Research Article

Effect of Replacing Increasing Proportions of *Panicum maximum* Instead of Alfalfa with Two Levels of Concentrate Feed on Nutritional Value and Digestibility Using Laboratory Methods

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Abstract | The study was conducted in the laboratories of Al-Qasim Green University and Razi University, Babil Governorate, from November 1, 2024, to May 4, 2025, with the objective of evaluating the effect of replacing alfalfa (AF) with *Panicum maximum* (PM) at two concentrate feed levels on the nutritional value and digestibility of lamb diets using laboratory methods. Treatments consisted of gradually replacing AF with increasing proportions of PM (0%, 25%, 50%, 75%, and 100%) and combining them with concentrate feed at ratios of 60% or 70%. Oat hay and PM were incorporated into the concentrate as a total mixed ration (TMR). Nutritional evaluation was performed using the *in sacco* and Theodorou methods. The parameters assessed included degradability (DB), effective degradability (ED), rapidly degradable fraction (a), slowly degradable fraction (b), degradation rate (c), rumen fluid pH, ammonia nitrogen (NH_3N), total volatile fatty acids (TVFA), total gas production (TGP), and *in vitro* organic matter digestibility (IVOMD). Results indicated that the inclusion of 50% PM and 50% AF with a higher concentration level (70%) enhanced DB ($P \leq 0.05$). Similarly, diets with 75% PM and 70% concentrate improved ED ($P \leq 0.05$) across passage rates of 0.02, 0.05, and 0.08. Furthermore, 100% PM replacement with either 70% or 60% concentrate increased the rapidly degradable fraction ($P \leq 0.05$). No significant effects of PM substitution were observed on rumen pH, NH_3-N, or TVFA. However, 75% PM inclusion with 70% concentration significantly improved IVOMD and increased TGP ($P \leq 0.05$). In conclusion, incorporating Panicum maximum (PM) at appropriate proportions within the roughage, particularly 50% PM with 50% alfalfa at a 70% concentrate level, or 75% PM with a 30:70 concentrate ratio enhanced degradability, effective degradability, the rapidly degradable fraction, IVOMD, and total gas production, while having no significant effect on rumen pH, NH_3–N, or TVFA.

Keywords | *In sacco* methods, *Panicum maximum,* Theodorou methods, Dry matter degradability

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INTRODUCTION

Forage is vital for the nourishment of ruminant animals; a lack of fresh or processed forage leads to reduced livestock populations and consequently lowers meat and milk production (Ahamed *et al.*, 2023). The increasing population and improved living conditions due to increased incomes are expected to boost the demand for livestock products, leading to the expansion of meat and dairy production efforts. This expansion has contributed to increased consumption of feedstuffs, especially grains and soybeans, raising concerns about competition for arable land used to grow essential food crops, which emphasizes the need to adopt more sustainable agricultural practices

(Wilkinson and Lee, 2018).

One of the major issues faced by the producers of livestock, particularly ruminant producers, is the costliness and scarcity of dry feed stuff. Roughage is of utmost importance in controlling the right number of microbes within the rumen because it helps in the digestion of tough plant fiber, aids digestion, and increases utilization of nutrients by the animal, leading to improved growth and production (Mui and Ledin, 2005). Providing roughage is critical to the bacteria in a ruminant's rumen because it helps break down plant cell walls and supports digestion, enabling the animal to achieve maximum utilization of feed and improve production. One effective means of enhancing feed availability in poor-resource countries is to explore the utilization of alternative and new fodder crops, i.e., *Panicum maxima* cv. Mombasa, also referred to as Maximum or Mombasa grass, is a high-yielding annual grass of decent nutritional quality, widely grown in most countries (Al-Mubarak and Al-Shammari, 2019).

Panicum maximum (PM) contains approximately 16% protein and yields approximately 10 tons of dry matter per hectare yearly. Studies demonstrate that providing cattle with this grass improves milk yield and benefits the performance of sheep and goats (Sabatier et al., 2015). The plant exhibits exceptional drought and heat tolerance, necessitating about half the irrigation water of alfalfa, yet its production may reach double that of alfalfa (Hare *et al*., 2014). It possesses robust roots that augment its resilience to severe climatic circumstances and can to a height of approximately three meters; it is recognized for its substantial yield and acceptable palatability for animals (Muir *et al*., 2001). It can be offered as fresh forage immediately after harvest or dried for no more than five days to preserve it in bale form, which is more efficient for fattening animals (Al-Mubarak and Al-Shammari, 2019).

This study aims to explore the effects of using more PM instead of Alfalfa (AF) in two types of concentrate feed on the nutritional value and digestibility of mixed lamb diets, utilizing laboratory methods to determine if PM can serve as a sustainable alternative forage for ruminant nutrition.

MATERIALS AND METHODS

In current study, all animal experimental procedures were approved and carried out in line with ethical standard (ethical approval NO. qgec/25/2025). In this experiment, samples of PM 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. The ingredients were mixed according to the specified proportions, as shown in Table 1.

Table 1: Composition of the concentrate feed and their percentages.

Ingredient	Percentage (%)
Barley	37.2
Corn	34.5
Wheat bran	10.3
Soybean meal	13.7
Sodium bicarbonate	1.9
Mineral-vitamin premix	1.2
Slow-release urea	0.6
Salt	0.6
Total	100 %

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 (T6, T7, T8, T9, T10) the proportion of concentrate feed was 70% and roughage 30%, as follows:
T1: 60% concentrate, 40% roughage (100% AF, 0% PM)
T2: 60% concentrate, 40% roughage (75% AF, 25% PM)
T3: 60% concentrate, 40% roughage (50% AF, 50% PM)
T4: 60% concentrate, 40% roughage (25% AF, 75% PM)
T5: 60% concentrate, 40% roughage (0% AF, 100% PM)
T6: 70% concentrate, 30% roughage (100% AF, 0% PM)
T7: 70% concentrate, 30% roughage (75% AF, 25% PM)
T8: 70% concentrate, 30% roughage (50% AF, 50% PM)
T9: 70% concentrate, 30% roughage (25% AF, 75% PM)
T10: 70% concentrate, 30% roughage (0% AF, 100% PM)

Chemical tests according to the *in sacco* method

The dry matter degradability (DMD) was estimated using the nylon bag (*in sacco*) technique with three Taleshi sheep. Samples of the (TMR) were ground using a 2–3 mm screen and dried at 65°C for 48 hours. They were then placed in nylon bags (50 µm pore size, 18 × 12 cm) following the method of Ørskov and McDonald (1979). The bags were inserted into the rumen through a fistula after being weighted to ensure complete submersion and incubated for 0, 2, 4, 8, 16, 24, 36, 48, 72, and 96 hours. At each incubation period, the bags were removed, rinsed with cold water to stop fermentation and remove residues, washed mechanically for 15 minutes, dried at 65°C for 24 hours, and then at 105°C for another 24 hours to determine dry matter content. The Ørskov and McDonald (1979) model was applied to calculate the decomposition coefficients:

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

Effective degradability was found through the following equation:

$$ED = \frac{a + b \times c}{c + k}$$

ED refers to the actual degradability of the feed. The fraction "a" represents the rapidly degradable part, while "b" is the slowly degradable part. The parameter "c" is the degradation rate constant of fraction b expressed as a percentage per hour. The symbol "K" denotes the passage rate of particles from the rumen, also expressed as a percentage per hour. ED values such as ED 0.02, ED 0.05, and ED 0.08 correspond to the ED at passage rates of 0.02, 0.05, and 0.08 per hour, respectively.

Chemical tests according to the Theodorou method

TGP (ml/500 mg dry matter), total volatile fatty acids (TVFA), ionized energy (ME), and NH_3N (mmol/100 ml) were measured after 24 h incubation according to the method of Theodorou *et al*. (1994). 500 mg of sample was mixed with 40 ml of rumen fluid in closed bottles under anaerobic conditions and incubated at 39°C for 24 h using a manometer (Testo 512, Germany). The bottles containing the samples were then cooled to stop the fermentation process. The pH of the rumen fluid was measured using a PH-0091 electronic device, and samples were drawn from the liquid portion of the bottles to determine ammonia nitrogen and TVFA. TGP was estimated in the laboratory according to the method of Menke (1988). NH_3N was determined after precipitating impurities by centrifugation, reacting samples with MgO and $CaCl_2$, and measuring the volume of acid used for titration according to the AOAC (2005) method using the equation:

$$NH_3\text{-}N\ (mg/100\ mL) = ((14.01 \times 0.5 \times Blank) - volume\ of\ acid\ used) \div sample\ volume \times 100$$

TVFA were measured using a Markham apparatus according to the equation:

$$Volatile\ fatty\ acid\ concentration\ (mmol/L) = (0.01 \times volume\ of\ buffer \div volume\ of\ incubation\ fluid) \times 1000$$

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

In sacco method

Effect of concentrate-to-roughage ratio on DMD

The results in Table 2 showed significant differences ($P < 0.05$) in DMD in the rumen due to varying concentrate-to-roughage ratios using the *in sacco* method. The 30:70 (roughage: concentrate) ratio recorded the highest values for all studied parameters compared to the 40:60 R:C ratio. Initial DB was 24.84% versus 22.65%, and ED values at passage rates of 0.02, 0.05, and 0.08 per hour improved in favor of the 70:30 R:C ratio, reaching 79.65, 73.03, and 69.36, respectively, compared to 77.33, 70.94, and 67.61 in the 40:60 R:C ratio.

Table 2: Effect of concentrate-to-roughage ratio on dry matter degradability in the rumen using the *in sacco* method.

Treatment	DB (%)	ED at 0.02	ED at 0.05	ED at 0.08
C: R 60:40	22.65[b]	77.33[b]	70.94[b]	67.61[b]
C: R 70:30	24.84[a]	79.65[a]	73.03[a]	69.36[a]
SEM	0.2497	0.2825	0.2851	0.2461
P-Value	0.0001	0.0001	0.0001	0.0001

Values in a column with different letters (a, b) indicate significant differences ($P < 0.05$).

These results indicate that increasing the concentrate ratio in the diet to 70% versus 30% roughage improves the rumen environment and enhances microbial activity, which positively affects DMD. This is attributed to the higher content of readily digestible organic matter in the concentrate feed, such as starch and degradable protein, which provides energy and increases the population of rumen microorganisms. Conversely, a higher roughage ratio, rich in slowly degradable fiber and low in energy, takes longer (up to 96 hours) for complete degradation (Pamungkas and Utomo, 2017). Both Arelovich *et al*. (2008) and Mediksa (2017) reported that the higher degradable protein content in concentrate feeds compared to natural grass hay or roughage improves the efficiency of rumen microbes, enhancing fiber degradation and digestion, and increasing feed intake. Additionally, Elghandour *et al.* (2020), Amin and Mao (2021), and Chen *et al.* (2021) confirmed that concentrate feeds provide additional nutritional sources supporting microbial growth in the rumen, leading to increased protozoa populations in rumen fluid, thus enhancing fermentation and improving DMD. Consistently, studies by Kang *et al.* (2016) and Phesatcha *et al.* (2020) showed that increasing concentrate levels in the diet improves DMD. The present study's findings also agree with Al-Galbi and Majeed (2022), who recorded the highest DMD rates in treatments with higher concentrate levels. In contrast, our results differ from those of Phesatcha *et al.* (2021), who found that the 60:40 (concentrate: roughage) ratio recorded the highest degradability of dry matter, organic matter, and crude protein.

Effect of increasing replacement levels of PM for AF on DMD

The results in Table 3 showed that increasing replacement levels of PM for AF in the diet did not cause significant differences in overall DMD, with treatments T1 to T5 recording similar values. This indicates that the replacement did not affect the rapidly degradable fraction or the total degradable matter. However, significant differences ($P \leq 0.05$) were observed in ED at different passage rates (0.02, 0.05, 0.08), where treatment T1 outperformed the others, and the values gradually decreased as the proportion of *P. maximum* replacement increased.

Table 3: Effect of increasing replacement levels of *Panicum maximum* for alfalfa on dry matter degradability in the rumen using the *in sacco* method.

Treatment	DB (%)	ED at 0.02	ED at 0.05	ED at 0.08
T1	23.83^{a}	79.46^{a}	73.20^{a}	69.58^{a}
T2	23.83^{a}	79.01^{ab}	72.56^{ab}	68.98^{ab}
T3	23.96^{a}	78.25^{abc}	71.55^{bc}	68.06^{b}
T4	23.30^{a}	78.05^{bc}	71.56^{bc}	68.13^{b}
T5	23.78^{a}	77.68^{c}	71.03^{c}	67.66^{b}
SEM	0.2497	0.2825	0.2851	0.2461
P-Value	0.4862	0.0036	0.0023	0.0026

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

The study findings showed no statistically significant influence on degradability between AF and PM as their respective NDF and ADF fiber content was similar, making the difference not sufficient enough to record a statistically significant effect. For ED at different passage rates (0.02, 0.05, 0.08), however, there was a reduction of the values with the increase of the proportion of *P. maximum* replacement. This is due to the chemical composition difference between PM and AF; PM contains a greater percentage of indigestible fiber and less degradable protein content. This can decrease the activity strength of rumen microorganisms' activity, degrading efficiency. AF has less fiber content and therefore is quick degradable and does not require a lengthy duration. Therefore, a rate of 0.08 per hour may not be sufficient to fully degrade more resistant PM fibers and lower feed usage generally. These findings agree with Meteab *et al.* (2025), who reported that increased replacement of AF with PM lowered DMD, which was attributed to greater contents of crude fiber (CF), NDF, ADF, and ash and lower organic matter content and higher tannin and total phenolic content in PM compared to AF. Yasmin *et al.* (2008) and Zhong *et al.* (2021) described how DMD was enhanced in feeds that contained low lignin and NDF levels because high lignin level inhibits the action of fiber-degrading microbes in the rumen. It may also be caused by anti-nutritional factors (ANFs), which are the secondary metabolites that reduce nutrient availability and reduce ruminant feed intake. Common ANFs found in feeds include tannins, glucosides, flavonoids, alkaloids, terpenoids, cyanides, coumarins, nitrates, oxalates, and organic acids. High levels of ANFs in the diet inhibit microbial and fungal growth in the rumen, which affects digestion and nutrient absorption (Abu Hafsa et al., 2022; Yasmin *et al.*, 2008; Acamovic and Brooke, 2005). Nwakaego (2023), Shaghaleh *et al.* (2024), and Meteab *et al.* (2025) studies also indicated that PM contains several secondary metabolites or ANFs. Previous research underscored that the nutrient profile of the diet, specifically fiber and protein-to-fermentable carbohydrate ratio, plays a role in controlling DMD in the rumen (Elghandour *et al.*, 2020; Amin and Mao, 2021; Chen *et al.*, 2021). Therefore, replacing AF with PM fully may not be the best practice if one aims to achieve maximum dry matter digestion efficiency in the rumen.

Effect of the interaction between the concentrate-to-roughage ratio and the proportion of PM replacement instead of AF on DMD

Table 4 shows that the interaction between concentrate-to-roughage ratios and the levels of PM replacement for AF had a clear effect on DMD in the rumen using the *in sacco* method. Ten treatments were studied, differing in two concentrate-to-roughage levels (40:60 and 30:70) and five levels of PM replacement (0%, 25%, 50%, 75%, 100%). The results revealed highly significant differences ($P < 0.01$) among treatments for all studied traits. Treatments T6, T8, and T9, which belonged to the higher concentrate level (70%), showed a clear significant superiority ($P < 0.01$) in most traits, especially treatment T6 (100% AF, 0% PM), which recorded the highest ED at all passage rates (80.60% at 1%, 74.40% at 2%, and 70.66% at 3%). Treatment T9 did not differ significantly from T6 in degradability and ED. Treatment T8 (50% AF, 50% PM) recorded the highest degradability (24.80%). Conversely, treatments T3, T4, and T5, which were within the lower concentrate level (60%) and had high PM replacement (50% or more), showed a highly significant decrease ($P < 0.01$) in ED (76.80, 76.40, 76.60 at 1%; 70.43, 69.96, 70.20 at 2%; and 67.20, 66.80, 67.0 at 3%).

Table 4: Effect of the interaction between concentrate to -roughage ratio and the proportion of *Panicum maximum* replacing alfalfa on dry matter degradability in the rumen using the *in sacco* method.

Treatment	DB (%)	ED at 0.02	ED at 0.05	ED at 0.08
T1	23.13^{cde}	78.33^{bcd}	72.0 ^{abcd}	68.50 ^{abcd}
T2	23.43^{bcde}	78.53^{abcd}	72.10 ^{abcd}	68.56 ^{abcd}
T3	22.13^{e}	76.80^{cd}	70.43 ^{cd}	67.20 ^{cd}
T4	22.16^{e}	76.40^{d}	69.96 ^{d}	66.80 ^{d}
T5	22.36^{de}	76.60^{cd}	70.20 ^{cd}	67.0 ^{d}
T6	24.53^{abc}	80.60^{a}	74.40 ^{a}	70.66 ^{a}
T7	24.23^{abcd}	79.50^{ab}	73.03 ^{ab}	69.40 ^{abc}
T8	24.80^{a}	79.70^{ab}	72.66 ^{abc}	68.93 ^{abcd}
T9	24.43^{abc}	79.70^{ab}	73.16 ^{ab}	69.46 ^{ab}
T0	25.20^{ab}	78.76^{abc}	71.86 ^{bcd}	68.33 ^{bcd}
SEM	0.2497	0.2825	0.2851	0.2461
P-Value	0.0001	0.0001	0.0001	0.0001

Values in a column with different letters indicate significant differences ($P < 0.01$).

The results showed the superiority of treatments T6 and T9 in ED, which is attributed to the slow passage rate allowing maximum utilization of all degradable components. Both treatments, T6 and T9, contain 70% rapidly degradable concentrate rich in energy necessary for the activity of rumen microorganisms, as well as Guinea grass, which contains a high proportion of digestible protein. These findings agree with Santra and Karim (2009), who reported that the digestibility of dry matter and crude protein increased with higher concentrate proportions in ruminant diets. Similarly, Shem *et al.* (2003) and Tessema and Bars (2004) stated that adding concentrates improved the utilization of low-quality feeds by ruminants by enhancing the effectiveness and potential degradation of dry matter through stimulating rumen fermentation activity. However, our results contrast with Castrillo *et al.* (1995), who mentioned that adding concentrates to roughage diets (C:R) decreases the ED of dry matter. The superiority of treatment T8 in degradability is attributed to the equal ratio of PM and AF, where PM acts as a rumination stimulator that increases microbial activity in the rumen, while AF provides high-quality protein. On the other hand, high concentrations of feed provide a quick source of energy for rumen microbes, stimulating overall microbial activity (Leamkrajang *et al.*, 2024). Protein content also affects dry matter digestibility due to the different solubility and resistance to degradation of protein sources. Improved digestibility and efficiency of microbial utilization of nutrients in the rumen reflect the quality of the diet (Sutardi, 1980). Tonga *et al.* (2017) also added that feed intake, gut passage rate, and diet type all contribute critical roles in affecting dry matter digestibility. The above observations agree with Meteab *et al.* (2025), who determined that the addition of equal rates of PM and AF yielded maximum *in vitro* DMD.

The decrease in ED in treatments T3, T4, and T5 can be attributed to the higher fiber composition of PM, which contains high levels of indigestible fiber and anti-nutrient compounds such as tannins and phenols that inhibit microbial growth in the rumen. This agrees with Meteab *et al.* (2025) who reported reduced DMD with increased levels of PM. Garcez *et al.* (2020) attributed the decline in DMD to reduced degradation rates of nitrogen fractions, which lowers ammonia nitrogen availability in the rumen. Ammonia nitrogen is the primary nitrogen source for microbes to synthesize amino acids, build cells, and sustain growth. Consequently, this deficiency reduces microbial activity, negatively affecting the efficiency of dry matter degradation in the rumen (Dentinho *et al.*, 2019).

Table 5: The effect of replacing increasing proportions of *Panicum maximum* instead of alfalfa on the consequences of dry matter degradability in rumen.

Treat-ment	Rapidly degradable fraction (a)	Slowly degra-dable fraction (b)	Degradation rate of the slowly degrada-ble fraction (c)
T1	35.73 ^{c}	50.75 ^{a}	0.095 ^{a}
T2	37.10 ^{bc}	49.43 ^{b}	0.086 ^{ab}
T3	37.51 ^{b}	49.15 ^{b}	0.074 ^{bc}
T4	36.11 ^{bc}	49.83 ^{b}	0.079 ^{b}
T5	41.96 ^{a}	44.48 ^{c}	0.065 ^{c}
SEM	0.6029	0.4629	0.0024
P-Value	0.0001	0.0001	0.0001

Values in a column with different letters indicate significant differences ($P < 0.01$).

Effect of replacing PM with AF in the diet on DMD

The results of Table 5 showed significant differences in the parameters of DMD in the rumen when increasing levels of PM replaced AF. Treatment T5 (100% PM) recorded a significant ($P<0.01$) superiority in the rapidly degradable fraction (a), with the highest value of 41.96%, while treatment T1 (100% AF) recorded the lowest value of 35.73%. Regarding the slowly degradable fraction (b), T1 had the highest value (50.75%), which gradually decreased with increasing PM levels, reaching the lowest value in T5 (44.48%). As for the degradation rate of the slowly degradable fraction (c), T1 achieved the highest rate (0.095 fraction/hour), while the lowest value was recorded in T5 (0.065 fraction/hour).

The results of the current study indicated that gradually replacing AF with PM led to a significant improvement in the rapidly degradable fraction (a), particularly in treatment T5 (0% AF and 100% PM). This improvement may be attributed to the high content of soluble sugars and non-structural carbohydrates in PM, especially during its early growth stages. In addition, the physical structure of PM allows for rapid swelling of the vegetative tissues and faster water absorption after being introduced into the rumen environment. These findings are consistent with Burns and Fisher (2013), who reported a significant improvement in DMD with increased PM proportions relative to AF, with this improvement declining when the AF proportion increased. The results also agree with Rashid (2022), who found a significant superiority in DMD in the treatment containing a high concentrate level (25%) with PM at 75%, compared to other ratios (25% PM + 75% concentrate) and (50% PM + 50% concentrate).

Conversely, the slowly degradable fraction (b) and its degradation rate (c) decreased with increasing PM proportion. This may indicate that the insoluble fibers in PM are less degradable or more complex than those in AF. Furthermore, the structural nature of PM fibers and its high content of lignin (ADL) a plant component that is indigestible by rumen microbes reduces digestion efficiency (Mertens, 1997). These findings are supported by Hassan *et al.* (2022), who noted that replacing high proportions of PM (75% and 100%) instead of AF reduced dry matter digestibility. Rodrigues et al. (2014) also reported that as PM matures, its fiber content particularly NDF, ADF, and ADL increases, while crude protein (CP) content decreases, which contributes to the decline in ruminal feed digestibility. Similarly, both Neto *et al.* (2020) and Pereira *et al.* (2022) found high levels of NDF, ADF, and ADL in PM forage, which negatively affect the slowly degradable fraction and the degradation rate of dry matter.

Effect of the interaction between concentrate-to-roughage ratio and the replacement level of PM with AF on DMD

The results presented in Table 6 showed a significant interaction effect (P ≤ 0.01) between the concentrate-to-roughage ratio and the replacement level of PM for AF on DMD parameters in the rumen, measured by the *in sacco* method. It was observed that the rapidly degradable fraction (a) increased with higher levels of concentrate and greater proportions of *Panicum maximum*, with treatment T10 (70% concentrate, 100% PM) recording the highest value at 44.96%, while treatment T3 (60% concentrate, 50% PM) recorded the lowest value at 34.23%. This improvement is attributed to the increased energy concentration and the ease of digestion of some soluble components in PM. These findings align with the study by Alalade et al. (2013), who reported significant differences in dry matter digestibility (DM, 76.84 g/kg) and crude protein (CP, 74.20 g/kg) for animals fed PM intercropped with three rows of legumes. They attributed this to the higher crude protein content in the forage, which enhanced crude protein digestibility, possibly linked to increased bacterial production in the rumen at the expense of protozoal growth.

Table 6: Effect of the interaction between the concentrate-to-roughage ratio and the replacement level of *Panicum maximum* for alfalfa on dry matter degradability parameters in the rumen.

Treatment	Rapidly degradable fraction (a)	Slowly degradable fraction (b)	Degradation rate of the slowly degradable fraction (c)
T1	34.96 f	50.80 a	0.086 ab
T2	35.50 ef	50.63 ab	0.085 abc
T3	34.23 f	50.60 ab	0.076 bc
T4	34.50 f	50.30 ab	0.070 bc
T5	38.96 bc	46.10 e	0.067 bc
T6	36.50 def	50.70 a	0.104 a
T7	38.70 bcd	48.23 cd	0.087 ab
T8	40.80 b	47.70 d	0.073 bc
T9	37.73 cde	49.36 bc	0.089 ab
T0	44.96 a	42.86 f	0.063 c
SEM	0.6029	0.4629	0.0024
P-Value	0.0001	0.0001	0.0001

Values in a column with different letters indicate significant differences (P < 0.01).

Conversely, the slowly degradable fraction (b) decreased with increasing levels of PM replacement, where treatments T1 and T6 (100% AF) recorded the highest values (~50.8%), while T10 recorded the lowest value at 42.86%. This decline is due to the higher lignin (ADL) content in PM, which inhibits the degradation of cell wall components, negatively affecting the slowly degradable fraction (Hassan *et al.*, 2022). Additionally, the degradation rate of the slowly degradable fraction (c) was negatively affected by increasing PM levels, gradually decreasing from 0.104 in T6 to 0.063 in T10. This indicates that AF exhibits a faster degradation rate compared to PM, likely due to its tissue composition and lower content of digestion-resistant compounds. Álvarez-Rodriguez *et al.* (2012) noted that the higher degradation rate of the slowly degradable fraction in AF is attributed to the slower passage rate of forage crops in the rumen, providing microbes more time to digest cellulose. Reid *et al.* (1990) and Coleman *et al.* (2003) also reported that AF not only contains higher crude protein (CP) concentrations but generally has lower acid detergent fiber (ADF) levels, which enhances organic matter intake and improves DMD.

Theodorou method

Effect of concentrate-to-roughage ratio on pH, TVFA concentration, NH_3N, TGP concentration, and IVOMD

The results of Table 7 showed the effect of the concentrate-to-roughage ratio on pH, TVFA concentration (mmol/100 ml), and NH_3N (mg/100 ml) after 24 hours of incubation using the Theodorou method. There was no significant difference between the 60% concentrate:40% roughage and 70% concentrate: 30% roughage levels in pH, TVFA concentration, and NH_3N. These results agree with Chen *et al.* (2015) but differ from Kim *et al.* (2025) and Van Dung *et al.* (2014). Conversely, the results showed no significant difference among treatments in TGP volume (ml/500 mg dry matter) and IVOMD. These findings are consistent with Kumar et al. (2013) but contrast with several other studies (Sampaio *et al.*, 2024; Reddy *et al.*, 2016).

Table 7: Effect of concentrate-to-roughage ratio on rumen fluid pH, NH3N (mg/100 ml), total volatile fatty acids concentration (mmol/100 ml), total gas production volume (ml/500 mg dry matter), and vitro organic matter digestibility after 24-hour incubation.

Treatment	Rumen Fluid pH (pH)	NH_3N (mg/100 ml)	TVFA (mmol/ 100 ml)	TGP (ml/500 mg DM)	IVO-MD (%)
C: R 60:40	6.74 [a]	209.10 [a]	41.66 [a]	54.06 [a]	64.06 [a]
C: R 70:30	6.75 [a]	224.64 [a]	43.16 [a]	55.43 [a]	65.25 [a]
SEM	0.02006	3.9648	2.5214	1.2884	1.1478
P-Value	0.8462	0.056	0.775	0.5127	0.5243

Effect of substituting increasing proportions of PM instead of AF on pH, TVFA concentration and NH_3N

Table 8 shows the effect of progressively replacing AF with PM on pH, TVFA concentration (mmol/100 ml), and NH_3N (mg/100 ml) after a 24-hour incubation period using the Theodorou method. No significant differences were observed among treatments for pH, TVFA, or NH_3N. These results agree with Sujani *et al.* (2015), who reported no significant differences in pH after 24 hours. However, they differ from findings by Jahan *et al.* (2018), Wang *et al.* (2020), and Meteab *et al.* (2025), which showed significant differences in some chemical indicators depending on the type and concentration of feed used.

Table 8: Effect of progressively replacing alfalfa with *Panicum maximum* on pH, total volatile fatty acids concentration (mmol/100 ml), and ammonia nitrogen (mg/100 ml) after a 24-hour incubation period.

Treat-ment	Rumen Fluid pH (pH)	NH_3N (mg/100 ml)	TVFA (mmol/ 100 ml)	TGP (ml/500 mg DM)	IVO-MD (%)
T1	6.690 [a]	212.01 [a]	37.917 [a]	58.96 [a]	68.46 [a]
T2	6.836 [a]	223.34 [a]	40.833 [a]	55.01 [ab]	64.75 [ab]
T3	6.768 [a]	214.73 [a]	42.917 [a]	46.08 [b]	56.88 [b]
T4	6.698 [a]	230.37 [a]	52.500 [a]	59.59 [a]	68.96 [a]
T5	6.745 [a]	203.92 [a]	37.917 [a]	54.07 [ab]	64.23 [ab]
SEM	0.02006	3.9648	2.5214	1.2884	1.1478
P-Value	0.1293	0.2599	0.3922	0.0038	0.0035

Values in a column with different letters indicate significant differences ($P < 0.05$).

Moreover, Table 8 indicates a significant effect ($P \le 0.05$) of progressively replacing AF with PM on digestibility. Treatments T4 (59.59) and T1 (58.96) showed significantly higher TGP (ml/500 mg dry matter) compared to T3 (46.08). This advantage is attributed to treatment T1 containing high levels of slowly degradable structural fibers (NDF) with fermentable protein fractions, which stimulate fiber-degrading bacteria, leading to increased gas accumulation. Treatment T4 produced large amounts of gas initially due to a high content of soluble sugars and rapidly fermentable carbohydrates along with sufficient slowly degradable structural fibers (Torres-Velázquez *et al.*, 2025; Scott *et al.*, 2025). These findings partially agree with Meteab *et al.* (2025), who noted that replacing AF with increasing levels of PM decreased organic matter digestibility (IVOMD) and gas production at high replacement levels ($p < 0.01$), while NH_3-N production increased to a certain point before declining at the highest replacement level.

Conversely, these results contradict those of Odedire and Babayemi (2008), Hassan *et al.* (2022), and Rashid (2022), who reported that increasing the amount of green forage led to a decrease in TGP because the green forage (PM) supplied digestible energy to the rumen, thereby reducing the energy expended in gas production by shortening the fermentation time of nutrients.

Furthermore, Table 8 reveals a significant increase ($P \le 0.05$) in IVOMD for treatments T4 (68.96) and T1 (68.46) compared to T3 (56.88). The superiority of T1 is linked to AF's richness in protein and moderate levels of structural fibers and soluble sugars, while T4 is characterized by higher soluble sugar content alongside slowly degradable fibers. De Ondarza *et al.* (2017) noted that increasing soluble sugars in the diet stimulates microbial activity and enhances TVFA production, thereby improving organic matter digestibility. Similarly, Belanche *et al.* (2023) reported that optimal ratios of rapidly degradable sugars (RDS) and rapidly degradable protein (RDP) significantly improve ruminal bacterial efficiency, leading to better feed digestibility and overall organic digestion.

The effect of the interaction between concentrate to roughage ratio and the replacement level of PM for AF on pH, TVFA concentration, and NH_3N

The results in Table 9 showed that the interaction between the concentrate to roughage ratio and the replacement level of PM for AF had no significant effect on pH, TVFA concentration (mmol/100 ml), and NH_3N (mg/100 ml) after 24 hours of incubation. These results differ from the study by Leamkrajang *et al.* (2024), which indicated that a 70:30 roughage to concentrate ratio increased ammonia concentration, TVFA, and pH. The results also contradict those of Santra and Karim (2009) and Van Dung *et al.* (2014). The difference in our results compared to previous studies may be due to the fact that the diets used were balanced in terms of crude protein, fiber, and energy content, which minimized the effects of changes in replacement ratios or concentrate to roughage ratios on the rumen environment.

Table 9: Effect of the Interaction Between Concentrate to Roughage Ratio and the Replacement Level of *Panicum maximum* for alfalfa on pH, total volatile fatty acids (mmol/100 ml), and ammonia nitrogen (mg/100 ml) After 24 Hours of Incubation.

Treat-ment	Rumen Fluid pH (pH)	NH_3N (mg/100 ml)	TVFA (mmol/ 100 ml)	TGP (ml/500 mg DM)	IVO-MD (%)
T1	6.72 ^{a}	208.01 ^{a}	32.50 ^{a}	57.008 ^{ab}	66.70 ^{ab}
T2	6.79 ^{a}	215.17 ^{a}	41.67 ^{a}	57.008 ^{ab}	66.54 ^{ab}
T3	6.81 ^{a}	199.22 ^{a}	50.00 ^{a}	43.05 ^{b}	54.28 ^{b}
T4	6.70 ^{a}	222.77 ^{a}	47.50 ^{a}	59.16 ^{ab}	68.58 ^{ab}
T5	6.68 ^{a}	200.37 ^{a}	36.67 ^{a}	54.06 ^{ab}	64.21 ^{ab}
T6	6.66 ^{a}	216 ^{a}	43.33 ^{a}	60.92 ^{a}	70.22 ^{a}
T7	6.87 ^{a}	231.51 ^{a}	40.00 ^{a}	53.01 ab	62.96 ^{ab}
T8	6.71 ^{a}	230.24 ^{a}	35.83 ^{a}	49.12 ^{ab}	59.48 ^{ab}
T9	6.69 ^{a}	237.97 ^{a}	57.50 ^{a}	60.03 ^{a}	69.35 ^{a}
T0	6.81 ^{a}	207.48 ^{a}	39.17 ^{a}	54.09 ^{ab}	64.24 ^{ab}
SEM	0.020061	3.964895	2.5214	1.2884	1.1478
P-Value	0.2142	0.3301	0.5934	0.0267	0.0254

Values in a column with different letters indicate significant differences ($P < 0.05$).

Additionally, Table 9 shows that the TGP volume (ml/500 mg dry matter) had a significant superiority ($P \leq 0.05$) for treatments T6 (60.92) and T9 (60.03) compared to treatment T3 (43.05). Likewise, results showed a significant increase ($P \leq 0.05$) in the IVOMD for treatments T6 (70.22) and T9 (69.35) compared to T3 (54.28). This superiority is attributed to the good nutritional balance of these two treatments, which were characterized by a higher proportion of soluble carbohydrates and easily degradable fibers, increasing microbial activity in the rumen and enhancing TVFA production. Consequently, this positively affected the values of organic matter digestibility and metabolizable energy.

These findings agree with Saini *et al.* (2012), who reported that higher concentrate levels yielded greater gas production and higher digestibility for both NDF and true organic matter, in addition to providing more available energy. They also align with Leamkrajang *et al.* (2024), which indicated that a 30:70 roughage to concentrate ratio can notably improve rumen fermentation characteristics, including increases in bacterial, fungal, and core microbial populations, thereby improving TVFA production efficiency. Similarly, Ramos *et al.* (2009) reported that diets with a high concentrate ratio (70%) resulted in higher organic matter digestibility and better microbial protein synthesis efficiency. Falola and Olufayo (2017) also found that *Panicum* improved crude protein, organic matter digestibility (OMD), short-chain fatty acids (SCFA), and metabolizable energy (ME). On the other hand, Meteab *et al.* (2025) reported that increasing *Panicum maximum* reduced gas production and organic matter digestibility rates.

CONCLUSIONS

In conclusion, the adding PM grass at 50% of the roughage portion combined with 50% AF roughage, along with a high concentrate level of 70%, led to an increase in degradability. A high concentrate ratio of 30:70 combined with 75% PM in the roughage improved ED with passage rates of 0.02, 0.05, and 0.08. Additionally, adding 100% PM as roughage with a high concentrate level of 30:70 and a lower concentrate level of 40:60 increased the rapidly degradable fraction. The inclusion of PM did not significantly affect rumen fluid pH, NH3N (mmol/100 ml), or TVFA concentration. However, adding 75% PM in the roughage with a high concentrate level of 30:70 improved the IVOMD and increased TGP.

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AUTHOR'S CONTRIBUTION

HAM: Conceptualization, data curation, investigation, methodology, project administration, resources, software, writing original draft, writing review and editing. AAAQ: Conceptualization, formal analysis, recourses, investigation, software, supervision, writing original draft, writing review and editing.

Generative AI and AI-assisted technology statement

AI-assisted tools were used for language and formatting support only. The authors take full responsibility for the content of this work.

Conflict of interest

The authors have declared no conflict of interest.

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