measured using a pH meter (Hanna, China).

Statistical Analysis

All samples were analyzed in triplicate. Differences among the control diet and PDs were assessed using one-way ANOVA in SPSS 22. Tukey's post-hoc test was applied to compare means among diets, with significance declared at P≤0.05 and tendency toward significance at P≤0.10. Then, data were presented as mean ± standard deviation.

Table 2: Nutrient profiles of phytogenic supplements used in the *in vitro* study.

Proximate components	Sage	Ivy gourd	Lemongrass
% DM on fresh basis	13.76	14.21	25.91
% components on DM basis			
Organic matter	87.30	84.26	91.32
Crude protein	6.78	14.25	5.04
Crude fibre	18.79	20.08	25.11
Ether extract	3.76	6.60	5.35
Ash	13.75	14.48	8.68
Nitrogen free extract	56.92	44.59	55.82
Neutral detergent fibre	33.81	35.93	67.86
Acid detergent fibre	16.54	13.59	39.47
Nonstructural carbohydrate	27.90	28.74	13.07
#TPC (mg GAE/g dry sample)	85.1	4.17	19.83
#TFC (mg QE/g dry sample)	9.07	6.27	10.51

% DM: Percentage dry matter; #Reference value: Rahman *et al*., 2021; Rahman *et al*., 2024b; TPC: Total phenolic contents; GAE: Gallic acid equivalent; TFC: Total flavonoid contents; QE: Quercetin equivalent.

RESULTS

Nutrient Profiles of PSs

Lemongrass showed higher DM (25.91%) content, followed by ivy gourd (14.21%) and sage (13.76%; Table 2) on fresh basis. On a DM basis, OM was higher in lemongrass, followed by sage and ivy gourd. CP content was 14.25% in ivy gourd and 6.78% in sage, with lemongrass showed the lowest (5.04%) value. Lemongrass also had the highest CF (25.11%), followed by ivy gourd (20.08%) and sage (18.79%). Then, EE (6.60%) and ash (14.48%) contents were highest in ivy gourd among three PSs. Lemongrass had higher level of nitrogen free extract (NFE), NDF, and ADF, followed by sage and ivy gourd, while NSC was highest in ivy gourd (28.74%) and lowest in lemongrass (13.07%).

Fibre Fractions of PSs

The cellulose content was highest in lemongrass (30.93%), followed closely by ivy gourd (22.34%), while sage had the lowest cellulose content (17.50%; Figure 1). Besides, the hemicellulose content was highest in lemongrass compared to ivy gourd and sage. The lignin content was highest in lemongrass (8.54%), followed by ivy gourd (3.88%) and sage (3.04%).

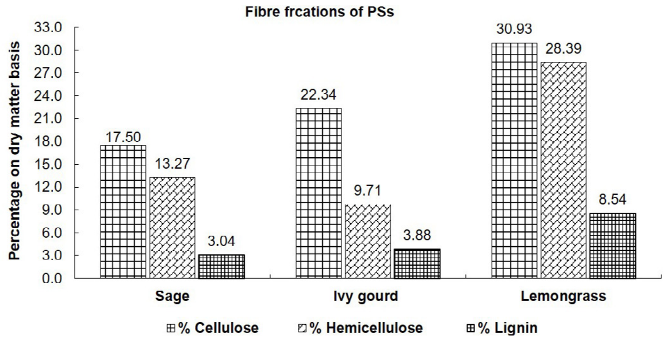

Figure 1: Crude fibre fractions of phytogenic supplements used in *In vitro* study.

In vitro Fermentation Kinetics

pH of rumen fluid exhibited no significant variation across all phytogenic diets (PDs; Table 3). The PDs (sage, ivy gourd, or lemongrass) showed a tendency for higher OM degradability, DE, ME, SCFAs, and MP synthesis compared to the control diet (P=0.063). However, there was no significant differences among the PDs for these parameters (P>0.05). Compared to the control diet, the PDs demonstrated a 7–8% increase in OM degradability, a 7% enhancement in MP synthesis, an 8% improvement in DE, a 10–11% increase in ME, and a 14–17% higher production of SCFAs.

In vitro Gas Production

Compared to the control diet, the PDs namely sage, ivy gourd, or lemongrass showed a propensity of 17–18% higher net gas production in 24 hours or each hour (P=0.06), while there was no variation among all PDs (P>0.05; Table 4). The sage-based PD significantly reduced CH_4 production over 24 hours (Table 4) and on an hourly basis (Figure 2) compared to the control and ivy gourd diet (P<0.05), and no significant difference was observed between the lemongrass and sage diets (P>0.05). Additionally, CH_4 as a percentage of net gas production was 16–30% lower in PDs than in the control, with the lowest value recorded for the sage diet (P<0.01). Similarly, CH_4 production per mg of NDF was decreased by 16–30% in all PDs compared to the control (P<0.01), with sage exhibiting the greatest reduction (Figure 3).

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Table 3: Effects of phytogenic supplements on *in vitro* rumen fermentation kinetics.

Variables	Control diet	Phytogenic diets			SEM	P-value
		Sage	Ivy gourd	Lemongrass		
pH	6.29±0.07	6.31±0.06	6.30±0.05	6.35±0.08	0.02	0.44
OMD (%)	57.33^{b}±0.89	61.58^{a}±2.53	61.91^{a}±3.00	61.87^{a}±2.98	0.75	0.063
DE (MJ/kg DM)	9.93^{b}±0.16	10.70^{a}±0.46	10.76^{a}±0.55	10.75^{a}±0.54	0.14	0.063
ME (MJ/kg DM)	7.80^{b}±0.17	8.61^{a}±0.49	8.68^{a}±0.57	8.67^{a}±0.57	0.14	0.063
SCFAs (mmoL)	0.76^{b}±0.03	0.88^{a}±0.07	0.89^{a}±0.09	0.89^{a}±0.09	0.02	0.063
MP (g/kg DM) synthesis	1.11^{b}±0.02	1.19^{a}±0.05	1.19^{a}±0.06	1.19^{a}±0.06	0.01	0.063

DE: Digestible energy; MJ/kg **DM:** Mega joule per kilogram dry matter; **ME:** Metabolizable energy; **OMD:** Organic matter digestibility; **%:** Percentage; **SCFAs:** Short-chain fatty acids; **mmoL:** Milli moles; **MP:** Microbial protein; g/kg **DM:** Gram per kilogram dry matter.

Table 4: Effects of phytogenic supplements on *in vitro* gas production.

Variables	Control diet	Phytogenic diets			SEM	P-value
		Sage	Ivy gourd	Lemongrass		
Gas production (mL) in 24 hours						
Net gas (mL/200 mg DM)	34.38^{b}±1.17	39.98^{a}±3.33	40.41^{a}±3.95	40.37^{a}±3.92	0.99	0.06
CH_4 (mL/200 mg DM)	6.21^{a}±0.18	5.08^{b}±0.74	6.10^{a}±0.53	5.93^{ab}±0.79	0.18	0.08
% CH_4 of net gas	18.08^{a}±0.73	12.66^{c}±0.85	15.15^{b}±1.32	14.73^{b}±1.64	0.57	0.01
Gas production per hour						
Net gas (mL/200 mg DM)	1.43^{b}±0.05	1.67^{a}±0.14	1.69^{a}±0.16	1.68^{a}±0.17	0.04	0.06
% CH_4 of net gas	0.75^{a}±0.03	0.53^{c}±0.03	0.63^{b}±0.05	0.62^{b}±0.07	0.02	0.01

mL: Milliliter; **mg DM:** Milligram dry matter.

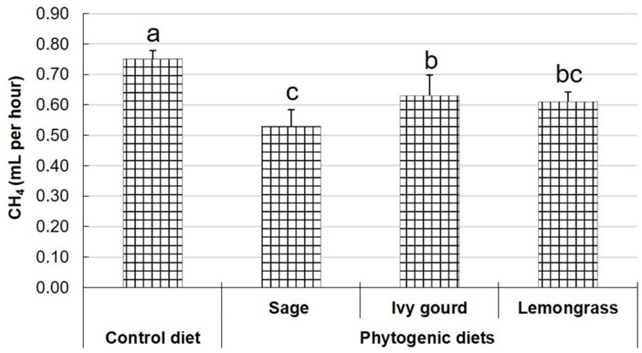

Figure 2: Effects of phytogenic supplements on methane production in *in vitro* system.

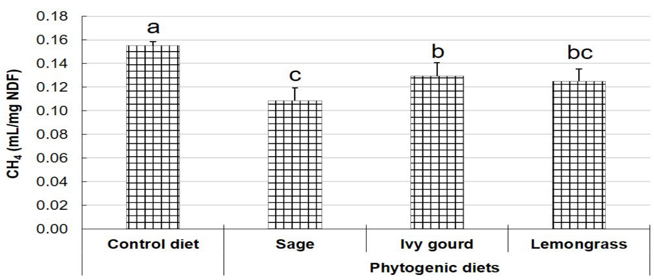

Figure 3: Effects of phytogenic supplements on methane production (mL) per mg NDF in *in vitro*.

DISCUSSIONS

Sage shrub was reported to contain 82.1% OM, 5.6% CP, 9.4% EE, 36.5% NDF, 17.7% ADF, and 30.6% NSC in Egypt (Kholif *et al*., 2024). However, the present study displayed different findings, with higher OM and CP contents but lower EE and ash values compared to previous finding. These variations are likely due to differences in geographical location, climatic conditions, and cultivation practices between Bangladesh and Ethiopia. Bangladesh is characterized by fertile alluvial soils and a humid monsoon climate, whereas Ethiopia features diverse volcanic soils and a climate ranging from arid to temperate. These environmental factors significantly contribute to nutritional differences in shrub (Yalew *et al*., 2020). On the contrary, the chemical composition of lemongrass in the present study nearly aligns with previous finding (Rahman *et al*., 2022), particularly in terms of DM, OM, CF, EE, NFE, ADF, and NSC. This similarity can be attributed to the fact that lemongrass in both studies was cultivated under identical regional conditions, on the same land, and following the same cultivation practices (Yalew *et al*., 2020). However, higher NDF and ADL levels were observed in the current study, which could be attributed to delayed harvesting, leading to increased lignification. Furthermore, the nutritional profile of ivy gourd analyzed in this study exhibited notable differences from the findings of Rahman *et al*. (2021), who assessed only the proximate composition of the leaves. The variations in DM, CF, EE, and NFE contents-except for CP and ash-may be due to differences in fibre fractions, particularly cellulose, hemicellulose, and lignin, which are present in variable concentrations across different plant parts. In addition, data on the cellulose, hemicellulose, and lignin content of sage and ivy gourd specific to this subcontinent were not available. However, their composition may vary depending on land type, cultivation practices, and harvesting processes. Besides, the total phenolic and flavonoid contents in these PSs are relatively high, as reported by Rahman *et al.* (2021, 2024b).

These primary nutrients and secondary metabolites especially phenolic and flavonoids, significantly influence rumen fermentation kinetics, nutrient utilization, gas production, and particularly CH_4 emissions in both *in vivo* and *in vitro* studies (Martínez-Fernández *et al.*, 2013). However, these effects are dose-dependent like more than 1% sage supplements reduce the nutrient degradability (Benchaar *et al.*, 2008). Consistent with the present findings, supplementation of 1% sage (Kholif *et al.*, 2024), or 1% lemongrass combined with mangosteen peel (1%; Prachumchai *et al.*, 2024), or 2% individually (Totakul *et al.*, 2024), improved gas production, nutrient degradability, and rumen fermentation while reducing CH_4 emissions. These current effects may be attributed to the enhancement of beneficial rumen microbes by secondary metabolites, contributing to improved OM degradability, energy yield (DE and ME), MP synthesis, SCFAs, and overall fermentation efficiency (Wanapat *et al.*, 2008; Cieslak *et al.*, 2013). These PSs enhance the activity and population of cellulolytic and amylolytic bacteria (Wanapat *et al.*, 2008; Prachumchai *et al.*, 2024), thereby improving feed degradability which may lead to increased OM degradability, energy yield (DE and ME), SCFAs, and net gas production. In addition, these PSs contain phenolic, flavonoids, tannin, saponin, and terpenoids which are responsible for inhibiting hyper-ammonia-producing bacteria, thus conserving nitrogen for microbial growth and improve the MP synthesis in the current study (Patra and Yu, 2015; Bodas *et al.*, 2012; Oskoueian *et al.*, 2013).

Additionally, the secondary metabolites present in sage (1,8-cineole, thujone, camphor, and other terpenoids) or lemongrass (citral, limonene, and myrcene) were proven to reduce protozoal populations and shift rumen fermentation patterns, leading to increased propionate production (Kholif *et al.*, 2024; Wanapat *et al.*, 2008). This greater availability of propionate helps to utilize hydrogen, which would otherwise be used to produce CH_4, thereby contributing to a reduction in CH_4 production by 18% with sage and 5% with lemongrass in the current study. Previous studies indicated that supplementation with 1% sage (Kholif *et al.*, 2024) and 0.50–0.75 mg/L sage essential oil (Kahvand and Malecky, 2018) has been shown to reduce CH_4 production by approximately 10.34% and 9–13%, respectively. Likewise, the inclusion of 1–2% lemongrass individually or 1% lemongrass with mangosteen peel resulted in a 2–5% and 21.0% reduction in CH_4 production under *in vitro* conditions (Totakul *et al.*, 2024; Prachumchai *et al.*, 2024) which aligns with the current study. Although the present study observed a relatively modest (5%) CH_4 reduction, this outcome is significant given the practical advantages of lemongrass, such as its lower cost, higher biomass yield, and greater DM content compared to sage and ivy gourd (Rahman *et al.*, 2024b). Additionally, low-dose inclusion of lemongrass has been reported to improve dairy cow productivity, immunity, and milk desirable fatty acids, and zinc content (Rahman *et al.*, 2024b, 2024c). In contrast to the findings of the present study, Glorio Patrucco *et al.* (2025) reported that lemongrass supplementation at 0.07% did not significantly reduce *in vitro* CH_4 production, which may be attributed to the relatively low doses of lemongrass essential oil used (Benchaar *et al.*, 2008). Supplementation with either sage or lemongrass at a 1% inclusion level has been shown to reduce the abundance of methanogens (Kholif *et al.*, 2024; Prachumchai *et al.*, 2024). This effect is primarily attributed to key secondary metabolites: citral in lemongrass (Fidriyanto *et al.*, 2021), and thujone and 1,8-cineole in sage (Patra and Yu, 2015), which are known to disrupt the integrity of methanogenic cell membranes, thereby contributing to decreased ruminal CH_4 production in the existing study. Moreover, additional *in vivo* studies are necessary to validate their applicability and confirm their effectiveness under practical feeding conditions. Besides, in the current study, ivy gourd supplementation significantly improved percentage of CH_4 of net gas production but directly failed to mitigate ruminal CH_4 production. This outcome may be attributed to suboptimal concentrations (on DM basis) of anti-methanogenic secondary metabolites, such as tannins (1.2–3.5%) and saponins (0.8–2.2%), present in ivy gourd (Benchaar *et al.*, 2008). Notably, previous study (Goel and Makkar, 2012) has reported that dietary inclusion of tannins at 2.5% and saponins at 0.75% can exert significant anti-methanogenic effects in ruminants.

CONCLUSIONS AND RECOMMENDATIONS

Lemongrass, sage, and ivy gourd showed distinct nutrient profiles, with lemongrass rich in dry matter and organic matter (OM), sage high in nitrogen-free extract and low in fibre, and ivy gourd highest in ash and ether extract. In an *in vitro* study, supplementation of phytogenic diets (PDs) with sage, ivy gourd, or lemongrass at 1% inclusion level exhibited a suggestive trend (P=0.063) toward enhancing OM degradability, microbial protein synthesis, feed energy values, short-chain fatty acids, and net gas production compared to the control diet. Among these PDs, sage demonstrated a notable reduction in CH_4 production,

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whereas lemongrass achieved a modest reduction, which hold practical significance when considering cost-effectiveness and better performance in cattle. The percentage of CH_4 relative to net gas volume was significantly reduced across all PDs, with the sage diet exhibiting the most substantial mitigation effect.

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NOVELTY STATEMENTS

This study provides a comprehensive analysis of the fibre composition including cellulose, hemicellulose, and lignin content of sage, ivy gourd, and lemongrass cultivated in Bangladesh. Notably, it is the first to investigate the methane mitigation potential of ivy gourd as a phytogenic supplement using an in vitro rumen fermentation system. In contrast, the inclusion of either sage or lemongrass at the same inclusion level with the basal diet resulted in significant improvements in rumen fermentation kinetics, along with notable reductions in methane production.

AUTHOR'S CONTRIBUTIONS

Md. Aliar Rahman: Conceptualization, Methodology, Data Collection and Analysis, Writing Original Draft and Editing.
Rakhi Chowdhury: Supervision, Editing and Review.

Conflict of Interest

The authors have declared no conflict of interest.

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