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Replacement of *Panicum maximum* for Alfalfa and its effect on nutritional

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Abstract
The study was conducted in the laboratories of Razi University in Kermanshah Governorate and Al-Qasim Green University laboratories in Babylon Governorate during the period from 1/11/2024 to 4/5/2025. The aim was to evaluate the effect of gradually replacing alfalfa (AF) with Panicum maximum (PM) at two levels of concentrate feed on gas production. Increasing proportions of PM (100%, 75%, 50%, 25%, 0%) were used to replace AF and mixed with concentrate feed. The concentrate feed proportion in the first five treatments was 60%, while in the remaining treatments it was 70%. The oat hay and PM were mixed with the concentrate feed as a total mixed ration (TMR). Laboratory experiments were then conducted to Parameters estimated included methane production (CH_4), Metabolizable energy (ME), net energy (NE), microbial protein (MP), and short-chain fatty acids (SCFA). The results revealed that the inclusion of PM at 100% of the roughage portion with concentrate-to-forage ratios of 60:40 and 70:30 led to a decline in ME, NE, MP, SCFA, and CH_4. In contrast, increasing the concentrate level to 70% with 30% roughage improved ME, NE, MP, SCFA, and CH_4 values. Furthermore, replacing AF with 75% PM at both concentrate levels enhanced the studied parameters compared to other substitution levels.

Keywords: Gas production, *Panicum Maximum*, volatile fatty acids, net energy

Introduction

Gas production techniques have been extensively applied to evaluate the nutritional value of ruminant feeds, particularly in estimating fermentation characteristics of forages and mixed diets (Getachew *et al*., 2000) [12]. This method allows simultaneous assessment of multiple feedstuffs by measuring gas volume at different incubation times, reflecting the microbial degradation of organic matter and production of volatile fatty acids (VFAs) as well as metabolic gases such as methane (CH_4) and carbon dioxide (CO_2) (Amanzougarene & Fondevila, 2020) [2]. The rate and volume of gas production are closely linked to feed chemical composition and physical structure, with rapidly fermentable carbohydrates promoting higher gas and VFA output, whereas protein contributes partially through ammonia and CO_2 interactions, and fat contributes minimally to gas formation. Volatile fatty acids, including acetate (C2), propionate (C3), and butyrate (C4), are the primary energy source for ruminants, accounting for up to 75% of ME, with acetate representing approximately 70%, propionate 20%, and butyrate 10% (Dijkstra *et al*., 2005; Eom *et al*., 2018) [7, 8].

Methane is predominantly produced in the rumen (87-93% of total methane) through three main pathways: hydrogenotrophic, methylotrophic, and acetoclastic, under anaerobic conditions where microbial populations utilise hydrogen, carbon dioxide, and one-carbon compounds to generate CH_4 (Kebreab, 2006) [17]. Methane emission is influenced by dietary composition, microbial activity, volatile fatty acid ratios, and the efficiency of fermentation; diets high in acetate and butyrate increase CH_4, while propionate reduces it (Brask *et al*., 2015; Reichardt *et al*., 2014; Williams *et al*., 2019) [5, 30, 35].

The composition of ruminant diets also affects the production of Metabolizable energy (ME), net energy (NE), microbial protein (MP), and short-chain fatty acids (SCFAs), which are crucial indicators of feed efficiency and animal performance. Diets with higher concentrate content typically enhance fermentation, gas production, and energy availability compared to high-fibre diets.

Replacing forages like alfalfa with alternative grasses such as Panicum maximum may alter fermentation dynamics, gas production patterns, and microbial protein synthesis, providing opportunities to optimise ruminant nutrition and reduce methane emissions. This study aims to evaluate the effects of gradually replacing increasing proportions of Panicum maximum instead of alfalfa with two levels of concentrated feed to roughage on gas production, volatile fatty acids, methane emission, metabolisable and net energy, and microbial protein synthesis in ruminants, establishing the link between fermentation activity and key nutritional parameters.

Material and Methods

In this experiment, samples of Panicum maxima and AF plants grown in the Jablah area, Babylon province, were used. The samples were collected from various locations in the field, and the crops were harvested at a height of 5 cm above the soil surface to avoid contamination. The collection of Panicum maxima and AF samples was carried out during the seventh cut. Chemical analyses of the forage samples were conducted in the laboratories of the College of Veterinary Medicine and the College of Agriculture at Al-Qasim Green University, in addition to *in vitro* digestion trials performed at the laboratories of Razi University. As for the raw materials that make up the concentrated feed, they were prepared from one of the feed manufacturing plants in Al-Mahaweel District, affiliated with Babil Governorate, and the ingredients included (wheat bran, barley, corn, and soybean meal), in addition to some ingredients from the local market, such as (table salt, sodium bicarbonate, slow-release urea, and a vitamin mineral supplement).

Preparation of Experimental Rations

After preparing the concentrate feed from the mentioned raw materials, it was mixed with different proportions of PM and AF. In the first five treatments, the proportion of concentrate feed was 60% and roughage 40%, while in the remaining treatments (T_6, T_7, T_8, T_9, T_{10}) the proportion of concentrate feed was 70% and roughage 30%, as follows:

- T_1: 60% concentrate, 40% roughage (100% AF, 0% PM)
- T_2: 60% concentrate, 40% roughage (75% AF, 25% PM)
- T_3: 60% concentrate, 40% roughage (50% AF, 50% PM)
- T_4: 60% concentrate, 40% roughage (25% AF, 75% PM)
- T_5: 60% concentrate, 40% roughage (0% AF, 100% PM)
- T_6: 70% concentrate, 30% roughage (100% AF, 0% PM)
- T_7: 70% concentrate, 30% roughage (75% AF, 25% PM)
- T_8: 70% concentrate, 30% roughage (50% AF, 50% PM)
- T_9: 70% concentrate, 30% roughage (25% AF, 75% PM)
- T_{10}: 70% concentrate, 30% roughage (0% AF, 100% PM)

Estimation of Methane Production (CH_4)

Total gas production was measured following Menke (1988) [22] using TMR with graded replacement of Alfalfa by Panicum maximum (100, 75, 50, 25, 0%) combined with concentrate. Samples (200 mg) were incubated in 100 mL syringes with 20 mL artificial saliva and 10 mL filtered rumen fluid from freshly slaughtered sheep. CO_2 was added to ensure anaerobic conditions. Syringes were incubated in a water bath at 39 °C, and gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours. Four replicates per treatment plus four blanks were used. pH was measured, and 4 mL of 4% NaOH was added to two syringes to determine methane (Fievez, 2005). IVOMD (%) and SCFA (mmol/100 mL) were calculated after 96 h incubation using Menke (1988) [22] equations. Gas components were estimated using Orskov and McDonald (1979) [26]:

$$P = a + b(1 - e^{-ct})$$

$$\mathrm{IVOMD}\ (\%) = 14.88 + 0.889\mathrm{GV} + 0.45 \times \mathrm{CP}$$

$$\mathrm{SCFA} = 0.00425 + (0.0222 \times \mathrm{Gas}\ 24)$$

P represents the amount of gas produced at time *t*, a is the gas produced from the rapidly fermentable fraction, and b is the gas produced from the slowly fermentable fraction. C denotes the fermentation rate and gas production of fraction *b*, and t is the time divided by Euler's number e (approximately 2.718). SCFA: Short-chain fatty acids (mmol/100 mL).

Artificial saliva was prepared from: KCl 0.77 g, Na_2HPO_4 2.77 g, $NaHCO_3$ 9.8 g, $MgCl_2 \cdot 7H_2O$ 2.16 g, $CaCl_2 \cdot 2H_2O$ 16 g, NaCl 0.47 g, dissolved in 1 L distilled water.

Measurement of Metabolizable Energy (ME)

Metabolizable energy (ME, MJ/kg DM) was calculated using the following equation according to Menke (1988) [22]:

$$\mathrm{ME} = 2.20 + 0.136\mathrm{GP} + 0.057\mathrm{CP} + 0.0029\mathrm{EE}$$

ME: Metabolizable energy (MJ/kg dry matter), GP: Gas volume produced after 96 hours of incubation (mL/200 mg dry matter), CP: Crude protein (g/kg dry matter), EE: Ether extract (%)

Net Energy (NE)

$$\mathrm{NE} = 0.54 + 0.096\,\mathrm{GAS} + 0.0038\mathrm{CP} + 0.000173\mathrm{EE}$$

Microbial Protein (MP)

The amount of microbial protein produced from the incubated samples was calculated using the equation (Makkar and Becker, 2010):

$$\mathrm{MCP} = \mathrm{OMD} - (\mathrm{GP} \times 2.2)$$

MCP: Microbial protein produced (mg/g of dry matter), GP: Gas volume produced over 96 hours (mL/200 mg of dry matter)

Statistical Analysis

Data were analyzed using SAS software (2018) in a 2×5 factorial completely randomized design. Analysis of variance (ANOVA) was performed, and means were compared using Tukey's test. The statistical model used was Yijk = μ + Ai + Bj + (A*B)ij + eijk. where: Yijk = observed value at the ith level of factor A, jth level of factor B, and kth replication. μ = overall mean. Ai = effect of factor A at level i (i = 1, 2). Bj = effect of factor B at level j (j = 1, 2, 3, 4, 5). (A*B) ij = interaction effect between factors A and B at levels i and j. eijk = random error, assumed normally distributed with mean zero and constant variance.

Results and Discussion

Effect of Concentrate-to-Forage Ratio on Nutritional Value

Table (1) shows the effect of different concentrate-to-forage ratios on several nutritional parameters. The 30:70 treatment showed a significant superiority ($p<0.01$) in all measured traits compared to the 40:60 treatment. ME increased to 11.22 MJ/kg DM, NE to 6.87 MJ/kg DM, and microbial protein to 144.93 mg/100 mL. In addition, short-chain fatty acid (SCFA) concentration rose to 1.31 mmol/100 mL, and CH_4 reached 11.32 mL/200 mg DM.

This improvement is attributed to the higher content of rapidly fermentable carbohydrates and lower content of slowly fermentable fibers in the diet, which enhanced the rate of organic matter degradation and increased volatile fatty acid production, providing a favorable environment for microbial fermentation in the rumen. This also contributed to higher microbial protein synthesis and energy derived from fermentation. The increase in CH_4 is associated with the greater release of hydrogen, which methanogenic microbes utilize to produce methane.

These results are consistent with the findings of Saini *et al.* (2012) [31], Nagadi (2019) [25], and Ramos *et al.* (2021) [28], who reported increases in ME, NE microbial protein, and SCFA concentration in diets with higher concentrate content than forage. Similarly, Suharti *et al.* (2011) [32] and Kumar *et al.* (2013) [18] observed a significant effect of increased concentrate on total volatile fatty acids. Conversely, these findings differ from Iqbal *et al.* (2008) [15] and Nagadi (2019) [25], who reported a decrease in methane (CH_4) production with higher concentrate levels and Panicum maximum inclusion.

Table 1: Effect of Concentrate-to-Forage Ratio on Nutritional Value (Energy and Protein)

Treatment	ME (MJ/kg dry matter)	NE (MJ/kg dry matter)	MP (mg/100 mL)	SCFA (mmol/100 mL)	CH_4 (mL/200 mg dry matter)
C: R 60:40	10.73 b	6.52 b	138.57 b	1.25 b	10.73 b
C: R 70:30	11.22 a	6.87 a	144.93 a	1.31 a	11.32 a
SEM	0.0531	0.0372	0.6799	0.0081	0.0693
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001

Values with different letters (a, b) indicate significant differences ($p<0.01$).

Effect of Gradually Replacing Alfalfa with Panicum maximum on Nutritional Value

The results in Table (2) show the effect of gradually replacing Alfalfa with Panicum maximum on nutritional parameters (energy and protein). Significant increases ($p<0.05$) in ME were observed in treatments T_1, T_2, T_3, and T_4, with values of 11.07, 10.97, 11.07, and 10.96 MJ/kg DM, respectively, compared to T_5, which recorded 10.79 MJ/kg DM. The lower value in T_5 is attributed to the slower fermentation of PM and insufficient synchronization between energy and nitrogen availability. These results align with Hassan *et al.* (2022) [14], who reported increased ME in diets containing a concentrate mixture with 100% AF, and with Meteab *et al.* (2025) [23], who observed higher ME in diets with 100% AF compared to other Panicum maximum replacement levels (50%, 75%, and 100%). NE also showed significant superiority ($p<0.05$) in T_1, T_2, T_3, and T_4, with values of 6.77, 6.70, 6.76, and 6.69 MJ/kg DM, respectively, compared to T_5 (6.57 MJ/kg DM), due to higher digestibility of organic matter and increased volatile fatty acid production. Regarding microbial protein (MP), treatments T_1 and T_3 were significantly superior ($p<0.05$), reaching 142.89 and 143.04 mg/100 mL, respectively, compared to T_5 (139.57 mg/100 mL). The superiority of T_3 is attributed to the combination of rapidly fermentable energy from jet hay and sustained energy from Panicum, providing a temporal gradient that allowed rumen microbes to use nitrogen more efficiently for microbial protein synthesis. The superiority of T_1 is linked to greater microbial diversity and complementary interactions among species. These results are consistent with Hassan *et al.* (2022) [14], who reported that replacing 25-50% of clover with Panicum maximum optimized rumen fermentation and microbial protein production, and with Uzegbu and Ukonu (2022) [33], who observed increased microbial protein with Panicum supplementation.

For SCFA, treatments T_1-T_4 were significantly higher ($p<0.05$), recording 1.30, 1.28, 1.29, and 1.28 mmol/100 mL, respectively, compared to T_5 (1.24 mmol/100 mL). This is attributed to a more favorable rumen environment and the availability of degradable nitrogen, enhancing organic matter fermentation and gas production. These results agree with Abdelrahman *et al.* (2017) [1], Ma *et al.* (2014, 2015) [19, 20], who showed that including clover in lamb diets increases volatile fatty acids, particularly acetate and propionate, improving rumen fermentation efficiency and providing a rapid energy source compared to high-fiber hay.

Regarding methane (CH_4) production, treatments T_1-T_4 recorded significantly higher values ($p<0.05$) of 11.21, 11.04, 11.13, and 11.06 mL/200 mg DM, respectively, compared to T_5 (10.70 mL/200 mg DM). This increase is attributed to the availability of rapidly fermentable carbohydrates in T_1-T_4 due to clover inclusion (100-25%), which increased hydrogen release utilized by methanogenic microbes for CH_4. In contrast, T_5 contained 0% jet hay and 100% Panicum maximum. These findings align with Meteab *et al.* (2025) [23], who reported decreased gas production with increased replacement of clover hay by graded levels of Panicum maximum. They differ from Phesatcha *et al.* (2020) [27], Kang *et al.* (2016) [16], and Anantasook and Wanapat (2012) [3], who reported reduced calculated CH_4 production with higher concentrate levels in the diet.

Table 2: Effect of Increasing Replacement Levels of PM for Jut on DMD in the Rumen Using the in sacco Method

Treatment	ME (MJ/kg dry matter)	NE (MJ/kg dry matter)	MP (mg/100 mL)	SCFA (mmol/100 mL)	CH_4 (mL/200 mg dry matter)
T_1	11.07 a	6.77 a	142.89 a	1.30 a	11.21 a
T_2	10.97 a	6.70 a	141.51 ab	1.28 a	11.04 a
T_3	11.07 a	6.76 a	143.04 a	1.29 a	11.13 a
T_4	10.96 a	6.69 a	141.51 ab	1.28 a	11.06 a
T_5	10.79 b	6.57 b	139.57 b	1.24 b	10.70 b
SEM	0.0531	0.0372	0.6799	0.0081	0.0693
P-Value	0.0004	0.0004	0.0007	0.0001	0.0001

Values with different letters (a, b, c) indicate significant differences ($p<0.05$).

Effect of the Interaction between Concentrate-to-Forage Ratio and Panicum Maximum Replacement for Alfalfa on Nutritional Value

The results in Table (3) indicate that the interaction between concentrate-to-forage ratio and the replacement level of Panicum Maximum for Alfalfa had a significant effect on nutritional parameters (energy and protein). Treatment T_6 showed a significant superiority ($p<0.05$) in ME, reaching 11.40 MJ/kg DM, compared to T_1, T_2, T_3, T_4, T_5, and T_{10}, which recorded lower values of 10.74, 10.73, 10.93, 10.76, 10.48, and 11.10 MJ/kg DM, respectively. This superiority is attributed to the higher content of rapidly fermentable starch at the 70% concentrate level, combined with increased degradable nitrogen, which enhanced microbial mass and efficiency, thereby increasing volatile fatty acid and total gas production. These results differ from Fuller *et al*. (2020) [11], who reported that increases in ME are directly proportional to decreases in feed concentration.

T_6 also exhibited significant superiority in NE, reaching 7.00 MJ/kg DM, compared to other treatments (6.54, 6.53, 6.66, 6.55, 6.35, 6.78). This is due to the abundance of rapidly soluble carbohydrates at the higher concentrate level, providing a quick energy source, while the forage component contributed degradable nitrogen in the rumen, creating optimal synchronization between energy and protein. This enhanced microbial growth and efficiency, accelerating fermentation and energy production.

Microbial protein (MP) was highest in T_6, at 147.16 mg/100 mL, compared to 135.69-143.45 mg/100 mL in other treatments. This improvement is attributed to the ideal synchronization between energy and rumen-degradable protein, allowing optimal nitrogen utilization without losses as ammonia, thus promoting microbial growth and protein synthesis. These findings are consistent with Ramos *et al*. (2009) [29], Broderick (2001) [6], Guo *et al*. (2025) [13], and Mohammadzadeh *et al*. (2021) [24], who showed that increasing concentrate and clover levels or rumen-degradable starch improved microbial protein synthesis and rumen nitrogen utilization efficiency.

SCFA were significantly higher in T_6 (1.35 mmol/100 mL) compared to 1.19-1.29 mmol/100 mL in other treatments. This is associated with high levels of rapidly fermentable starch at the 70% concentrate level, combined with highly digestible forage, which stimulated rumen microbes to accelerate fermentation and total gas production. These results agree with Kumar *et al*. (2013) [18], Phesatcha *et al*. (2020) [27], and Hassan *et al*. (2022) [14], who observed increased SCFA production with higher concentrate diets and inclusion of clover.

Finally, methane (CH_4) production was significantly higher in T_6 (11.59 mL/200 mg DM) compared to 10.28-11.12 mL/200 mg DM in other treatments. This is attributed to increased total fermentation from higher volatile fatty acids, hydrogen release, and microbial proliferation, especially methanogens, which enhanced CH_4 despite relative shifts to propionate. These results are consistent with Eun *et al*. (2004) [9] and Hassan *et al*. (2022) [14], who reported higher CH_4 with high (70%) concentrate diets. Asikin *et al*. (2018) also noted that adequate neutral detergent fiber (NDF) in forage supports digestion and increases gas production over incubation time, aligning with the observed rise in methane due to improved microbial fermentation efficiency. However, these findings differ from other studies (Meteab *et al*., 2025; Van Wyngaard *et al*., 2018) [23, 34].

Table 3: Effect of the Interaction between Concentrate-to-Forage Ratio and Panicum Maximum Replacement for Alfalfa on Nutritional Value (Energy and Protein)

Treatment	ME (MJ/kg dry matter)	NE (MJ/kg dry matter)	MP (mg/100 mL)	SCFA (mmol/100 mL)	CH_4 (mL/200 mg dry matter)
T_1	10.74 de	6.54 de	138.62 de	1.26 c	10.83 d
T_2	10.73 de	6.53 de	138.61 de	1.25 c	10.74 d
T_3	10.93 cd	6.66 cd	141.13 cd	1.28 bc	10.97 bcd
T_4	10.76 de	6.55 de	138.79 de	1.26 c	10.86 cd
T_5	10.48 e	6.35 e	135.69 e	1.19 d	10.28 e
T_6	11.40 a	7.00 a	147.16 a	1.35 a	11.59 a
T_7	11.22 ab	6.87 ab	144.84 ab	1.32 ab	11.34 ab
T_8	11.22 ab	6.87 ab	144.95 ab	1.31 ab	11.29 ab
T_9	11.17 abc	6.83 abc	144.23 abc	1.31 ab	11.27 abc
T_{10}	11.10 bc	6.78 bc	143.45 bc	1.29 bc	11.12 bcd
SEM	0.0531	0.0372	0.6799	0.0081	0.0693
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001

Values with different letters indicate significant differences ($p<0.05$).

Conclusion

The inclusion of PM at 100% of the roughage portion, under concentrate-to-forage ratios of 60% concentrate with 40% roughage and 70% concentrate with 30% roughage, led to a deterioration in metabolizable energy (ME), net energy (NE), microbial protein (MP), short-chain fatty acids (SCFA), and methane gas (CH_4). In contrast, higher concentrate levels (70% concentrate and 30% roughage) resulted in improvements in ME, NE, MP, SCFA, and CH_4. Moreover, the inclusion of Panicum at 75% of the roughage portion with both concentrate levels improved the studied parameters.

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