#### RESEARCH ARTICLE

# Assessing deoiled plants biomass of lemongrass and palmarosa as novel feed resources under *in vitro* conditions

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Abstract: The feasibility of newer feed resources must be analyzed for sustainable livestock production addressing food security, climate change, and greenhouse gas emissions concerns. This study aimed to evaluate the nutritional value of lemongrass and palmarosa grass residues after their essential oils are extracted, which are otherwise discarded as wastes and contribute to environmental pollution. Chemical composition, in vitro gas production, in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), and methane production parameters were analyzed for the graded levels of lemongrass and palmarosa grass residues replacing wheat straw up to 50% in the total mixed ration (TMR). In vitro total gas production, IVDMD, IVOMD, and methane production did not differ among the graded inclusion levels of lemongrass residues. In case of palmarosa grass residues, IVOMD was highest at the 20% inclusion level however no significant (p > 0.05) changes were observed. IVDMD and gas production did not differ among the treatments. Methane share was found to be lowest at the 50% inclusion level as compared to the other treatments but no discernible differences (p > 0.05) were observed. These results demonstrate that after the extraction of essential oils, the leftover biomass residues or spent grass can potentially be incorporated in the cattle feed, alleviating dry fodder shortages to some extent and may help achieve sustainability in the livestock production system and reduce its environmental impacts.

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**Keywords:** Cymbopogon flexuosus; Cymbopogon martinii; in vitro dry and organic matter degradability; Microbial biomass production

#### Introduction

Agriculture and animal husbandry are intricately ingrained in human society's cultural, religious, and economic fabric as mixed farming and livestock rearing are fundamental components of rural life (Dagar et al. 2017). Agriculture remains the main source for 70 percent of rural households, with 82 percent of farmers being small-scale operators. On just 2.29% of the world's land area, India caters to approximately 10.7% of the world's livestock population (DADF 2019) and about 17.7% of the human population (UN 2022). These high human and animal populations fight tooth and nail for land resources for food and fodder production. For the last three decades, the area under fodder production has remained stagnant, with only 4-5% of the total cultivated area. Consequently, this caused a shortage of 11.24% green fodder, 23.4% dry fodder, and 28.9% concentrates (Ministry of Agriculture and Farmer Welfare, 2023). Despite these huge shortages, India is the largest milk producer in the world. Total milk production in the country during 2022-23 was 230.6 million tons (DADF 2022). The deoiled biomass was generated (whole plants after essential oil extraction) during the distillation of essential oil from aromatic biomass. Overall it was observed that the average essential oil content in aromatic plants is below 5 % (w/w) which generates a substantial amount of solid biomass of no commercial or environmental friendly use. It has been estimated that annually near about 200,000 tons of deoiled biomass are generated worldwide during extraction of essential oil from essential oil bearing plants (Saha and Basak, 2020).

If strategies like the inclusion of newer feed resources are adopted, there is still room to increase production. The feasibility of newer feed resources must be scrutinized for sustainable livestock production that addresses food security and environmental concerns. Medicinal and aromatic plants (MAPs), such as *Cymbopogon flexuous* (Lemongrass) and *Cymbopogon martinii* (Palmarosa grass), are well-known for their rich tapestry of bioactive compounds such as essential oils, flavonoids and phenolic compounds (Wifek et al. 2016; Hjouji et al. 2024). However, after the essential oils are extracted, the substantial

organic waste is largely discarded and thrown into the water bodies, increasing the biological oxygen demand (BOD). These left biomass residues are often overlooked despite their notable crude fiber and protein content, present considerable opportunities to be incorporated into cattle feed (Manurung et al. 2015). In this context, this research investigated the potential of lemongrass and palmarosa grass residues to be incorporated into cattle feed, alleviating dry fodder shortages to some extent and may help achieve sustainability in the livestock system and reduce its environmental impact. Therefore, this study aimed to evaluate the effects of incorporating these medicinal and aromatic plants residues in cattle diet on gas and methane production, dry matter, and organic matter degradability during *in vitro* fermentation.

#### Materials and methods

### Location of study area and ethical procedure

The experiment was carried out in the Animal Nutrition Division of ICAR-National Dairy Research Institute, Karnal, Haryana – 132 001 with 29.704°N and 76.982°E at an altitude of 245 meters above the main sea level in the Indo-Gangetic alluvial plain. The experimental plan of work was approved by the Institutional Animal Ethics Committee (IAEC) of the Indian Council of Agriculture (ICAR) - National Dairy Research Institute

constituted as per article 13 of the CPCSEA rules laid down by the government of India (IAEC Approval No. - 51-IAEC-24-24 dated 03/03/24).

# Sample collection and chemical analysis of medicinal and aromatic plants residues

The samples of two important and commercially grown aromatic grass like lemongrass (*Cymbopogon flexuosus*) deoiled biomass (LGR) and palmarosa (*Cymbopogon martinii*) deoiled biomass (PGR) were collected after essential oils extraction through the steam distillation. Steam distillation is carried out by passing dry steam through the aromatic biomass, whereby the steam volatile compounds (secondary metabolites) are volatilized, condensed, and collected in oil receivers. Steam distillation is considered a traditional technology for essential oil extraction (Elyemni et al. 2019). The lemongrass and palmarosa grass residues were dried at 60 °C in a hot air oven for 72 h and ground via a 1mm mesh before the chemical analysis and in vitro assays. The chemical composition of the residues is shown in Table 1.

# Chemical and mineral composition of the medicinal and aromatic planta residues

The medicinal and aromatic grass residues received were analyzed for their chemical composition (Goering 1970; Paez et al. 2016).

Table 1 Chemical composition of lemongrass and palmarosa grass residues in comparison to wheat straw

	Lemongrass residues		Palmarosa grass	residues	Wheat straw
	L1	L2	P1	P2	
DM %	$98.65^{a} \pm 0.13$	$98.78^{a} \pm 0.14$	$98.78^{a} \pm 0.10$	$98.49^{a} \pm 0.09$	$90.48^{b}\pm0.41$
CP %	$3.64\pm0.74$	$3.50\pm0.18$	$4.15\pm0.13$	$4.72 \pm 0.18$	$2.92\pm0.09$
EE %	$1.88^{\mathrm{ab}} \pm 0.04$	$2.06^a \pm 0.025$	$2.02^{a} \pm 0.04$	$1.70^{\rm b} \pm 0.05$	$1.91^{ab} \pm 0.09$
NDF %	$79.77^{a} \pm 1.02$	$78.02^a \pm 0.74$	$77.30^a \pm 0.52$	$77.04^a \pm 1.18$	$71.56^{b} \pm 1.07$
ADF %	$39.90^{b} \pm 0.52$	$46.71^{a} \pm 1.12$	$48.66^{a} \pm 1.18$	$47.07^a \pm 1.31$	$42.75^{ab} \pm 1.42$
ADL %	$8.45^{bc} \pm 0.44$	$9.61^{b} \pm 0.24$	$12.13^a \pm 0.14$	$11.30^a \pm 0.41$	$7.49^{\circ} \pm 0.30$
HC %	$39.86^a \pm 0.49$	$31.31^{b} \pm 0.37$	$27.20^{d} \pm 0.66$	$29.97^{bc} \pm 0.33$	$28.81^{cd} \pm 0.48$
Cellulose%	$38.31 \pm 0.55$	$37.64 \pm 1.32$	$38.53 \pm 1.11$	$39.92 \pm 1.03$	$38.50 \pm 0.62$
TA%	$3.10 \pm 0.43$	$5.73 \pm 1.28$	$6.75 \pm 0.54$	$6.14 \pm 1.19$	$8.96 \pm 0.16$

DM = Dry matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; HC = Hemicellulose; TA = Total ash. Different superscripts (a. b, c and d) in a row indicates difference at 5% level of significance.

**Table 2** Mineral concentrations in the medicinal and aromatic grass residues

Minerals	Lemongrass Residues	Palmarosa Grass Residues	
Ca%	$0.80 \pm 0.07$	$0.58 \pm 0.09$	
Mg%	$0.35 \pm 0.04$	$0.35 \pm 0.03$	
Cu (mg/kg DM)	$7.04 \pm 0.35$	$6.88 \pm 0.52$	
Mn (mg/kg DM)	$45.38 \pm 2.36$	$69.05 \pm 4.06$	
Zn (mg/kg DM)	$44.48 \pm 2.93$	$37.78 \pm 5.88$	
Na%	Below level of quantification	Below level of quantification	
K%	$0.66 \pm 0.07$	$1.01 \pm 0.04$	

Each value is the mean of three replicate determinations  $\pm$  standard error mean.

The mineral profile of the medicinal and aromatic grass residues was analyzed by Atomic Absorption Spectroscopy (Hitachi Model z-5000 with Zeeman correction). The calibration of AAS was done with the working standards prepared from commercially available mineral standard solutions (1000  $\mu g/mL$ , Merck, Germany). Specific hollow cathode lamps were used for the determination of the minerals, air as oxidant, and acetylene gas as fuel. The mineral composition of the medicinal and aromatic grass residues is presented in Table 2.

### Nutritional composition of the experimental treatments

Eleven TMRs were evaluated: (CON) concentrate + maize green + wheat straw, (35:35:30% DM), and the LGR and PGR were included at graded levels replacing wheat straw by up to 50% in the treatment groups during the *in vitro* studies. The nutritional composition of the experimental treatments is presented in Table 3.

# Chemical composition of ingredients used for making total mixed ration (TMR)

The total mixed ration was prepared to have forage and concentrate in a 65:35 ratio, with green maize as green fodder, wheat straw as dry fodder, and concentrate mixture. The nutritional composition of various feed ingredients used to formulate TMR for in vitro experiments is shown in Table 4.

#### In vitro studies

Two/ three sets of in vitro trials were conducted to study the rumen fermentation pattern in the substrate having concentrate, green maize, and wheat straw at 35: 35: 30. Lemongrass and palmarosa grass residues were added at inclusion levels of 0, 10, 20, 30, 40 and 50% replacing wheat straw. The trials were conducted in triplicates to estimate parameters viz., total gas production, IVDMD, IVOMD, microbial biomass production, and partitioning factor. These trials were conducted along with respective blanks in triplicates. The substrates used were 200 mg of air-equilibrated samples of TMRs. The incubations were carried out in 100 mL calibrated glass syringes as described by Menke and Steingass (Menke, and Steingass 1988). The substrate was weighed on a plastic boat with a removable stem and was placed into the bottom of the glass syringe without sticking to the sides of the syringe. The piston was lubricated with petroleum jelly and pushed inside the glass syringe. The syringes were incubated in the water bath at a temperature of  $39 \pm 0.5$  °C for 24 hours.

#### In vitro gas and methane production parameters

In vitro gas production (mL/200 mg substrate) was measured by subtracting the final and initial piston readings during the 24 hours. The piston level was recorded (initial reading) and the syringes were placed in the water bath pre-adjusted at  $39 \pm 0.5^{\circ}$ C. The syringes were shaken every 30 minutes for the first 2 h from

the start of the incubation and thereafter every 2 h up to 6 h of incubation. The total incubation period was 24 hours and the piston level was again recorded (final reading). After 24 h incubation, a suitable aliquot of gas was withdrawn from the tip of the incubation syringe using a gas-tight syringe and analyzed for its methane concentration with the help of a Gas chromatograph (Nucon 5700, India) fitted with a stainless-steel column packed with Porapak-N and Flame Ionization Detector (FID).

#### In vitro dry matter and organic matter degradability

After incubation of 24 h, fermentation was arrested by chilling at 4°C, followed by the collection of suitable aliquot of gas for CH<sub>4</sub> estimation; the syringe contents were then transferred to centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes and the pellets were used to estimate *in vitro* degradability of dry matter and organic matter. *In vitro* dry matter digestibility (IVDMD) and true organic matter degradability (TOMD) were calculated from the disappearances of dry matter and organic matter. Partitioning factor (PF) and microbial biomass production (MBP) were calculated based on truly degraded organic matter (TDOM) as described by Blummel et al. (1999) and Blummel et al. (2005) respectively.

#### **Statistical Analysis**

All the data obtained were subjected to a completely randomized design, and the significance of the differences between the means was determined using Tukey's multiple-range test. The study consisted of eleven treatments with three replications. Differences at p < 0.05 were considered statistically significant. All analyses were performed using SAS software, using the following linear statistical model:

$$Yij = \mu + \tau i + \varepsilon ij$$

Where:

*Yij*= dependent variable (%);  $\mu$ = average mean;  $\tau i$ = additives effect of treatment i;  $\varepsilon ij$  = experiment error.

#### **Results and Discussion**

### Chemical composition of medicinal and aromatic plants residues

The proximate composition of lemongrass and palmarosa plants residues is presented in Table 1. Dry matter was higher in both the LGR and PGR samples (L1, L2, P1, and P2) as compared to the wheat straw. Neutral detergent fiber and acid detergent lignin were higher in the medicinal and aromatic plants residues than wheat straw (p > 0.05). Acid detergent fiber and hemicellulose were found to be variable among the two different samples. However, no discernible differences were between these residues and wheat straw. (p > 0.05).

Crude protein and ether extract contents were similar in these medicinal and aromatic plants residues in comparison to wheat straw (p > 0.05).

#### In vitro gas and methane production

No significant differences were observed for total gas production (mL/200 mg DM) among the six graded levels (0%, 10%, 20%, 30%, 40%, 50%) of lemongrass residues replacing wheat straw in the TMR (Table 5). This can be attributed to the proximate composition of lemongrass residues being similar to wheat straw. The total gas production observed among the graded levels varied from 93 to 102 (mL/200mg DM) in different levels of inclusion.

The CH<sub>4</sub> percentage was  $13.69 \pm 0.36$  in the control group of TMR  $13.75 \pm 0.15$  at a 10% inclusion level, followed ( $13.91 \pm 0.34$  at 20%). At the 30% inclusion of LGR, a reduction in CH<sub>4</sub>% was noted ( $12.83 \pm 0.84$ ), and minimal fluctuations for 40 and 50% inclusion levels were there, suggesting variable relationship between the inclusion of lemongrass residues and methane percentage of the total gas produced. However, no differences were noted among the treatment groups (p > 0.05).

During the *in vitro* studies for the inclusion of PGR, at 0% (CON) inclusion of palmarosa grass residues, the total gas production was varied from 80.39 to 93.06% mL/ 200 mg at 10% inclusion level, however, with increased inclusion level, the gas production decreased (P>0.05). While some variability was observed, no significant variations were noted (p>0.05) for total gas production during the *in vitro* assay.

In the control group (CON), methane production was  $13.69 \pm 0.36$ . It slightly decreased to  $12.96 \pm 0.32$  at the (PGR10) 10% inclusion level, followed by a further decrease in methane production at the (PGR20) 20% inclusion level ( $11.99 \pm 0.26$ ). Increased methane percentage was observed in the further increasing inclusion levels of palmarosa grass residues. At the (PGR50) 50% inclusion level of palmarosa grass residues replacing wheat straw, the methane production decreased to  $11.14 \pm 0.10\%$ . However, no significant differences were found (p > 0.05) among the treatment groups.

### In vitro dry matter and organic matter degradability

**Table 3:** TMR composition of the experimental groups during *in vitro* studies

Treatment	Green maize (g/kg DM)	Concentrate mix	Wheat straw (g/kg DM)	Lemongrass residues	Palmarosa grass residues (g/kg DM)
		(g/kg DM)		(g/kg DM)	
CON	350	350	300	-	-
LGR10	350	350	270	30	-
LGR20	350	350	240	60	-
LGR30	350	350	210	90	-
LGR40	350	350	180	120	-
LGR50	350	350	150	150	-
PGR10	350	350	270	-	30
PGR20	350	350	240	-	60
PGR30	350	350	210	-	90
PGR40	350	350	180	-	120
PGR50	350	350	150	-	150

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; LGR10 – same as Control with 10% of wheat straw replaced by lemongrass residues; LGR20 - same as Control with 20% of wheat straw replaced by lemongrass residues; LGR30 - same as Control with 30% of wheat straw replaced by lemongrass residues; LGR40 - same as Control with 40% of wheat straw replaced by lemongrass residues; LGR50 - same as Control with 50% of wheat straw replaced by lemongrass residues; PGR10 – same as Control with 10% of wheat straw replaced by palmarosa grass residues; PGR20 - same as Control with 30% of wheat straw replaced by palmarosa grass residues; PGR40 - same as Control with 40% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues.

**Table 4:** Chemical composition of ingredients used for making total mixed ration (TMR)

Ingredients	CP%	EE%	NDF%	ADF%	TA%	
Maize green	$7.15 \pm 0.26$	$4.95\pm0.08$	$62.09 \pm 1.08$	$36.22 \pm 1.33$	$7.99 \pm 0.11$	
Wheat straw	$2.92 \pm 0.09$	$1.91 \pm 0.09$	$71.56 \pm 1.07$	$42.75 \pm 1.42$	$11.31 \pm 0.30$	
Concentrate mix	$18.7\pm0.53$	$2.95\pm0.24$	$31.42\pm1.23$	$15.15 \pm 0.72$	$8.96 \pm 0.16$	

Each value is the mean of three replicate determinations  $\pm$  standard error mean

(CP- crude protein, EE- ether extract, NDF- neutral detergent fiber, ADF- acid detergent fiber, TA- total ash)

There were no differences among treatments in IVDMD values for the inclusion of both the medicinal and aromatic plants residues replacing wheat straw up to 50% (Table 7). The values for IVOMD also did not show any discernible differences with the graded inclusion levels of both LGR and PGR (p < 0.05) (Tables 7 and 8).

Partitioning factor values ranged from 4.33 (CON) to 5.48 (LGR50) and from 4.33 to 5.54 (CON) to  $5.54\pm0.56$  (PGR50). While some variability existed, no significant variations were found among the treatment groups, indicating no potential changes in microbial activity and fermentation efficiency. Likewise, no differences were found in microbial biomass

Table 5 In vitro total gas parameters and methane production of experimental treatments (Lemongrass residues)

Attributes	CON	LGR10	LGR20	LGR30	LGR40	LGR50	P-value
Gas (mL/200mg	93.06	101.12	102.12±11.59	100.54	96.58	82.48	0.661
DM)	$\pm 7.47$	$\pm 11.80$		$\pm 8.64$	$\pm 10.12$	$\pm 4.37$	
Methane%	13.69	$13.75 \pm 0.15$	$13.91 \pm 0.34$	$12.83 \pm$	$12.80 \pm$	$12.84 \pm 0.2$	0.232
	$\pm 0.36$			0.84	0.17		
Methane mL	$2.56 \pm 0.26$	$2.80 \pm 0.33$	$2.89 \pm 0.39$	$2.67\pm0.39$	$2.52\pm0.29$	$2.14 \pm 0.13$	0.617

F:C = 65:35

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; LGR10 – same as Control with 10% of wheat straw replaced by lemongrass residues; LGR20 - same as Control with 20% of wheat straw replaced by lemongrass residues; LGR40

**Table 6.** In vitro total gas parameters and methane production of experimental treatments (Palmarosa grass residues)

Attributes	CON	PGR10	PGR20	PGR30	PGR40	PGR50	P-
							value
Gas (mL/200mg	93.06 ±	89.45 ±	87.58 ±	68.59 ±	92.63 ±	80.39 ±	0.150
DM)	7.47	4.74	6.07	10.26	3.60	3.88	
Methane%	$13.69 \pm$	$12.96 \pm$	$11.99 \pm$	$11.94\pm0.07$	$12.37 \pm$	$11.14 \pm$	0.065
	0.36	0.32	0.26		0.88	0.10	
Methane mL	$2.56 \pm 0.26$	$2.20\pm0.05$	$2.11 \pm 0.17$	$1.67 \pm 0.26$	$2.29 \pm 0.10$	$1.81 \pm 0.94$	0.054

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; **PGR10** – same as Control with 10% of wheat straw replaced by palmarosa grass residues; **PGR20** - same as Control with 20% of wheat straw replaced by palmarosa grass residues; **PGR30** - same as Control with 30% of wheat straw replaced by palmarosa grass residues; **PGR40** - same as Control with 40% of wheat straw replaced by palmarosa grass residues; **PGR50** - same as Control with 50% of wheat straw replaced by palmarosa grass residues.

**Table 7** *In vitro* dry matter and organic matter degradability, and microbial biomass production of experimental treatments (Lemongrass residues)

Attributes	CON	LGR10	LGR20	LGR30	LGR40	LGR50	P-value
IVDMD	44.55 ±	45.97 ±	44.65 ±	45.11 ±	51.42 ±	48.54 ±	0.336
	0.39	2.44	4.82	0.58	0.81	2.21	
IVOMD	$44.29 \pm$	$46.80 \pm$	$46.46 \pm$	$45.40 \pm$	$49.65 \pm$	$49.86 \pm$	0.667
	0.42	2.84	5.23	0.79	0.31	3.26	
Partitioning factor	$4.33 \pm 0.34$	$3.65 \pm 0.11$	$4.33 \pm 1.05$	$4.13 \pm 0.41$	$4.73 \pm 0.52$	$5.48 \pm 0.56$	0.389
Microbial	$38.92 \pm$	$39.92 \pm$	$39.19 \pm$	$38.53 \pm$	$47.73 \pm$	$53.99 \pm$	0.696
biomass production	3.19	8.97	14.65	5.32	4.74	6.76	

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; LGR10 – same as Control with 10% of wheat straw replaced by lemongrass residues; LGR20 - same as Control with 20% of wheat straw replaced by lemongrass residues; LGR40 - same as Control with 40% of wheat straw replaced by lemongrass residues; LGR50 - same as Control with 50% of wheat straw replaced by lemongrass residues; LGR50 - same as Control with 50% of wheat straw replaced by lemongrass residues.

**Table 8** *In vitro* dry matter and organic matter degradability, and microbial biomass production of experimental treatments (Palmarosa grass residues)

Attributes	CON	PGR10	PGR20	PGR30	PGR40	PGR50	P-
							value
IVDMD	44.55 ±	$48.90 \pm$	51.59 ±	45.09 ±	$44.19 \pm$	44.21 ±	0.060
	0.39	1.37	0.90	2.70	0.56	2.01	
IVOMD	$44.29 \pm$	$51.25 \pm$	$54.15 \pm$	$50.43 \pm$	$50.17 \pm$	$49.09 \pm$	0.053
	0.42	1.80	0.99	2.94	0.07	2.70	
Partitioning factor	$4.33 \pm 0.34$	$5.15 \pm 0.01$	$5.60 \pm 0.35$	$7.07 \pm 1.54$	$4.88 \pm 0.13$	$5.54 \pm 0.56$	0.238
Microbial biomass	$38.92 \pm$	$53.22 \pm 1.3$	$59.46 \pm$	$61.87 \pm$	$49.92 \pm$	$53.73 \pm$	0.108
production	3.19		2.80	9.65	0.79	6.34	

F:C = 65:34

Control – 200mg total mixed ration [Concentrate mixture (35): green maize(35): wheat straw(30)]; PGR10 – same as Control with 10% of wheat straw replaced by palmarosa grass residues; PGR20 - same as Control with 20% of wheat straw replaced by palmarosa grass residues; PGR30 - same as Control with 30% of wheat straw replaced by palmarosa grass residues; PGR40 - same as Control with 40% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced by palmarosa grass residues; PGR50 - same as Control with 50% of wheat straw replaced

production among the treatments due to the inclusion of both the lemongrass and palmarosa grass residues (p > 0.05).

### In vitro gas and methane production

Low *in vitro* gas production parameters observed in the LGR40 and LGR50 groups could be partly explained by the higher acid detergent lignin on ruminal fermentation. The higher indigestible lignin contents could have resulted in more unavailable carbohydrates causing reduced fermentative efficiency (Chesson 1988). However, the inclusion of lemongrass residues was not as much; the effect *on in vitro* gas production did not present any discernible difference. Similarly, when palmarosa grass residues replaced wheat straw, in vitro gas production did not vary with the graded inclusion levels. The findings of the current study are in agreement with those of Fidriyanto et al. (2021) who reported similar gas production when the lemongrass residues replaced 50% of the paddy straw.

The essential oils in the medicinal and aromatic plants are responsible for the reduction in methane emissions but after these essential oils are extracted via steam distillation method, the left biomass residues have inadequate ether content, which did not present any methane reductions in the current investigation. These results align with the findings of Manurung et al. (2015) and Fidriyanto et al. (2021) who found no significant differences on lemongrass waste substitution (p>0.05).

## In Vitro degradability parameters of dry matter and organic matter

In vitro DMD presented no differences on the inclusion of these medicinal and aromatic plants (MAPs) residues replacing wheat straw at graded levels up to 50%. This may be attributed to the similar chemical composition of these MAPs residues and wheat straw and comparatively their lesser incorporation in the total mixed ration. Similar findings were reported for in vitro OMD on

replacing wheat straw with lemongrass and palmarosa grass residues. These results comply with those of Fidriyanto et al. (2021) investigation findings.

The partitioning factor was found to be similar among all treatment groups of the lemongrass and palmarosa grass residues, indicating no potential changes in microbial activity and fermentative efficiency. The microbial biomass production also presented no discernible differences suggesting no potential changes in microbial growth and activity during the *in vitro* assays.

### **Conclusions**

The results revealed that the inclusion of lemongrass and palmarosa grass residues in the replacement of wheat straw as a forage source did not alter the degradability parameters in terms of dry and organic matter, *in vitro* gas, and methane production. The partitioning factor and microbial biomass production were similar in the treatment groups. The results obtained in this experiment indicate that both these unconventional forage sources can be incorporated into ruminant diets. However, further research and *in vivo* trials are required to validate the effectiveness of the inclusion.

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