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In vitro and ruminal characteristics of the three selected Nigerian herbs: Phyllantus amarus, Ocimum gratissimum and Lactuca taraxacifolia as feed additives in ruminant production

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Abstract

The study was conducted to evaluate the effects of herbal feed additives on the in vitro and ruminal fermentation by West African dwarf goats. A total of 20 goats were subjected to five dietary treatments for a 12- week feeding study in a completely randomized design: Feed only (control) (T1), Feed + *Ocimun gratissimum* leaf meal (T2), Feed + *Phyllantus amarus* leaf meal (T3), Feed + *Lactuca taraxacifolia* leaf meal (T4), Feed + P. amarus + O. gratissimum + L. taraxacifolia leaf meal (T5). Phytochemical Screening of the three herbs were carried out. Herbs were added at 3g/kg feed and were given at 5% body weight of the animal. The feed samples were subjected to 24 hours incubation. Gas volume were measured at the interval of 3hrs. At the end of incubation, methane volume was estimated. The gas volume produced was used to calculate metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acid (SCFA) concentrations. At the end of the feeding trial, rumen liquor was collected to determine ruminal characteristics and the microbial population as may be affected by the medical plants. The results showed that the leaves of the herbs were rich in saponin, tannin and alkaloid. The three medicinal plants were relatively high in crude protein (CP) and moderate in carbohydrate (CHO) content. Treatment containing the mixture of *O. gratissimum, P. amarus and L. taxifolia* (T5) contained more CP than the rest of the treatments while T2 had the highest CF. There was apparent increase in the value of potential gas production as the incubation period increases in *in-vitro* study. T1 and T4 produced more gas than the rest of the treatments. Methane production was lower in T3 and T5 compared to other treatments. T1 and T4 equally had the highest ME, OMD and SCFA concentrations. However, T3 and T5 improved the ruminal characteristics and microbial population in the rumen as the population was significantly modified. It is therefore concluded that T3 (*Phallantus amarus*) and the combination of the three herbs (T5) be used as feed additives for mitigating methane emission and improve rumen ecology of the animals for enhanced ruminant productivity.

Introduction

Adopting feeding strategies to mitigate the amount of energy lost as methane and reduce ammonia concentration in the rumen can enhance feed conversion efficiency, improve animal productivity and reduce global warming (Gebrehiwot, 2014). Researchers have shown that manipulation of rumen microbial fermentation to decrease methane and ammonia production from ruminant livestock using extracts/meal of herbs and spices has proven to be a potentially useful strategy in improving production efficiency in ruminants (Benchaar *et al.*, 2008; Benchaar and Greathead, 2011). Methane expelled by ruminants is one of the most important sources of global warming, having a great impact on the environment. Greenhouse gas emission, especially methane from ruminants, has been recognized as an important issue worldwide as it is a driver for global warming and climate change (Kim *et al.*, 2012). It has been reported that livestock production and its by-products are responsible for at least 51 percent of global warming gases (Goodland and Anhang, 2009). Gas emissions from the livestock sector are estimated at between 4.1 and 7.1 billion tonnes of carbon dioxide equivalents per year, equating to 15-24% of total global anthropogenic greenhouse gas emissions (Wanapat *et al.*, 2013).
Phytogenics, are natural bioactive compounds that are derived from plants and incorporated into animal feed to enhance productivity (Gadde *et al* 2017) and reduction in methane emission to the environment. The incorporation of synthetic antibiotics in the ration of livestock has raised public concerns because of the development of drug-resistant bacteria. However, the use of some herbs and spices has proved to influence the rumen environment (Szumacher-Strabel and Cieślak, 2010; Cieslak *et al.*, 2013). Rezaei and Pour (2012) reported that the addition of thyme methanolic extracts had a reducing effect on the degradability of soybean meal using an in vitro gas production technique. Chaudhry and Khan (2012) in an in vitro rumen fermentation study with five curry spices which include coriander, turmeric, cumin, clove and cinnamon revealed that the spices acted as natural antibiotics, destroying methane-producing bacteria in the animal’s gut which led to a reduction in methane production.

Dong *et al.* (2010) reported a reduction in methane production, increased propionate production and decreased protozoa numbers when luzerne extract, *Artemisiae annuae* extract and mixed herbal medicine were included in goat diets. In another study conducted to assess the effect of ginger (*Zingiber officinale*) on the in vitro rumen ecosystem of sheep, Mohammad and Moeini (2015) reported that ginger supplementation improved ruminal fermentation due to a reduction in ammonia, reduction in methane loss, reduction in acetate to propionate ratio and beneficial changes in protozoa population. In addition, Tag El-Din *et al.* (2012) also concluded that the addition of garlic juice and ginger improved rumen fermentation, increased gas production and reduced methane production. Busquet *et al.* (2005) also reported that garlic oil altered rumen fermentation by reducing the acetate to propionate ratio like what is obtainable using monensin in a continuous culture. Kim *et al.* (2012) also reported that plant extracts were shown to have properties to reduce methane production, reduce acetate to propionate ratio, decrease methanogen population and increase fibrolytic bacteria population.

Meanwhile, Mariam *et al.* (2014) investigated the potential impacts of different levels of essential oils blend (eucalyptus, cinnamon, peppermint, thyme and lemon) on rumen fermentation parameters and nutrient utilization in barki sheep and reported no significant effects on *in vitro* gas production, methane production, short chain fatty acids (SCFA), NH_3-N concentration and protozoa count. Notwithstanding, Arhab *et al.* (2013) observed a significant reduction in methane production, rumen fermentation traits and decreased ammonia N concentration when essential oils extracted from *Juniperus phoenicea*, *Satureja calamintha* and *Mentha pulegium* were used in vetch oat hay *in vitro*. To mitigate climate change, there is a need to reduce greenhouse gas (GHG) emissions from all the sectors. Reisinger *et al*. (2021) observed that reduction in global warming to below 2º C cannot be realistic without reducing emissions from the agricultural sector

Studies reported that dietary supplementation with 10 g/DM of plantain herb and/or 10 g/DM of *Allium sativum* (garlic leaf) resulted in enhanced crude protein intake, dry matter intake, feed conversion ratio and body weight gain compared with the control diet (Redoy *et al.*, 2020).

Similarly, dietary *Andrographis paniculata* leaves and stems increase feed intake and growth performance in goats (Yusuf, 2015). It has also been reported that the dietary supplement with *Nigella sativa* seeds or *Rosmarinus officinalis* leaves at 1 % concentration enhanced nutrient digestibility in lambs (Odhaib *et al.*, 2018). Moreover, the addition of *N. sativa* leaf meal up to 66.7 % as a replacement for cottonseed meal in the diets of sheep resulted in improved feed utilization and growth performance of growing Farafra sheep (Abdullah and Farghaly, 2019).

Materials and Methods

Experimental Site

The experiment was conducted at the Small Ruminant Unit at Teaching and Research Farm, Kwara State University, Malete, Kwara State. The location is situated on Latitude 8.71^0N and Longitude 4.44^0E (Ogunbosoye *et al.,* 2015) in the southern Guinea Savannah agro-ecological zone of Nigeria.

Experimental Animals

The experimental animals comprised twenty (20) growing WAD goats, sourced within the Ilorin metropolis. They were neck tagged for identification and quarantined upon arrival at the Small Ruminant Unit of the Teaching and Research Farm. The animals were vaccinated against Pestes des Petit Ruminants (PPR) using the PPR vaccine at the rate of 1mL per animal to confer immunity, administered with Albendazole (dewormer at 12.5 mg/kg body weight), dusted with ectoraid against ectoparasites and treated with oxytetracycline HCl (a broad spectrum antibiotic) before the commencement of the experiment. The animals were housed in their pen with *ad libitum* access to clean water.

Procurement and Preparation of Herbs

Phyllantus amarus, *Ocimum gratissimum* and *Lactuca taraxacifolia* were harvested within the school farm in large quantities. They were air dried for 3 -5 days (by laying them over metal mesh rack dryers placed above the floor) at room temperature, then milled into fine powder, sieved through a 1mm gauge and preserved in tightly closed plastic jars until use.

Experimental Design and Diet Formulation

The animals were randomly allotted to five treatments in a completely randomized design (CRD) with four (4) goats in each of the treatments. The five experimental treatments were:
Treatment 1: Feed only (control).
Treatment 2: Feed + *Ocimun gratissimum*
Treatment 3: Feed *Phyllantus amarus*
Treatment 4: Feed + *Lactuca taraxacifolia*
Treatment 5: Feed + *Phyllantus amarus* + *Ocimun gratissimum* + *Lactuca taraxacifolia*

Herbs Administration

The herbs were administered to the goats at 3g/ kg feed as described by Abdelhamid *et al.* (2011). The procedures were repeated every week. The weighted content was served at 8.00 hr. and 16.00 hr. daily throughout the experimental period.

Chemical Composition

The moisture content of the diets was determined by drying in a forced-air oven at 60^0C until constant weight was achieved. After drying, samples were ground to pass a 2 mm sieve and then subjected to proximate analysis according to AOAC, (2005). The carbohydrate content was calculated from the differences in the sum of protein, fat, moisture, and ash content subtracted from 100. Neutral detergent soluble (NDS), Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin, Hemicelluloses and cellulose were analyzed according to Van Soest *et al.* (1994) procedure.

In vitro Fermentation

Rumen fluid was obtained from five West African dwarf (WAD) goats through the suction tube before the morning feed and sieved with four layered cheesecloth in continuous flushing with CO_2. To 200 mg sample in the syringe was added 30 ml inoculum that contained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57g KCl + 0.47g NaCl + 0.12g $MgSO_4$. $7H_20$ + 0.16g $CaCI_2$. $2H_2O$ in a ratio 1:4 v/v respectively. Incubation was carried out in 120 ml calibrated syringes in five replicates with blank syringes (no substrates) at 39 ± 1º C for 24 hrs. The gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24h (Menke and Steingass, 1988). The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Metabolizable energy (ME, MJ/Kg DM), organic matter digestibility (OMD %) and short-chain fatty acids (SCFA µmol) were estimated at 24h incubation

ME = 2.20 + 0.136 GV + 0.057 CP + 0.0029CF (Menke and Steingas, 1988).

OMD = 14.88 + 0.88GV + 0.45CP +0.651XA (Menke and Steingas, 1988).

SCFA = 0.0239GV – 0.0601 (Getachew *et al.*, 1999).

Where GV, CP, CF and XA are net gas production (ml/200mg, DM) at 24 h incubation time, crude protein, crude fibre and ash contents of incubated samples respectively.

Methane Production

At the end of 24 hours of incubation, 4 ml of NaOH (10 M) was introduced, following a procedure of Fievez *et al.* (2005). The content was inserted into the silicon tube, which was fastened to the syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration.

Determination of Rumen Liquor Parameters

pH and NH_3-N Analysis

At the end of the growth study, the goats fasted overnight but water was served *ad libitum*.100ml rumen fluids were collected from the West African Dwarf (WAD) goats according to the treatments using a stomach tube, sieved with a four-layered muslin cloth into a beaker. The pH of the rumen fluid was measured immediately using a pH meter. The collected rumen liquor samples were then strained, frozen and later thawed at room temperature and then shaken. The contents were transferred into glass tubes and centrifuged at 4000 rpm for 10 minutes. The supernatant was analyzed for ruminal ammonia-nitrogen concentration (NH_3-N) by the method of steam distillation with MgO using a Kjeltec distillate unit as described by AOAC (2012). The NH_3-N concentrations were calculated according to the following equation:

NH_3-N (mg/100ml) = [ml. of HCl titrate ×normality of HCl × 0.014 / volume of rumen liquor (1ml)] ×100 (AOAC, 2012)

Total Volatile Fatty Acids Analysis

Total volatile fatty acids (TVFAs) in the rumen liquor were estimated according to the steam distillation method (Samuel *et al.*, 1997). The TVFAs were calculated according to the following equation:

TVFA (mmol/L) = (ml. NaOH titrate ×normality of NaOH/volume of rumen liquor (1ml) ×1000) (Samuel *et al.*, 1997). The molar proportions of individual volatile fatty acids (VFA) were determined by chromatography technique using high-performance liquid chromatography according to the method described by (Filípek and Dvořák, 2009). The rumen fluids were sent to the laboratory to measure the individual volatile fatty acids; acetic acid, propionic acid and butyric acid.

Microbial Count Analysis

Rumen fluid was also used for total direct counts of bacterial, protozoa and fungal zoospores using methods of Galyean (1989) by haemacytometer. One (1) ml of rumen fluid was transferred to a plastic bottle to which 9 ml of 10 ml/L formalin solution (1:9 v/v, rumen fluid: 10 ml/L formalin) was added and then stored at 4°C for measurement of fungal zoospores and the protozoa population. Another 10 ml of the fluid was used for viable bacteria count (total viable bacteria count) according to the method of Aberu *et al.* (2004). Total viable counts of bacteria were determined in roll tubes on a complete medium, while numbers of cellulolytic bacteria were estimated as the most probable number using a cellulose medium.

Data Analysis

Data collected for all determined parameters were subjected to Analysis of Variance (ANOVA) and differences between treatment means were separated by Least Significance Difference using the General Linear Model procedure of Statistical Analysis System (SAS, 2011).

Results and Discussion

Phytochemical Qualitative Abundance of Leaves of Lactuca taraxacifolia, Phyllanthus amarus and Ocimum gratissimum

Table 1 shows the phytochemical qualitative abundance of leaves of *Lactuca taraxacifolia, Phyllanthus amarus* and *Ocimum gratissimum*. The results showed that there was abundant (high) presence of saponin (+++) in *Lactuca taraxacifolia* and *Ocimum gratissimum*, but was moderately (++) present in *Phyllanthus amarus*. Steroid (+) was relatively present in *Lactuca taraxacifolia* and *Phyllanthus amarus* but was absent (-) in *Ocimum gratissimum.* Tannin (+) was relatively present in *Latuca tarataxifolia* and *Phyllanthus amarus* but was moderately present (++) in *Ocimum gratissimum.* Terpenoid was relatively present in *Lactuca taraxacifolia* but was absent in both *Phyllanthus amarus* and *Ocimum gratissimum.* Flavonoids presence was moderate (++) in *L. taraxacifolia*, relative in *Ocimum gratissimum* and absent in *P.* amarus. Glycoside was only present in *L. taraxacifolia*. Phenol was present (+) in *L. taraxacifolia* and *O. gratissimum*, but absent (-) in *P.* amarus. Oxalate was absent (-) in all the medicinal plants while phytate was only relatively present in *O. gratissimum*

Phytochemicals are biologically active compounds (naturally occurring chemicals in plants), found in trace amounts, which are not established nutrients, but contribute significantly to protection against degenerative diseases and have disease-preventive properties (Ladipo *et al.*, 2010). The presence of tannins, saponins, steroids, flavonoids, cardiac glycosides and terpenoids has also been reported in *L. taraxacifolia* (Adinortey *et al.* (2012). Likewise, Rosine *et al.* (2022) reported the presence of phenols, flavonoids, tannins, anthraquinones, alkaloids, steroids and glycosides and absence of terpenoids and saponins in *Lactuca taraxacifolia.* The high presence of favonoid, glysosides and terpenoids, moderate presence of phenols, tannin and phatates, slight presence of alkanoid, saponin and steroid and the absence of oxalate was reported by Mgbeje *et al.* (2019) in *Ocimum gratissimum*. *Phyllanthus amarus* has been reported to contain tannins, saponins, flavonoids, terpenoids, alkaloids, steroids and glycosides in relative amounts (Alexandra, 2016).

Table 1. Phytochemical Screening of leaves of *Lactuca taraxacifolia* (LT), *Phyllanthus amarus* (PA) and *Ocimum gratissimum* (OG)

Phytoconstituents	LT	PA	OG
Saponin	+++	++	+++
Steroid	+	+	-
Tannin	+	+	++
Alkaloid	+	+	++
Terpenoid	+	-	-
Flavonoid	++	-	+
Glycoside	++	-	-
Phenol	+	-	+
Oxalate	-	-	-
Phytate	-	-	+

Key: present (+), moderately present (++), highly present (+++), not present (-)

Proximate Composition of Lactuca taraxacifolia, Phyllanthus amarus and Ocimum gratissimum

The proximate composition of *Lactuca taraxacifolia, Phyllanthus amarus* and *Ocimum gratissimum* is presented in Table 2. The crude protein (CP) contents of the herbs ranged between 15.87 and 19.44%, the ash content was lowest in ocimum (2.55%) but highest in latuca (20.34%), and crude fibre was also highest for latuca (14.39%). The quantity of CP of above 15 % recorded in the leaves suggests that the leaves have an appreciable amount of protein that may be made available to the rumen microbes which could lead to an increase in the digestibility of the feed, thereby enhances productivity of the ruminant. The crude protein contents obtained in all the leaves study are above the 10-12% recommendation for the growth or maintenance of small ruminants and are also above the minimum threshold level (7% CP) required for optimal rumen microbial activities (Norton, 2003). Carbohydrate contents of *Ocimum gratissimum* obtained in this study is comparable to the value of 66.34 obtained for *Ocimum gratissimum* by Gideon *et al.* (2018) but was higher than the one reported by Akinwumi and Omotayo (2016) of common vegetables in southwestern Nigeria. Likewise, carbohydrate contents of *Phyllanthus amarus* obtained in this study is higher than the value of 30.56 obtained for *Phyllanthus amarus* by Adinortey *et al.* (2012). This makes *O. gratissimum* and *P. amarus* a good source of energy for animal. Ash contents in *Lactuca taraxacifolia*, being high is an indication of its richness in minerals.

Table 2. Proximate Composition (%) of *Lactuca taraxacifolia*, *Phyllanthus amarus* and *Ocimum gratissimum*

Parameters	LT	PA	OG
Dry Matter	7.44	10.43	7.96
Crude Protein	19.44	15.87	15.98
Total Ash	20.34	5.73	2.55
Crude Fibre	14.27	7.83	9.85
Crude Fat	0.49	7.02	2.45
Carbohydrate	37.90	53.12	61.21

All the parameters measured for chemical composition of the experimental diets fed to WAD goats were significantly influenced (P<0.05) by dietary treatments (Table 3). The values ranged as follows; crude protein (13.47 – 17.27%), crude fibre (4.89 – 5.94%), ash (4.90 – 6.69%), ether extract (6.79 – 8.51%), carbohydrate (42.76 – 63.51%), Neutral Detergent Soluble NDS (9.57 – 11.41), Acid Detergent Fibre (37.83 – 39.98), Acid Detergent Lignin (29.01 – 30.90), hemicellulose (7.93 – 10.15), cellulose (22.86 – 31.11) and Neutral Detergent Fibre (61.94 – 69.88).

A high dry matter content diets makes the diets a good source of feed for animals in the dry season and will also encourage more feed intake. Moreover, diet containing the mixture of *Ocimum gratissimum*, *phyllantus amarus* and *Lactuca taraxacifolia* (T5) had the highest crude protein (17.27) than the rest of the diets. This suggest that the combination of the 3 herbs in the diet of the goats improved the nutritive capacity of the diets. Similar findings were reported by Amany *et al.* (2022) who also observed that the mixture of herbal plants improved the crude fibre and nutritive value of experimental diets. Diet with *Ocimum gratissimum* (T2) only also had appreciable nutritive value as the CP though slightly lower in value but was statistically similar to that of T5. This suggests that *Ocimum gratissimum* might be considered a veritable protein rich vegetable with potential to serve as substitutes for protein in diet of ruminants especially goats. This is consistent with the findings of Gideon *et al.* (2018) who describes *Ocimum gratissimum* as being very rich in proteins and can serve as substitutes for protein, especially among rural dwellers.

However, T2 had the highest crude fibre content with the least value recorded in T1. This suggests that the herbs especially *Ocimum gratissimum* contain appreciable level of fibre in their composition. There were more of carbohydrate in diets containing herbs compared to control diet with diet T3 having the highest CHO. This indicates that the addition of the herbs to the diets increases the energy content of the diets and thus could use as energy source in goats diet formulation. Fajohunbo and Egbeyale (2010) had earlier reported that carbohydrate is the dominant nutrient in *O. gratissimum* when compared to its crude fibre, ash and crude protein values. Ezeonu *et al.*, (2012) also reported that there is likelihood of prebiotics being contained in *O. gratissimum* as a green leafy vegetable and since prebiotics are carbohydrate complexes, it suggests that the plant could contain a good quantity of some classes of prebiotics. Cellulose and hemicellulose of diet with herbs were higher than the control diet, except in T5 which was similar statistically to control diet. This is expected as the herbs are plant-based feed materials which are expected to be made up of certain levels of cellulose and hemicellulose contents.

Table 3. Chemical Composition (%) of Experimental Diets with the Inclusion of Herbs

Parameters	T1	T2	T3	T4	T5	SEM
Dry matter	92.85^{a}	93.11^{c}	92.99^{b}	93.11^{c}	93.00^{b}	0.0006
Crude Protein	15.75^{ab}	16.08^{ab}	13.47^{c}	15.20^{b}	17.27^{a}	0.6884
Crude Fibre	4.89^{d}	5.94^{a}	5.51^{c}	5.64^{b}	5.67^{b}	0.0008
Ash	4.90^{d}	6.03^{c}	6.69^{a}	6.69^{a}	6.52^{b}	0.0011
Ether Extract	8.51^{a}	7.91^{b}	6.79^{d}	7.06^{c}	7.14^{c}	0.0436
CHO	42.76^{e}	57.15^{c}	63.51^{a}	58.53^{b}	56.41^{d}	0.0780
Neutral Detergent Soluble	11.41^{a}	10.53^{b}	10.82^{b}	9.57^{c}	10.36^{b}	0.0698
Acid Detergent Fibre	39.08^{b}	39.98^{a}	39.94^{a}	38.67^{c}	37.83^{d}	0.0329
Acid Detergent Lignin	30.18^{b}	30.90^{a}	29.43^{c}	29.01^{d}	29.90^{b}	0.0822
Hemicellulose	8.90^{c}	9.09^{b}	10.15^{a}	9.83^{a}	7.93^{c}	0.0822
Cellulose	22.86^{d}	28.31^{c}	29.97^{b}	31.05^{a}	31.11^{a}	0.0102
Neutral Detergent Fibre	61.94^{e}	68.29^{c}	67.93^{d}	69.88^{a}	68.94^{b}	0.0123

[abcde] Means along the same row with different superscripts are significantly different (P<0.05), SEM = Standard Error of Mean, CHO = carbohydrate, T1 = No herbs (Control), T2 = *Occimum gratissimum*, T3 = *Phyllantus amarus*, T4 = *Lactuca taraxacifolia*, T5 = *P. amarus* + *O. gratissimum* + *L. taraxacifolia*

In vitr Gas Production Pattern of the Experimental Diet

There was non-significant (P>0.05) variation in the gas production pattern of the experimental diets (Table 4). The volume produced under each treatment continue to increase at every three hours. The values for 3 hours incubation period ranged from 8.00 – 9.33 ml/200mg DM and continues to increase till 24th hour from 26.33-36.33 ml/200mg DM among the diets. This trend is an indication that more degradation is still possible after 24 hours of incubation due to rises in the gas at every three hours. Though the production of gas was not significantly (P>0.05) influenced by the incubation period, T1 and T4 diets produced more gas followed by T2 and then T3 while T5 had the least gas production. The production of more gas may be due to higher percentage of cellulose and hemicellulose in T4 diet and higher crude fibre content in *Lactuca taraxacifolia*, than others. Likewise the less gas production as observed in T5 may be due to lesser carbohydrate in the diet when compared with others. Gas production is a function of and a mirror of degradable carbohydrate and therefore, the amount depends on the nature of the carbohydrates (Blummel and Becker, 1997). Many factors may determine the amount of gas produced during fermentation, factor such as the nature and level of fibre, the presence of secondary metabolites and potency of the rumen liquor for incubation (Babayemi *et al.*, 2004). The trend of gas production in this study is consistent with the trend reported by Bunglavan *et al.* (2010) where supplementation of eight different herbs in ruminant diets also led to the continuous production of gas at every four hours incubation.

Table 4. *In vitro* Gas Production Volume of the Experimental Diets at Different Incubation Hours

Incubation hours	Treatments						
	T1	T2	T3	T4	T5	SEM	p-value
3	8.00	9.33	7.33	9.33	7.33	1.30	0.6678
6	15.33	14.00	12.00	16.67	13.33	1.79	0.4452
9	20.67	20.67	16.66	23.33	18.67	2.04	0.2771
12	24.67	24.00	21.33	27.33	22.00	1.63	0.1533
15	26.67	26.00	22.67	29.33	23.33	1.96	0.1878
18	28.67	28.00	26.33	31.33	25.33	2.01	0.3237
21	32.00	28.67	28.00	31.33	25.33	1.55	0.0712
24	36.33	31.33	29.67	34.00	26.33	1.27	0.0022

[abcde] Means along the same row with different superscripts are significantly different (p<0.05), SEM = Standard error mean, T1 = No herbs (Control), T2 = *Occimum gratissimum*, T3 = *Phyllantus amarus*, T4 = *Lactuca taraxacifolia*, T5 = *P. amarus* + *O. gratissimum* + *L. taraxacifolia*

Methane Production of the Experimental Diets

Presented in Figure 1 is the methane production of the experimental diets. There was significant (P>0.05) variation in the methane production. Methane (CH_4) production was highest in control diet T1 than other diets with T5 recording the lowest value. It varies from 7.67 ml/200 mg DM (T5) to 12.67 ml/200 mg DM (T1). The amount of methane produced depends on the level of fermentation. It was observed from this study that experimental diet with high gas production produces more methane. Similar findings have also been reported by and Babayemi and Bamikole (2009) and Ogunbosoye (2016). Methane production is a monumental waste but it is a parameter to measure feedstuff digestibility in the rumen of an animal (Njidda and Nasiru, 2010).

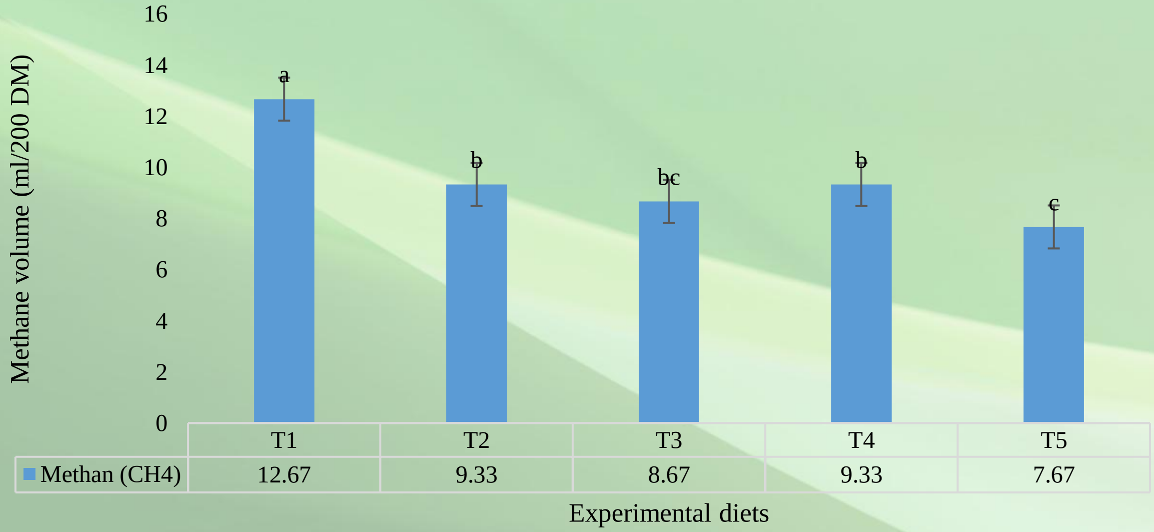

Fig 1. The methane (CH_4) production of experimental diets

Calculated OMD (%), ME (MJ/Kg) and SCFA (µmol) of Experimental Diets

Figures 2, 3 and 4 show the Organic Matter Digestibility (OMD), Metabolizable Energy (ME), and Short Chain Fatty Acids (SCFA) of the experimental diets, resoectively. All the parameters calculated in this study were significantly influenced by the experimental diets. The values for the OMD, ME and SCFA ranged from 48.56 (T5) to 57.45 (T1), 6.99 (T5) to 8.35 (T1) and 0.57 in T5 to 0.81 in T1, respectively. The range value of OMD recorded in this study indicates good digestibility of herbs despite the fact that the control diet recorded the highest digestibility potential. The values of metabolic energy yield reported in this study suggests the potential of the herbs especially *Lactuca taraxacifolia* as energy laden feed additives which can be incorporated in feed formulation for ruminant animals, as animal performance also depends on ME content present in the feed (Niemeläinen *et al.*, 2001). The levels of OMD and ME obtained in this study were similar to 40.12 to 67.06 % (OMD) and 5.58 to 10.32 MJ/Kg (ME) reported by Tag-El-Din *et al.* (2012) in ram.

The low SCFA estimated for T5 might be due to the lower gas production obtained from the diet. The appreciable estimated SCFA in T4 compared to T2, T3 and T5, further suggests the potential of *Lactuca taraxacifolia* to make energy available to the ruminants when consumed. Akinfemi and Ladipo (2014) had earlier reported that SCFA is an indicator of energy availability to the animal. The values of SCFA obtained in this study were slightly higher than the range of 0.23 to 0.60 µmol obtained by Ogunbosoye (2016) on forages fed to ruminant, but was similar to the level of 0.8 – 0.9 µmol reported by Mpanza *et al.* (2020) in ruminant. However, the values were lower than the range values of 6.07 – 6.97 µmol reported by Abd El Tawab *et al.* (2021) in ruminant fed two herbal plants based diets.

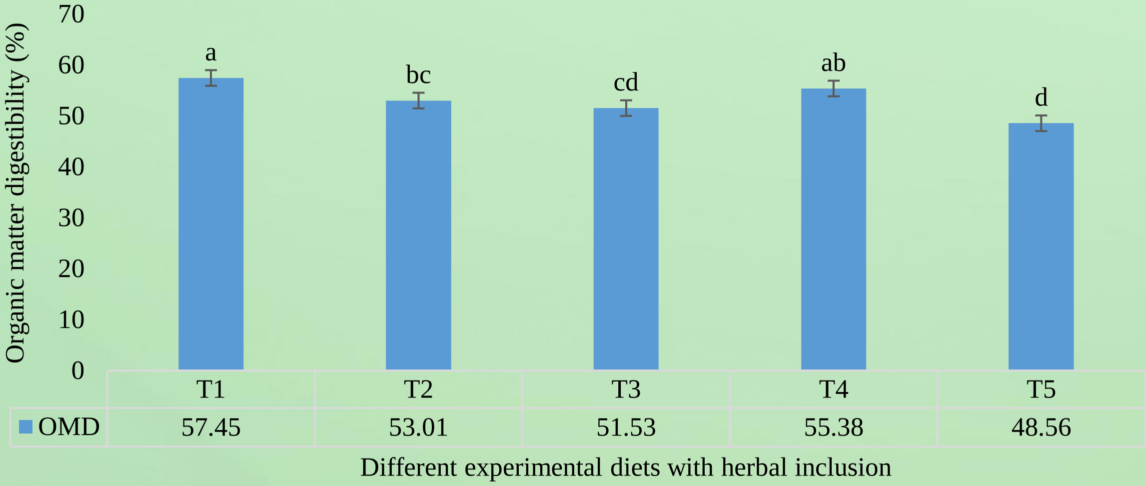

Fig 2. The Organic Matter Digestibility of the Experimental Diet (%)
T1 = No herbs (Control), T2 = *Occimum gratissimum*, T3 = *Phyllantus amarus*, T4 = *Lactuca taraxacifolia*, T5 = *P. amarus* + *O. gratissimum* + *L. taraxacifolia*

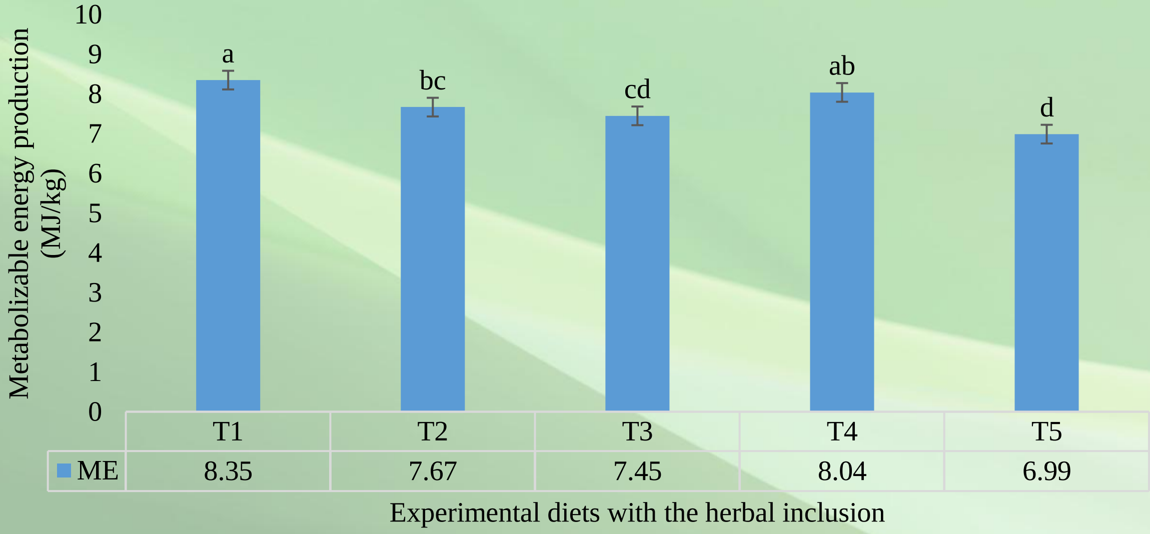

Fig 3. The Metabolisable Energy of the Experimental Diet (MJ/kg)

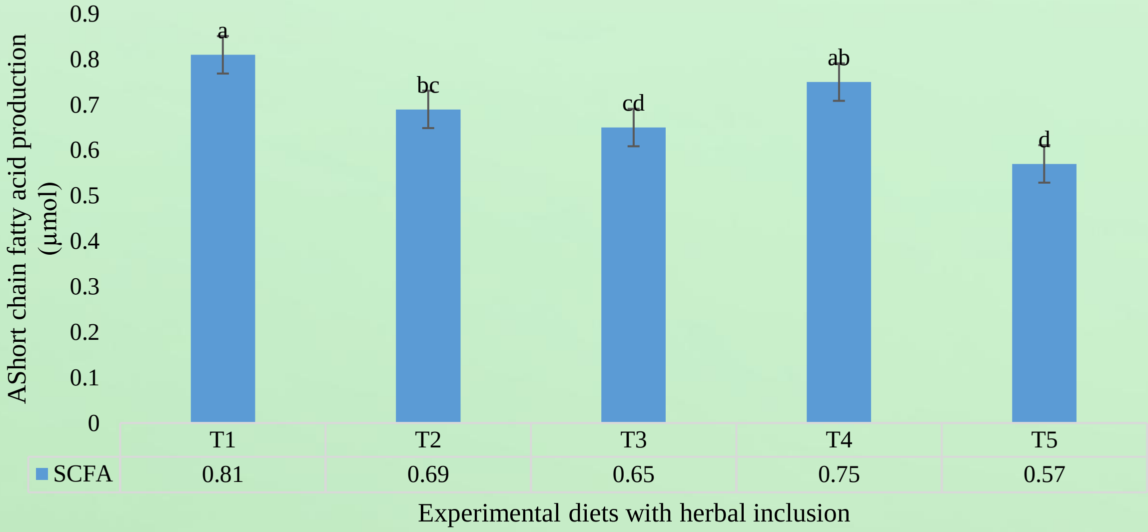

Fig 4.4: The Short Chain Fatty Acid of the Experimental Diet (µmol)

Ruminal Characteristics of West African Dwarf Goats Fed Herbal-Fortified Diets

The effect of different herbal supplementations on the ruminal characteristics of West African dwarf goats is presented in Table 5. All the parameters investigated were significantly (P<0.05) influenced by dietary treatments except rumen pH, acetic-propionic ratio (A/P) and rumen ammonia nitrogen (NH_3). The acetic acid values of the goats varied significantly across the treatment groups with goats fed *Phyllantus amarus* (T3) having the highest rumen acetic acid value of 36.45mm/100mL, which is statistically similar to 33.41mm/100mL recorded in goats fed T5, while the values of 28.72, 27.65 and 27.00 mm/100mL obtained in goats fed T2, T1 and T4, respectively were lower and statistically the same. A similar trend was observed in propionic acid content of the rumen. The butyric acid values were higher in all the goats fed the herbal supplements than the control group with goats fed T4 (8.39 mm/100mL) having the highest value, followed by goats on T3 (7.85 mm/100mL), T5 (7.56 mm/100mL) and T2 (7.29 mm/100mL), respectively while control group (5.98 mm/100mL) recorded the least value.

The concentration of acetic acid produced was higher than the concentration of butyric and propionic acid. This is attributed to the fact that cellulolytic bacteria produce acetic acid as major end product from fiber degradation, thus their concentration is always higher than other acids. The range values of acetic acid (27.00 - 36.45 mm/100mL) is lower to the value of 58.5 - 60.2 obtained by Seong *et al.* (2022) but higher than the values of 11.29 – 14.78 obtained by Kurniawati *et al.* (2018) in ruminant fed herbal supplements. The range values of butyric acid (5.98 - 8.39 mm/100mL) and propionic acid (6.52 - 10.42 mm/100mL) obtained in this study are consistent with the values of 5.33 - 5.96 and 9.50 - 11.62 obtained for butyric and propionic acid, respectively by Hassan *et al.* (2021)

Goats on control diet (64.59 mm/100mL), T4 (64.45 mm/100mL) and T2 (64.32 mm/100mL) diet had similar total volatile fatty acid (TVFA) values which were higher than those on T5 (61.00 mm/100mL) and T3 (58.43 mm/100mL) respectively. The range value of 58.43 – 64.45 mm/100mL obtained in this study were lower than the value of 73.14 - 90.57 mm/100mL recorded in goat fed herbal supplement by Wang *et al.* (2023). Rumen pH and TVFA content are essential index for assessing ruminal fermentation (Cui *et al.* 2022). According to Puniya *et al.* (2015), pH range of 5.5 to 6.9 is considered physiologically the normal pH that support normal feed fermentation in the rumen. In this study, supplementation of diet with herbs didn't have significant influence of the ruminal pH, besides the values were slightly lesser than the normal range values, however, TVFA changes significantly, though the values in diets supplemented with herbs were not better than the control diets. This indicates that the herbs supplementation did not improve the energy generation in the rumen of the goats as the herbs might not have aided properly the activities of ruminal cellulolytic bacterial which would have led to increased digestibility of the experimental diets for subsequent energy generation. This may be attributed to the quality of herbs in terms of their constituents and the stage at which they were harvested which might have influenced the way microbes in the rumen acted on them.

It has also been observed that the use of a lower dose of herbal supplement in ruminant diet led to the metabolic redundancy of the ruminal ecosystem, which resulted in lower concentration of TVFA in rumen (Patra *et al.*, 2019). TVFA plays an important role in maintaining and providing energy for the growth of ruminants. The structure, quality, microbial activity, and microflora of ruminant diets has been reported as factors that affect the composition and concentration of TVFA in the rumen (Sun *et al.*, 2019)

Table 5. Effect of Different Herbal Supplementation on Ruminal Characteristics of West African dwarf goats

Parameters	T1	T2	T3	T4	T5	SEM
pH	5.02	5.06	4.48	5.05	4.80	0.10
Acetic acid (mm/100mL)	27.65^{b}	28.72^{b}	36.45^{a}	27.00^{b}	33.41^{ab}	1.32
Butyric acid (mm/100mL)	5.98^{b}	7.29^{ab}	7.85^{ab}	8.39^{a}	7.56^{ab}	0.33
Propionic acid (mm/100mL)	6.69^{ab}	6.90^{ab}	10.42^{a}	6.52^{b}	8.47^{ab}	0.58
TVFA (mm/100mL)	64.59^{a}	64.32^{a}	58.43^{b}	64.45^{a}	61.00^{ab}	0.92
A/P	4.40	4.18	3.56	4.22	4.08	0.18
NH_3-N(mg/100mL)	5.30	5.06	4.48	5.05	4.80	0.10

^{a,b} Means along the same row with different superscripts are significantly different (P<0.05), SEM = Standard Error of Mean, TVFA= Total Volatile Fatty Acids, A/P= Acetic acid: Propionic acid, NH_3-N= Rumen ammonia Nitrogen, T1 = No herbs (Control), T2 = *Occimum gratissimum*, T3 = *Phyllantus amarus*, T4 = *Lactuca taraxacifolia*, T5 = *P. amarus* + *O. gratissimum* + *L. taraxacifolia*

Effect of Different Herbal Supplementation on the Rumen Microbial Count of West African Dwarf goats

The effect of different herbal supplementation on the rumen microbial count of West African Dwarf goats is presented in Table 6. Herbal supplementation had significant (P<0.05) effect on the microbial population of West African Dwarf goats observed in this study. The total bacterial count, fungal count and protozoan values of the experimental goats ranged from 65.33 - 190.00 × 10^{-4}cfu/mL, 5.67 – 11.00 sfu/mL and 12.67 – 28.67 x10^{-2}/mL respectively.

It is observed that the population of bacteria (190.00) and fungi (11.00) were highest in diet containing the mixture of *P. amarus, O. gratissimum and L. taraxacifolia*. This indicates that herbal mixtures as against sole supplementation perhaps had better impact on the ruminal cellulolytic bacterial activity by multiplying their number to ensure increased digestibility. Moreover, sole supplementation with *P. amarus, O. gratissimum and L. taraxacifolia* also increases the population of bacteria and fungi in the rumen of the goats compared to the control group. The increase population of cellulolytic bacterial is considered a positive impact as the rumen bacteria are known to involve in the degradation of plant polysaccharides to produce VFA as the main source of energy for animals (Mizrahi, 2012). Thus, cellulolytic bacterial activities in the rumen is beneficial to the animal as they help ensure increased digestibility of feed material, ensure availability of nutrient for absorption by the animal. The range values of bacteria obtained in this study were higher than the values of 2.06 – 3.05 obtained in WAD goats fed *O. gratissimum* as reported by Adebayo *et al.* (2019) and higher than 3.1 – 7.6 obtained by Wanapat *et al.* (2013). It is also observed that the population of protozoa were lower in diets supplemented with herbs compared to the control diet except in diet supplemented with *Lactuca taraxacifolia* (T4).

The least protozoa population was recorded in diet supplemented with *P. amarus* (T3). This indicates that other herbs and their combination were able to inhibit the destructive nature of protozoa in the rumen. The secondary plant metabolites in herbs especially saponins have been found to suppress or eliminate protozoa from the rumen and reduce methane and ammonia production (Anantasook and Wanapat, 2012). At appropriate dose, saponins or saponins containing plants have been shown to suppress protozoal population, increase bacteria and fungi population and decrease methanogenesis hence improve performance in ruminants (Sirohi *et al.*, 2009). Lila *et al.* (2003) had earlier reported that the elimination of ciliate protozoa from the rumen reduced methane emission by 30 to 45%. The range value of protozoa population obtained in this study was higher than the value of 4.8 – 7.8 x10^{-2}/mL obtained in ruminant by Wanapat *et al.* (2013) and the higher 0.25 – 0.68 x10^{-2}/mL obtained in WAD goat by Adebayo *et al.* (2019). This also explain why the diet with highest protozoa produced the highest methane.

Table 6. Effect of Different Herbal Supplementation on Rumen Microbial Count of West African Dwarf Goats

Parameters	T1	T2	T3	T4	T5	SEM
Total bacterial count (x10^{-4}cfu/mL)	65.33^{d}	71.67^{cd}	93.33^{b}	75.00^{c}	190.00^{a}	2.83
Total fungal count (sfu/mL)	5.67^{b}	5.83^{b}	9.50^{a}	6.67^{b}	11.00^{a}	0.51
Protozoan (x10^{-2}/mL)	19.00^{b}	13.33^{c}	12.67^{c}	28.67^{a}	17.50^{b}	0.90

SEM = Standard Error of Mean, T1 = No herbs (Control), T2 = *Occimum gratissimum*, T3 = *Phyllantus amarus*, T4 = *Lactuca taraxacifolia*, T5 = *P. amarus* + *O. gratissimum* + *L. taraxacifolia*

Conclusion

The results of this study demonstrate that the leaves of *Latuca taraxacifolia*, *Phyllanthus amarus*, and *Ocimum gratissimum* are rich in bioactive compounds, including saponin, tannin, and alkaloid. These phytochemicals significantly impacted methane production, with diets supplemented with *Phyllanthus amarus* and the combination of *Latuca taraxacifolia* + *Phyllanthus amarus* + *Ocimum gratissimum* exhibiting substantial reductions in methane production. Furthermore, the studied plant combinations improved ruminal characteristics and significantly modified the microbial population in the rumen, highlighting their potential as natural feed additives for mitigating methane emissions and promoting rumen health.

References

Abd El Tawab, A.M., Khattab, M.S.A., Hadhoud, F.I. and Shaaban, M.M. (2021). Effect of mixture of herbal plants on ruminal fermentation, degradability and gas production. *Acta Scientiarum. Animal Sciences*, 43: 1-8.

Abdel-Hamid, N.M., Nazmy, M.H., Mahmoud, A.W., Fawzy, M.A., Youssof, M. (2011). A Survey on herbal management of hepatocellular carcinoma. *World Journal of Hepatology* 3(7): 175-183

Abdullah, M.A.M. and Farghaly, M.M. (2019) Impact of Partial Replacement of Cotton Seed Meal by Nigella sativa Meal on Nutrients Digestibility, Rumen Fermentation, Blood Parameters, Growth Performance of Growing Lambs. *Egyptian Journal of Nutrition and Feeds*; 22(2): 11-20

Aberu, A., Carulla, J.E., Lascano, C.E., Diaz, T.E., Kreuzer, N. and Hess, H.D. (2004). Effect of fruits on runinal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legine. *Journal of Animal Science* 82: 1392- 1399.

Adebayo, K.O., Aderinboye, R.Y., Sanwo, K.A., Oyewusi, I.K. and Isah, O.A. (2019b). Microbial population and blood parameters of West African dwarf goats fed scent leaf (Ocimum gratissimum) as additive. *Nigerian Journal of Animal Production*. 46(1):225-235

Adinortey, M.B., Sarfo, J.K., Quayson, E.T., Weremfo, A., Adinortey, C.A. and Ekloh W. (2012). Phytochemical screening, proximate and mineral composition of *Launaea taraxacifolia* leaves. *Research Journal of Medicinal Plants*. 6(2):171–9.

Akinfemi, A. and Ladipo, M. K. (2014). Nutritional potential of some selected weed species in north central Nigeria Using in vitro gas production technique. *International Journal of Agric Science* Vol. 4(4): 214-217.

Akinwumi, O.A, and F.O. Omotayo (2016). Proximate analysis and nutritive values often common vegetables in South-West (Yoruba Land) Nigeria. *Community Applied Science*. 4(2):79-91.

Alexander, P. (2016). Phytochemical Screening and Mineral Composition of the Leaves of *Ocimum Gratissimum* (Scent Leaf). *International Journal of Applied Science* and Biotechnology, 4(2): 161-165.

Amany, A.K., El-Badawy, M. M and Hanaa S. S. (2022). Effect of Some Medical Herbs on Productive Performance of Lactating Zaraibi Goats. *Egyptian Journal of Nutrition and Feeds* 25 (3):303-321.

Anantasook, N. and Wanapat, M. (2012). Influence of rain tree pod meal supplementation on rice straw based diets using in vitro gas fermentation technique. *Asian-Australasian Journal of Animal Sciences*; 25(3): 325-331.

AOAC. (2012). Official Methods of Analysis, 19th ed. Association of Official Analytical Chemists: Gaithersburg, MD, USA.

Arhab, R., Karima, K., Nassima, L., Hakim, B., Besma, H. and Hacene, B. (2013). Effect of essential oils extracted from *Satureja calamintha, Mentha pulegium* and *Juniperus phoenicea* on *in vitro* methanogenesis and fermentation traits of vetch oat hay. *African Journal of Environmental Science and Technology*, 7: 140-144.

Babayemi, O. J., and Bamikole, M. A (2009). Nutrient Value and In Vitro Gas Production of African Wild Cocoyam (*Colocasia Esculentum*). *African Journal of Food Agriculture, Nutrition and Development.* 9(1): 593-607.

Babayemi, O. J., Demeyer, D., and Fievez, V. (2004). In vitro fermentation of tropical browse seeds in relation to their content of secondary metabolites. *Journal of Animal Feed and Technology.* 13(1): 31–34.

Benchaar, C. and Greathead, H. (2013). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. *Animal Feed Science and Technology*, 166:338-355.

Benchaar, C., McAllister, T. A. and Chouinard, P. Y. (2008). Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or yucca schidigera saponin extracts. *Journal of Dairy Science*, 91, 4765-4777.

Blummel, M. and Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by in vitro gas production and their relationship to voluntary feed intake. *British Journal of Nutrition*, 77: 757-768.

Bunglavan, S.J., Valli, C., Ramachandran, M. and Balakrishnan, V. (2010). Effect of supplementation of herbal extracts on methanogenesis in ruminants. *Livestock Research for Rural Development*, 22 (11): 76-83.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W. and Kamel, C. (2005). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. *Journal of Dairy Science*, 88, 2508-2516.

Chaudhry, A. S. and Khan, M. M. H. (2012). Impacts of different spices on in vitro rumen dry matter disappearance, fermentation and methane of wheat or ryegrass hay based substrates. *Livestock Science*, 146: 84-90.

Cieslak, A., Szumacher-Strabel, M., Stochmal, A. and Oleszek, W. (2013). Plant components with specific activities against rumen methanogens. *Animals*, 7:253-265.

Cui, Y., Liu, H., Gao, Z., Xu, J., Liu, B., Guo, M., Yang, X., Niu, J., Zhu, X., Ma, S., Li, D., Sun, Y and Shi, Y. (2022) Whole-plant corn silage improves rumen fermentation and growth performance of beef cattle by altering rumen microbiota. *Applied Microbiology and Biotechnology* 106:4187–4198.

Dong, G., Wang, X., Liu, Z. and Wang, F. (2010). Effects of phytogenic products on in vitro rumen fermentation and methane emission in goats. *Journal of Animal and Feed Sciences*, 19: 218-219.

Ezeonu, I. M., Akabueze, E.U. and Chah, K.F. (2012). Prebiotic Effects of Vernonia amygdalina and Ocimum gratissimum Aqueous Leaf Extracts in Rabbit. *African Journal of Biotechnology* 11(10): 2537-2547

Fajohunbo, G.O. and Egbeyale, I.T. (2010). Effect of Ocimum gratissimum Leaf Meal on the Haematological Parameters of Broilers. (Dissertation Submitted to University of Agriculture, Abeokuta.

Fievez, V., Babayemi, O.J. and Demeyer, D. (2005). Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production requiring minimal laboratory facilities. *Animal Feed Science and Technology*. (124-128), 197-210.

Filípek, J. and Dvořák. R. (2009). Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachophoresis. *Acta Veterinaria Brno*, 78: 627-633.

Gadde U, Kim WH, Oh ST, Lillehoj HS (2017) Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews*, 18:26–45

Gebrehiwot, T. (2014). Rumen manipulation for enhanced feed utilization and improved productivity performance of ruminants: A review. *Momona Ethiopian Journal of Science*, 6: 3-17.

Getachew, G.H, Makkar, P.S and Becker, K. (1999). Stoichiometric relationship between short chain fatty acid and in vitro gas production in the presence and absence of polyethylene glycol for tannin containing browses, EAAP Satellite Symposium, Gas production: Fermentation kinetics for feed evaluation and to assess microbial activity.18th-19th August, Wageningen, Netherlands.

Gideon, O.O., Mahboob A.J., Olusanya. A.O., Abdulfatai B.R and Adepeju O.O (2018). Proximate analysis, mineral composition, and antioxidant properties of bitter leaf and scent leaf. *International Journal of Vegetable Science*. pp 1-9.

Goodland, R. and Anhang, J. (2009) Livestock and Climate Change: What If the Key Actors in Climate Change Are Cows, Pigs, and Chicken? World Watch, November/December.

Hassan, F., Tang, Z., Ebeid, H.M., Li, M., Peng, K., Liang, X. and Yang, C. (2021). Consequences of herbal mixture supplementation on milk performance, ruminal fermentation, and bacterial diversity in water buffaloes. *Peer Journal* 9:112-121.

Kim, E.T., Kim, C.H., Min, K.S. and Lee, S.S. (2012). Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in *in vitro*. *Asian-Australasian Journal of Animal Sciences* 25: 806–11.

Kurniawati, A., Widodo, W.T.A and Yusiati, L.M. (2018). Study of local herb potency as rumen modifier: The Effect of Red Ginger (Zingiber officinale) on Parameters of Ruminal Fermentation in Vitro. IOP Conf. Series: *Earth Environmental Science* 119: 67-74.

Ladipo, M.K., Doherty, V.F. and Kanife, U.C. (2010). Phytochemical screening and antibacterial investigation of the extract of *Ocimum gratissimum* (Scent Leaf) on selected enterobacteriaceae. *Journal of Clinical Pathology*, 6(2):75 -84.

Lila, Z.A., Mohammed, N., Kandam S., Kamadam T. and Itabashi, H. (2003). Effect of Sarsaponin on Ruminal Fermentation with Particular Reference to Methane Production in vitro. *Journal of Dairy Science* 86:3330–3336.

Mariam, A. G., El-Zarkouny, S. Z., El-Shazly, K. A. and Sallam, S. M. A. (2014). Impact of essential oils blend on methane emission, rumen fermentation characteristics and nutrient digestibility in barki sheep. *Journal of Agricultural Science*, 6: 144.

Menke, K.H and Steingass H. (1988) Estimation of the Energetic Feed Value Obtained.from Chemical Analyses and Gas Production using Rumen Fluid. *Animal Research and Development* 28: 7-55.

Mgbeje, B.I.A. Umoh, E.U and Emmanuel-Ikpeme, C. (20-19). Comparative Analysis of Phytochemical Composition of Four Selected Tropical Medicinal Plants Namely: Ocimum gratissimum, Piper guineense, Gongronema latifolium and Vernonia amygdalina. *Journal of Complementary and Alternative Medical Research* 7(3): 1-11.

Mizrahi, I. (2012). The role of the rumen microbiota in determining the feed efficiency of dairy cows. In: Beneficial microorganisms in multicellular life forms. Berlin, Heidelberg: Springer, pp 203–210.

Mohammad, E. N. S. and Moeini, M. M. (2015). The influence of ginger (*Zingiber Officinale*) on in vitro rumen fermentation patterns. *Annual Research & Review in Biology*, 5: 54-63.

Mpanza, T.D.E., Hassen, A and Akanmu, A.M. (2020). Evaluation of *Stylosanthes scabra* Accessions as Forage Source for Ruminants: Growth Performance, Nutritive Value and *In Vitro* Ruminal Fermentation. *Animals* 10:1939-1949.

Njidda, A. A. and Nasiru, A. (2010). In vitro Gas Production and Dry Matter Digestibility of Tannin Containing Forges of Semi-Arid Region of North-Eastern Nigeria. *Pakistan Journal of Nutrition* 9 (1): 60- 66.

Norton, B. W. (2003). The nutritive value of tree legumes. In R. G. Gutteridge and H. M.Shelton (Eds.), Forage tree legumes in tropical agriculture. Retrieved on from http://www.fao.org/ag/AGP/AGPC/doc/Publicat/Guttshel/x5556e0j.htm#4.1

Odhaib, K. J., Adeyemi, K. D., Ahmed, M. A., Jahromi, M. F., Jusoh, S., Samsudin, A. A. and Sazili, A. Q. (2018) Influence of Nigella sativa seeds, Rosmarinus officinalis leaves and their combination on growth performance, immune response and rumen metabolism in Dorper lambs. *Tropical animal health and production*, 50(5): 1011-1023.

Ogunbosoye, D. O. (2016). In Vitro Gas Production and Acceptability Evaluation of Some Plant Species in Southern Guinea Savannah Zone of Nigeria by Red Sokoto Goats. *International Journal of Current Research* 8 (05): 31150-31155.

Ogunbosoye, D.O., Tona, G.O and Otukoya, F.K. (2015). Evaluation of the nutritive value of selected browse plant species in the southern guinea savannah of Nigeria for feeding to ruminant animals. *British Journal of Applied Science & Technology*, 7(4): 386-395.

Patra, A.K., Park, T., Braun, H.S., Geiger, S., Pieper, R. and Yu, Z (2019). Dietary bioactive lipid compounds rich in menthol alter interactions among members of ruminal microbiota in sheep. *Front Microbiology* 10: 1– 18.

Puniya, A.K., singh, R. and Kamra, D.N. (2015). Rumen Microbiology: From Evolution to Revolution. Ed 1. *Springer Publisher India, pp* 3 -10.

Redoy, M.R.A., Shuvo, A.A.S., Cheng, L. and Al-Mamun, M. (2020). Effect of herbal supplementation on growth, immunity, rumen histology, serum antioxidants and meat q quality of sheep. *Animals* 14(11): 2433–2441.

Reisinger A, Clark H, Cowie AL, Emmet-Booth J, Gonzalez Fischer C, Herrero M, Howden M, Leahy S (2021) How necessary and feasible are reductions of methane emissions from livestock to support stringent temperature goals? Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 379, 20200452. doi:10.1098/rsta.2020.0452.

Rezaei, N. and Pour, H. A. (2012). Evaluation effect of thyme extract on degradability of soybean meal with gas product technique. *Advances in Bioresearch*, 3: 45-47.

Rosine, B.T., Fidèle, N. and David, M.M. (2022). Pharmacological potential of Lactura taraxacifolia leaf on blood glucose levels, lipid profile, and oxidative stress parameters in diabetic rats. *Journal of Pharmacology and Biological Science* 10(1):29-36.

Samuel, M., Sagathewan, S., Thomas, J. and Mathen, G. (1997). An HPLC method for estimation of volatile fatty acids of ruminal fluid. *Indian Journal of Animal Science*, 67; 805–811.

Seong, U.J., Shin, J.L., Hyun, S.K., Jun, S.E., Youyoung, C., Da, S.O., Dongryeoul B and Sung S.L. (2022). Effects of oriental medicinal plants on the reduction of methaneproduction mediated by microbial population. *Italian Journal of Animal Science* 21(1): 522-531.

Sirohi, S.K., Pandey, N., Goel, N., Singh, B., Mohini, M., Pandey, P. and Chaudhry, P.P. (2009). Microbial Activity and Ruminal Methanogenesis as Affected by Plant Secondary Metabolites in Different Plant Extracts. *International Journal of Civil Environmental Engineer* 1:1-8.

Sun, S., Hou, Z., Dai, Q. and Wu, D. (2019). Effects of the forage type and chop length of Ramie silage on the composition of ruminal microbiota in black goats. *Animals* 9:177-185.

Szumacher-Strabel, M. and Cieślak, A. (2010). Potential of phytofactors to mitigate rumen ammonia and methane production. *Journal of Animal and Feed Sciences*, 19: 319-337.

Tag El-Din, A. E., Moharam, M. S., Nour, A. A. and Nasser, M. E. (2012). Effect of some herbs on the rumen fermentation: 1- Effect of ginger (*Zingiber officinale*) and garlic (allium sativum) on gas production, energy values, organic matter digestibility and methane emission, in vitro. *Journal of Agricultural and Environmental Science*, 11: 33-53.

Van Soest, P.J. (1994) Nutritional ecology of the ruminant. Ithaca (2.ed) Cornell University Press. p476

Wanapat, M., Kang, S., Khejornsart, P. and Wanapat, S. (2013). Effects of Plant Herb Combination Supplementation on Rumen Fermentation and Nutrient Digestibility in Beef Cattle. *Asian Australas Journal of Animal Science*. 26:1127-1136.

Wang, M., Wu, L., Guo, Y., Sun, J., Deng, M., Liu, G., Li, Y. and Sun, B. (2023). Effects of fermented herbal tea residue on meat quality, rumen fermentation parameters and microbes of black goats. AMB Express 13(106): 1-13.

Yusuf, A. L. (2015). Evaluation of dietary supplementation of *Andrographis paniculata* on growth performance and meat quality of Boer goats. Ph.D. Thesis, University of Putra, Malaysia. Retrieved from http://www.lib.upm.edu.my.