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EVALUATION OF ASSOCIATIVE EFFECTS OF *GLIRICIDIA SEPIUM* AND *MEGATHYRSUS MAXIMUS* COMBINATIONS ON FIBRE FRACTIONS, *IN-VITRO* CARBON DIOXIDE AND METHANE PRODUCTION

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Abstract: The voluntary feed intake and digestibility of forages are contingent upon the quality of the pasture, which in turn has an impact on ruminant productivity. There is limited research on the proximate/chemical composition, in-vitro gas production, and degradability of mixtures containing *Megathyrsus maximus* (MM) and *Gliricidia sepium* (GS). The study investigated the impact of different combinations of MM and GS on in-vitro gas production, degradability, and chemical composition. *Megathyrsus maximus* (MM) and *Gliricidia sepium* (GS) were combined as follows: T_1- 100%MM + 0%GS, T_2- 75%MM + 25%GS, T_3- 50%MM + 50%GS, T_4- 25%MM + 75%GS, and T_5- 0%MM + 100%GS. The treatments were assayed using standard procedures. The proximate composition of the treatments was also determined using standard procedures. Data were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. Initial gas produced in T_1 and T_2 and net gas volume (NGV) observed in T_1 and T_2 were significantly lower compared to other treatments. In addition, the CO_2 gas produced in T_3, T_4, and T_5 was significantly higher than that produced in T_1 and T_2. However, T_1 had the lowest CO_2 gas production. The treatment with 100% legume (T_5) had the highest methane (CH_4) production, followed by T_4 and T_3. Treatments containing 100% (T_1) and 75% (T_2) grass had the lowest CH_4 production. In terms of degradability, it was observed that T_5 had significantly higher organic matter degradability (OMD) compared to other dietary treatments. The crude protein observed in T_5 was also significantly higher than other treatments. In conclusion, mixtures with a high content of soluble carbohydrates presented the lowest gas production. It was determined that a mixture of 75% *Megathyrsus maximus* and 25% *Gliricidia sepium* has increased carbohydrate, ash, lower moisture content, and in vitro gas production and can be utilized by ruminant farmers as a cheap and readily available source of nutrition for their animals.

Keywords: *In-vitro* gas production, Chemical composition, Organic Matter Degradability, Ruminant.

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1. Introduction

Ruminant production in developing countries, including Nigeria, faces significant constraints due to the scarcity and fluctuating quality of year-round feed, particularly forages. This challenge is exacerbated by the tropical climate, where forage growth and quality closely mirror the seasonal distribution of rainfall, leading to acute feed shortages during the dry season (Preston, 1995; Babayemi and Bamikole, 2006). Compounding the issue is the high population density in Nigeria, which intensifies land-use competition between pasture for livestock and food crop cultivation. Addressing these challenges to ensure adequate, high-quality feed for ruminants is critical for sustaining livestock productivity and remains a focal concern for agricultural scientists and policymakers (Anim-Jnr et al., 2023).

Forage is herbaceous plants or herbaceous plant parts made available for animal consumption. Forages form the basal diet of ruminants, encompassing a wide array of plant materials consumed by grazing livestock, including grasses, legumes, and crop residues. It can also be defined as 'edible parts of plants, apart from separated grain, that can provide feed for grazing animals or that can be harvested for feeding' (Forage and Grazing Terminology Committee, 1991; Barnes and Baylor, 1995). Grasses, primarily from the *Poaceae* family, are essential forage components, with species such as *Megathyrsus maximus* (syn. *Panicum maximum*, commonly known as guinea grass) demonstrating high adaptability and production potential under local conditions (Ajayi and Babayemi, 2008). Guinea grass is versatile, suitable for cutting, silage, or grazing, and offers higher protein yield and dry matter content compared to other tropical grasses like elephant grass (*Pennisetum purpureum*) (Man and Wiktorsson, 2003). Ajayi and Babayemi (2008) stated that *P. maximum* is one of the most common grasses in the savanna region of Nigeria. Under adequate conditions, its nutritional value is high, having up to 12.5% crude protein, Total Digestible Nutrients (TDN) of 10.20 %, and some minerals such as calcium, phosphorus, and magnesium. Other researchers (Aderinola et al., 2014) affirmed that *P. maximum* has been classified among the best forage grasses due to its high nutritive value. Also, *P. maximum* produces a high yield of palatable fodder and is suited for grazing, but rapidly declines in nutritive value with age and could also die off if continually grazed close to the ground.

Odeyinka (2001) mentioned that ruminants cannot meet their maintenance needs on grass alone. Bamikole and Babayemi (2004) stated that although ruminants relish *P. maximum*, this grass becomes scarce during the dry season, and this causes nomads to travel long distances with their livestock in search of greener pastures and in the process, they cause damage to farmlands, lose their animals to snakebites and exposure to extremes of weather, and destroy lives and properties of farmers. These findings led to the search for leguminous forages, which are more palatable and well-accepted by ruminants all year round (Odeyinka, 2001). One such shrub in Nigeria is *Gliricidia sepium*, which is a medium-sized, semi-deciduous tree that grows up to between 10 and 15 m high. *Gliricidia sepium*, which contains an average of 22.3% crude protein, is described as a suitable feed for ruminants (Bawala et al., 2006). Meanwhile, the integration of mixed agricultural systems such as agro-pastoralism and silvo-pastoralism in providing suitable tropical forages for ruminants has been an effective strategy in combating feed shortages whilst reducing environmental footprint (Arango et al., 2020).

Furthermore, the combined use of grasses and legumes, for instance, guinea grass and *Glyricidia sepium*, in feeding ruminants is beneficial because the legume fixes nitrogen to the soil, which can be utilized by grasses and thereby increase their crude protein content (Cook et al., 2020). It also provides fermentable nitrogen, other nutrients for rumen microbes, readily fermentable cellulose, and bypass protein. In addition, *Glyricidia sepium* is commonly used as fencing material (Mbah et al., 2024), and it grows all year round; this makes it a legume of great choice for ruminant farmers.

There are many ways in which feed/diets can be evaluated. It can be carried out by proximate or chemical analysis (AOAC, 1990), *in vitro* method (Menke et al., 1979 and Babayemi, 2007), and *in vivo* method, in which the diet is introduced to animals whilst considering feed intake. Although there are other methods used to carry out *in vitro* fermentation assessment, the gas method is mostly used.

Altering the mix of dietary components is frequently suggested as a tactic that farmers could utilize to diminish the amount of energy expended by animals through eructated gasses (methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O)) and to enhance feed and energy efficiency. Methane is one of the ruminal fermentation end-products synthesized by methanogenic archaebacteria from CO_2 and H_2 derived from the fermentation of carbon-containing substrates. It is now widely understood that methane is a potential greenhouse gas capable of causing even more global warming than carbon dioxide over time (IPCC, 2013). In ruminants, the production of enteric gas is mostly determined by nutrition and feed intake. Usually, several factors such as forage processing, type of carbohydrate, additives, fat source, degradability, and amount of H_2 produced can influence gas production (Janssen, 2010). In vitro approaches can yield substantial insights into the mechanisms of digestive interactions among different feed sources. Furthermore, evaluating forages individually and in association should yield insights into a plant's capacity to influence nutrient utilization from another plant (Zhang et al., 2017).

Despite these developments, limited research exists on the associative effects of *M. maximus* and *G. sepium* mixtures on proximate composition, in vitro gas production, and nutrient degradability. This study aims to fill this knowledge gap by investigating the impact of varying grass-to-legume ratios on fermentation dynamics, nutrient digestibility, and greenhouse gas emissions. The findings will provide critical insights into optimizing forage mixtures for sustainable ruminant production systems, especially in low-income countries.

2. Materials and Methods

The experiment was carried out in 2018 at the Teaching and Research farm of the University of Ibadan, Oyo State, Nigeria. It is situated in the derived savanna vegetation belt (Latitude 7° 27' N and 3° 45'E) and at an altitude between 200m and 300m above sea level; mean temperature of 25-29°C with an average annual rainfall of about 1250mm. The soil is well-drained and belongs to the alfisol (Babayemi et al., 2003). The analyses were, however, carried out in the Laboratories of the Department of Animal Science, University of Ibadan.

2.1. Experimental Design and Substrates

The samples used for the experiment were derived from plant materials at the Teaching and Research farm and surroundings of the University of Ibadan, Oyo State, Nigeria. Fresh blades of guinea grass and tender stems and leaves of gliricidia were cut from matured plants. Gliricidia contains approximately 25% DM, and guinea grass contains 23% DM levels. The grass regrowth was cut at 15 cm height above the ground, while the legume was cut at 30 cm from the branch tip. Each fresh sample consisting of leaves and tender stem harvested was sun dried for a week and ground. MM and GS were combined as follows: T_1- 100%MM + 0%GS, T_2- 75%MM + 25%GS, T_3- 50%MM + 50%GS, T_4- 25%MM + 75%GS, and T_5- 0%MM + 100%GS.

2.2. Chemical Composition

The ground samples were oven-dried to constant weight at 105 °C. Further proximate analysis (ash, ether extract, crude protein, and crude fiber determination) of all the samples was carried out according to the procedures laid down by the Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were assayed by the method of Van Soest et al. (1991).

2.3. In Vitro Fermentation Procedure and Measurement of Gas Production

The rumen fluid was collected prior to early morning feeding. *In vitro* gas production analyses were performed based on the procedure described by Menke and Steingass (1988). The rumen fluid was collected through the suction method from three West African Dwarf goats under the same feeding regime and condition with the use of a suction tube as described by Babayemi and Bamikole (2006). The animals were fed with 40% concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell, and 0.25% fish meal) and 60% browse plants. The fluid was then filtered through a four-layered cheesecloth into a warm flask, flushed with carbon dioxide (CO_2) gas, and kept in a water bath previously heated to 39 °C. The mixture was intermittently stirred using an automatic Two hundred milligrams (200mg) DM of each dried and ground sample was carefully weighed into 100 ml calibrated syringes with pistons lubricated with Vaseline and thereafter, the syringes were filled with 30ml of medium consisting of 10ml of rumen fluid and 20ml of buffered mineral solution (NaHCO3+3Na2HPO4+KCl+NaCl+MgSO4.7H2O+CaCl2.2H 2O) and each sample was replicated three (3) times. The syringes were tightly capped and carefully arranged in an incubator maintained at 39±1^0 C along with three (3) blank syringes containing 30ml of medium (inoculums and buffer) only as control. The gas production was recorded at 3, 6, 9, 12, 15, 18, 21, and 24 hours. The gas produced was read by measuring the space formed between the top of the piston and the liquid in the syringe. The net gas produced was recorded as the gas produced (in ml) at 24 hours of incubation. After every reading (every 3 hours), the content of the syringes was shaken properly to allow for proper mixing of the substrate and the liquid. After incubation time, 4ml of 10M NaOH solution was introduced to estimate methane (CH_4) production according to Fievez et al. (2005). Mixing of the contents with NaOH allowed absorption of CO_2, with the gas volume remaining in the syringe considered to be CH_4 (Demeyer et al., 1988). Graphs of the volume of gas produced every 3-hour interval of the three replicates of each sample were plotted against the incubation time.

From the graph, the degradation characteristics were estimated as defined in the equation 1:

Y= a+ b (1-ect) (Orskov and Mc Donald, 1979) where
Y= degradability at time (t)
a= intercept (or initial gas produced)
b= potentially degradable of b
c= rate of degradation of b
t= incubation time (1)

The asymptote represents (a+b) of the potential degradability. The intercept of the curve is represented by a and given the DMD value at a time (zero hours). The b value was calculated as the difference between the asymptotic DMD and the intercept a, i.e. (a+ b) – a. To get a good estimate of c, Y was selected (DMD% at the time) when the curve changed rapidly. The gas produced on incubation, together with the levels of other chemical constituents, was used to predict the digestibility of organic matter (equations 2 and 3).

OMD (percent) =14.88 + 0.889Gp + 0.45CP + 0.0651XA, R2=0.92 (2)

Partition Factor (PF) = OMD/Gp (3)

Where,
DM dry matter,
OMD organic matter digestibility.
CP, crude protein in percent.
XA, ash in percent.
Gp, the net gas production in ml from 200 mg dry sample after 24 h of incubation and after correction for the day-to -day variation in the activation of rumen liquor using the Hohenheim standard.

2.4. Statistical Analysis

Data obtained was subjected to a one-way Analysis of Variance using SAS software (1990), and the significant differences among the means were separated using the Duncan Multiple Range Test.

3. Results

3.1. In vitro Gas Production

The in vitro gas production characteristics of forage mixtures are presented in Table 1. A graph showing methane gas production as affected by different proportions of grass-legume mixtures is also presented in Figure 1.

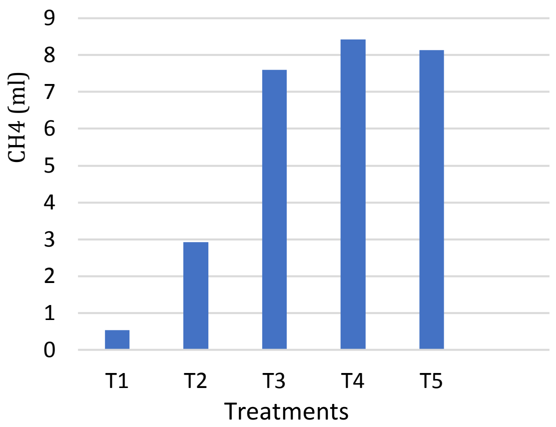

Figure 1. Methane gas production as affected by different proportions of forage mixtures. T_1= 100% Megathyrsus maximus +0% Gliricidia; T_2= 75% Megathyrsus maximus +25% Gliricidia; T_3= 50% Megathyrsus maximus +50% Gliricidia; T_4= 25% Megathyrsus maximus +75% Gliricidia; T_5= 0% Megathyrsus maximus +100% Gliricidia

It was observed that initial gas (a) produced in T_1 (3.00) and T_2 (2.50) were similar and significantly ($P<0.05$) lower compared to other treatments. The potentially degradable fraction (b) observed in T_3 (7.67) and T_5 (10.33) were significantly ($P<0.05$) higher compared to other treatments. However, the lowest ($P<0.05$) potentially degradable fraction was observed in T_1 (1.67). Higher ($P<0.05$) potential gas production (a+b) was observed in T_3 (17.00), T_4 (16.33), and T_5 (17.67) compared to other treatments. The lowest ($P<0.05$) potential gas production was observed in T_1 (1.33). The T_4 (0.13) had a significantly ($P<0.05$) higher rate of degradation compared to other treatments. However, T_1 (0.00) and T_3 (0.01) were significantly ($P<0.05$) lower compared to other treatments. Higher ($P<0.05$) incubation time (t) was observed in T_2 (16.50) compared to T_1 (7.50) and T_3 (10.00) but did not differ significantly ($P<0.05$) from T_4 (11.00) and T_5 (15.00). The degradability at t (Y) was significantly ($P<0.05$) affected by different proportions of forage mixtures. It was observed that degradability in T_4 (12.66) and T_5 (15.22) was significantly ($P<0.05$) higher compared to T_1 (4.67) and T_2 (4.17).

Table 1. *In vitro* Gas production characteristics of the forage mixtures

Parameters	T1	T2	T3	T4	T5	SEM	P value
inital gas produced	3.00^{b}	2.50^{b}	9.33^{a}	11.00^{a}	11.00^{a}	2.80	0.02
potentially degradable fraction	1.67^{c}	3.33^{bc}	7.67^{a}	5.35^{b}	10.33^{a}	1.50	0.04
potential gas production	1.33^{c}	5.83^{b}	17.00^{a}	16.33^{a}	17.67^{a}	0.83	0.01
rate of degradation	0.00^{c}	0.04^{bc}	0.01^{c}	0.13^{a}	0.07^{b}	0.02	0.02
incubation time	7.50^{b}	16.50^{a}	10.00^{b}	11.00^{ab}	15.00^{ab}	3.03	0.04
degradability at time (t)	4.67^{b}	4.17^{b}	10.22^{ab}	12.66^{a}	15.22^{a}	2.71	0.04

^{abc} Means for treatments along x axis with different superscripts differed significantly ($P<0.05$)
T_1= 100% Megathyrsus maximus +0% Gliricidia; T_2= 75% Megathyrsus maximus +25% Gliricidia; T_3= 50% Megathyrsus maximus +50% Gliricidia; T_4= 25% Megathyrsus maximus +75% Gliricidia; T_5= 0% Megathyrsus maximus +100% Gliricidia.

3.2. In- vitro Fermentation Parameters

Table 2 shows the in vitro fermentation parameters of grass-legume mixtures. The net gas volume (NGV) observed in T_1 (1.33) and T_2 (5.83) was significantly ($P<0.05$) lower compared to other treatments. The CH_4 gas produced in T_1 (0.53) was the lowest when compared to other treatments and was not significantly different from T_2 (2.93). However, T4 (8.43) had the highest ($p < 0.05$) CH_4 gas production. Different proportions of forage mixtures did not significantly ($P<0.05$) affect net methane (NM) to net gas (NG) ratio and ranged from 0.40 (T_1) to 0.53 (T_4). The CO_2 to organic matter digestibility (OMD) ratios observed in T_3 (0.25), T_4 (0.28), and T_5 (0.26) were similar and significantly ($P<0.05$) higher compared to other treatments. The T_1 (0.02) had the lowest ($P<0.05$) CO_2/OMD.

Table 2. *In vitro* fermentation parameters of the grass and legume mixtures

Parameters	T1	T2	T3	T4	T5	SEM	P value
NGV	1.33^{b}	5.83^{b}	17.00^{a}	16.33^{a}	17.67^{a}	2.54	0.02
CH_4	0.53^{b}	2.93^{b}	7.60^{a}	8.43^{a}	8.13^{a}	1.29	0.04
NM/NG	0.40	0.51	0.45	0.53	0.47	0.04	0.36
OMD	30.61^{b}	29.99^{d}	29.91^{d}	30.49^{c}	30.79^{a}	0.03	<0.0001
CO_2	0.80^{c}	2.90^{c}	9.40^{a}	7.90^{a}	9.53^{a}	0.80	0.02
CO_2/OMD	0.02^{c}	0.10^{b}	0.25^{a}	0.28^{a}	0.26^{a}	0.03	0.02
CH_4/OMD	0.03^{b}	0.09^{b}	0.31^{a}	0.26^{a}	0.31^{a}	0.03	0.03
PF	0.07^{a}	0.02^{b}	0.01^{c}	0.01^{c}	0.01^{c}	0.003	0.004

^{abcd} Means of treatments along a row with different superscript differed significantly ($P<0.05$), NGV= net gas volume, CH_4= methane, NM/NG- Net methane: Net gas. T_1= 100% *Megathyrsus maximus* +0% *Gliricidia;* T_2= 75% *Megathyrsus maximus* +25% *Gliricidia;* T_3= 50% *Megathyrsus maximus* +50% *Gliricidia;* T_4= 25% *Megathyrsus maximus* +75% *Gliricidia;* T_5= 0% *Megathyrsus maximus* +100% *Gliricidia.*

The T_1 (0.03) and T_2 (0.09) had significantly ($P<0.05$) lower CH_4 to OMD ratios compared to treatments. The CO_2 gas produced in T_3 (9.40), T_4 (7.90), and T_5 (9.53) were significantly ($P<0.05$) higher compared to other treatments. However, T_1 (0.80) had the lowest ($P<0.05$) CO_2 gas production. The net CO_2 to net gas ratio was not significantly ($P<0.05$) affected by different proportions of forage mixtures and ranged from 0.47 (T_4) to 0.60 (T_1).

3.3. Nutritional Composition of Grass-legume Mixtures

Table 3 shows the proximate composition of dietary treatments as influenced by different proportions of forage mixtures. Higher ($p<0.05$) Moisture Content (MC) was observed in T_5 (5.95%) compared to other dietary treatments. The T_2 (4.37%) had the lowest ($p<0.05$) MC. The Crude Protein (CP) observed in T_5 (21.48) was significantly ($p<0.05$) higher compared to other treatments. However, the lowest ($p<0.05$) CP was observed in T_1 (13.16). Higher ($p<0.05$) ether extract (EE) was observed in T_1 (3.29) compared to other treatments. The T_5 (1.98) had the lowest ($p<0.05$) EE but was similar to T_3 (2.01). The crude fiber (CF) observed in T_1 (25.99) was significantly ($p<0.05$) higher compared to other dietary treatments. Similar ($p<0.05$) CF values were observed in T_3 (22.60) and T_4 (22.59). However, T_3 (20.20) had the lowest ($p<0.05$) CF. Ash content observed in T_1 (9.13) was significantly ($p<0.05$) higher compared to other treatments. The lowest ($p<0.05$) ash content was observed in T_3 (5.55). The NFE observed in T_2 (71.46) was significantly ($p<0.05$) higher compared to other dietary treatments. The T_1 (69.71) and T_3 (69.72) had similar ($p<0.05$) NFE values. However, T_5 (65.04) had the lowest ($p<0.05$) NFE value. Higher ($P<0.05$) partitioning factor (PF) was observed in T1 (0.07) compared to other dietary treatments.

Table 3. Proximate composition of grass and legume mixtures

Parameters	T1	T2	T3	T4	T5	SEM	P value
Moisture content	4.70^{d}	4.37^{c}	5.42^{b}	4.84^{c}	5.95^{a}	0.02	<0.0001
CP	13.16^{c}	13.99^{d}	15.38^{c}	18.39^{b}	21.48^{a}	0.03	<0.0001
EE	3.29^{a}	2.01^{c}	2.01^{d}	2.09^{b}	1.98^{d}	0.01	<0.0001
CF	25.99^{a}	24.01^{b}	22.60^{c}	22.59^{c}	20.20^{d}	0.01	<0.0001
Ash	9.13^{a}	8.16^{b}	7.47^{c}	6.59^{d}	5.55^{c}	0.03	<0.0001
NFE	69.71^{b}	71.46^{a}	69.72^{b}	68.08^{c}	65.04^{d}	0.05	<0.0001

^{abcd} Means of treatment along a row with different superscript differed significantly CP= crude protein, EE- ($P<0.05$) ether extract, CF= crude fiber, NFE-Nitrogen free extract. T_1= 100% Megathyrsus maximus +0% Gliricidia; T_2= 75% Megathyrsus maximus +25% Gliricidia; T_3= 50% Megathyrsus maximus +50% Gliricidia; T_4= 25% Megathyrsus maximus +75% Gliricidia; T_5= 0% Megathyrsus maximus +100% Gliricidia.

3.4. Fiber Fractions of Dietary Treatments

The fiber fractions of dietary treatments as affected by different proportions of forage mixtures are shown in Table 4. İt was observed that the Neutral Detergent Fiber (NDF) was not significantly ($P<0.05$) affected by different proportions of forage mixtures and ranged from 58.90 (T_2) to 62.76 (T_4). Higher ($P<0.05$) acid detergent fiber (ADF) values were observed in T_4 (31.90) and T_5 (30.60) compared to other dietary treatments. The T_2 (27.41) had the lowest ($P<0.05$) ADF value compared to other dietary treatments. It was observed that the T_3 (16.60) had significantly ($P<0.05$) higher acid detergent lignin (ADL) value compared to other dietary treatments. Similarly ($P<0.05$) ADL values were observed in T_4 (15.80) and T_5 (15.53) and were higher ($P<0.05$) than in T_1 (14.60) and T_2 (14.20). However, the lowest ($P<0.05$) ADL value was observed in T_2 (14.20).

Table 4. Fiber fractions of dietary treatments as affected by different proportions of forage mixtures

Parameters	T1	T2	T3	T4	T5	SEM	P value
NDF	60.06	58.90	60.02	62.76	60.39	1.45	0.47
ADF	28.60^{c}	27.41^{d}	31.06^{a}	30.90^{b}	30.60^{b}	0.08	<0.0001
ADL	14.60^{c}	14.20^{d}	16.60^{a}	15.80^{b}	15.53^{b}	0.12	<0.0001

^{abcd} Means of treatments along a row with different superscript differed significantly ($P<0.05$). NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin. T_1= 100% Megathyrsus maximus +0% Gliricidia; T_2= 75% Megathyrsus maximus +25% Gliricidia; T_3= 50% Megathyrsus maximus +50% Gliricidia; T_4= 25% Megathyrsus maximus +75% Gliricidia; T_5= 0% Megathyrsus maximus +100% Gliricidia.

4. Discussion

Megathyrsus maximus is one of the most common grasses in the savanna region of Nigeria. Under adequate conditions, its nutritional value is high, having up to 12.5% crude protein, Total Digestible Nutrients (TDN) of 10.20%, and some minerals such as calcium, phosphorus, and magnesium (Ajayi and Babayemi, 2008). Aderinola et al. (2014), affirmed that *Megathyrsus maximus* has been classified among the best forage grasses due to its high nutritive value and produces high yield of palatable fodder suitable for grazing, but rapidly declines in nutritive value with age and could also die off if continually grazed close to the ground. These reports are consistent with the findings of the current study, as observed in T_1 with a crude protein content of 13.16%.

The result of the current study confirms the report of Bawala et al. (2006), who described *Gliricidia sepium* as a suitable feed for ruminants and that the combined use of grasses and legumes, for instance, guinea grass and *Gliricidia sepium* in feeding ruminants is beneficial because the legume fixes nitrogen to the soil which can be utilized by the grass and thereby increase its crude protein. It also provides fermentable nitrogen, other nutrients for the rumen microbes, readily fermentable cellulose, and bypass protein.

Kearny (2005), noted that fiber plays a fundamental role in ruminant nutrition, and it is the component in a feed that is not digested by mammalian enzymes. Some of these components are soluble under mild extraction procedures and thus result in 'soluble' and 'insoluble' fiber. Most constituents of soluble fiber (pectin, fructans, and β-glucans) are readily fermented in the rumen and may even be readily fermented in the large intestine of monogastric animals (Righi et al., 2008). From the current study, the NDF was not significantly affected by different proportions of forage mixtures and ranged from 58.90 (T_2) to 62.76 (T_4). Higher ADF values were observed in T_3 (31.06) and T_4 (30.90) compared to other dietary treatments. The T_2 (27.41) had the lowest ADF value. Merten (1997), stressed that the chemical definition of dietary fiber, such as neutral detergent fiber (NDF) or acid detergent fiber (ADF) content, was an inadequate description of the fiber content of a diet. The ADF fraction of feedstuffs includes cellulose and lignin as the primary components. Concentrations of ADF and lignin are correlated more with digestibility than with intake. It is an indicator of digestibility; as the ADF increases, digestibility decreases. NDF is a measure of cellulose, hemicelluloses ADF, and lignin fractions of feeds. NDF is more highly correlated with feed volume and chewing activity than ADF or crude fiber (CF) (Oba et al., 1999).

Some of the NDFs are highly digestible, but forage NDF is the best indicator of an animal's voluntary feed intake (VFI). As the NDF content of forage increases, the VFI decreases. Also, it has been shown several times that the digestibility of plant material in the rumen is related to the proportion and lignification of plant cell walls (NDF). Forage with high lignin contents is often of low digestibility (Norton, 2010). Merry et al. (2006), reported that when animals are fed a grass and legume mixture, digestive interactions can occur in the rumen between substrates contained in the different plants, and the response of the animal to the combination of forages can differ from the balanced median values of their components considered individually. These interactions, named associative effects, can modify the metabolic processes in the digestive tract, particularly in the rumen, so that the response of an animal to a combination of forages can differ from the balanced median values of its components considered individually. This kind of response can be synergistic or antagonistic with a possible impact in terms of nutrient use by the animal, N excretion, and methane emissions. Dhiman and Satter (1997) noted that the nutritional complementarities of plant species could contribute to integrating productivity and environmental requirements, as observed from the current study. The net gas volume produced was greatest in the mixtures ranging from 50%-100% Gliricidia. This may be due to two factors. One is the higher crude protein content in these combinations, as the greater availability of crude protein in the diet allows for greater microbial activity as it is not limiting in the diet.

Johnson et al. (1995) reported that the production of methane represents a loss of between 2 and 15% of the gross energy in the feed. The authors increased feed efficiency by production and inhibition of rumen methanogenesis because methane plays a role in the global warming phenomenon and the destruction of the ozone layer. Chynoweth (1996) reported that compared to other greenhouse gases, methane is an excellent candidate to reduce global warming in the near term. However, Houghton (1997) reported that because of the shorter lifetime of methane in the atmosphere (about 12 years compared with 100-200 years for CO2), only a relatively small reduction in the anthropogenic emission of CH4, about 8% would be required to stabilize its concentration at the current level. Leng (1993) concluded that enteric methane emission is one of the few global sources of methane that can be reduced relatively. The author further stated that it is easier to manipulate, for instance, methane produced from marshes or in rice production. Furthermore, methane reduction strategies from livestock will directly benefit the farmers by improving animal productivity. The result of the current study on methane production has shown that 100% *Megathyrsus maximus* or a combination of 75% *Megathyrsus maximus* and 25% *Gliricidia sepium* had the least methane production.

Furthermore, Beauchemin et al. (2009) reported that improving forage quality (i.e., increasing dietary starch content) through the supplementation of alternative forages, such as leguminous and non-leguminous shrubs, has the potential to reduce CH_4 emissions per kg animal products as a result of increased diet digestibility and a shortened duration of feeding. The authors further stated that dietary strategies such as this have been successful in manipulating methanogenesis, at least in the short term, through either direct inhibition of methanogens, reducing the production of hydrogen in the rumen, or providing alternative sinks for the disposal of hydrogen. Carulla et al. (2005) observed a similar report and stated that secondary plant compounds (e.g., condensed tannins and saponins) have been shown to reduce enteric CH_4 emissions through the direct inhibition of methanogens. Similarly, including high starch feedstuffs favors propionate production and reduces ruminal pH, thus inhibiting methanogen and protozoal growth (Boadi et al., 2004). From the current study, the degradability of forage mixtures was affected by different proportions of inclusion. The T_5 (30.79) had significantly higher OMD compared to other dietary treatments. Similar OMD values were observed in T_2 (29.99) and T_3 (29.91) and were significantly lower compared to other dietary treatments. The result of the present findings corroborates the report of Swan et al. (2006), who noted that the degradation of feed ingredients also depends on the distribution of starch granules within the substance. Qin et al. (2012) also added that starch granules of wheat endosperms seem to be floury and have a relatively small particle size. Consequently, the smaller starch granules have a larger surface area available for microbial and enzymatic starch hydrolysis, which results in rapid degradation. Bonhomme (1990) reported that increased methane emission can be observed as a result of the optimum symbiotic relationship between bacteria and protozoans and the efficient exchange of hydrogen between these microorganisms. Furthermore, methane production is affected by the type of carbohydrate fed to the animals (Moe and Tyrrell, 1979). Qin et al. (2012) reported that wheat had relatively higher effective degradability of dry matter (EDDM), which was more rapidly fermented by ruminal microbes. Thus, having a higher methane production of wheat may be attributed to EDDM. Moreover, low methane production of other feed ingredients might also be attributed to low EDDM and, thus, slow fermentation of ruminal microbes. Low EDDM might be due to the thickness of the protein matrix, which coats starch granules, and this matrix is relatively difficult to hydrolyze with water and enzymes (McAllister et al., 1996). The result of the present study contradicts the findings of Shibata (1992), who noted that providing ruminants with feed containing carbohydrates and high protein levels had a negative effect on methane emission while providing a diet rich in fiber resulted in an elevated volume of methane being produced. It was observed that T_5 has the highest methane production, followed by T_4 and T_3. T_1 and T_2 had the lowest methane production. T_1 had the lowest degradability, whereas T_5 had the highest degradability.

5. Conclusion

The study demonstrated that varying proportions of *Megathyrsus maximus* and *Gliricidia sepium* significantly influence nutrient composition, in vitro gas production, and organic matter degradability. Treatments with higher proportions of *Gliricidia sepium* exhibited elevated crude protein levels, methane, and CO_2 production, with T_5 (100% G. sepium) achieving the highest crude protein content (21.48%) and methane production (8.13 mL). Conversely, treatments with higher *M. maximus* content, such as T_1 (100% *M. maximus*), had higher crude fiber (25.99%) but lower methane production (0.53 mL), indicating reduced fermentability.

The degradability of organic matter significantly improved with increasing *G. sepium* inclusion, with T_5 recording the highest value (30.79%). However, methane emissions also increased with higher legume proportions, highlighting a trade-off between enhanced digestibility and environmental impact. The study suggests that a 75% *M. maximus* and 25% *G. sepium* mixture offers an optimal balance between nutrient availability, low methane emissions, and adequate degradability, making it a suitable option for sustainable ruminant feeding systems. This proportion minimizes greenhouse gas emissions while ensuring efficient nutrient utilization, supporting productivity in tropical livestock systems.

Author Contributions

The percentage of the authors' contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	G.A.I.	O.O.	U.Ş.
C	30	70	
D	30	70	
S		100	
DCP	70	30	
DAI	50	50	
L	90	10	
W	80		20
CR	30	40	30
SR	30	40	30
PM	50	50	
FA	100		

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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