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ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND ITS RELATIONSHIP WITH IN VITRO FERMENTATION AND METHANE MITIGATION POTENTIAL OF GRASS – LEGUMES DIET

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Abstract

The study evaluated the chemical composition and its relationship with in vitro total gas production at 24 hour (GP_{24}), in vitro fermentative parameters and nutritive values of Panicum maximum supplemented with either Daniellia oliveri or Afzelia africana leaves and their mixture. Chemical compositions were analyzed, while total GP volume was measured and methane estimated after 24 h incubation. Total GP_{24} produced by the experimented diets (T1, T2, T3 and T4) respectively steadily increased and was most pronounced ($P<0.05$) in T2 (24.30 mL/200 mg DM), and the lowest at T1 (18 mL/200 mg DM). Methane concentration varied ($P<0.05$) from 39.0 in T2 to 45.00% in T1. Methane reduction potential (MRP) followed the same trend as methane concentration. There were positive correlations (r = 0.001**, r = 0.05* respectively) between crude protein and MRP, ME, organic matter digestibility (OMD) and total GP_{24}, short chain fatty acid, (SCFA), total digestible nutrient and dry matter intake (DMI) respectively. non-fibre carbohydrates (NFC) contents of the diets and total gas production at 24 h incubation. Neutral detergent fibre and acid detergent fibre were negatively, (r = 0.001 and r = 0.050 respectively) correlated with total $GP_{24,}$ MRP, and all the nutritive values. Nonfibre carbohydrates was also positively (r = - 0.001 and r = - 0.010, respectively) associated with total GP_{24} and MRP and all the nutritive values evaluated. Crude protein (CP), and NFC had a more pronounced positive correlation (r = 0.001** and r = 0.000** respectively) with MRP, OMD and DMI. Results suggest that all the experimental diets except T1 contained adequate nutrients with potential to reduce methane production and positively impact rumen fermentation for improved nutritive values which was reflected most in T2 diet.

Key words: Wild legume leaves; enteric methane; climate change; sheep

Introduction

In the tropics, particularly the semi-arid tropical regions like Nigeria, fodder is not readily available through the year. Dry season in Nigeria is characterized by shortage of fresh fodders. This is really the major constraint to livestock production in the country. Supplementation of concentrates to roughage based diet enhances the animal performances, however the cost of concentrates are high. Farmers target reduction in the feed cost and sustaining good performances of animals, and one of the strategies to achieve this is to supplement guinea grass (Panicum maximum) with browse legume foliages. Although the animal performances were increased with the use of high quality exotic grass, the high cost of feed was still remaining because of concentrate supplementation. Legume forages are a cheap source of nitrogen to livestock when used as a supplement to low quality forages and improve feed intake and animal performances (Devendra, 2005). Daniellia oliveri and Afzelia africana foliages could be used as nitrogen sources with little or no detrimental effects in ruminants (Okunade *et al*., 2014). However, the supplementation of high quality legume forage to exotic grass on feed cost and animal performances are still remaining to investigate. The feeding trial is required to determine those parameters; however the feedstuffs and diets should be analyzed with in vitro trial prior to feeding trial to avoid undesirable feeding problems. The in vitro gas production method has been successfully applied at the aspects of feed evaluation, including organic matter digestibility, metabolizable energy (Menke and Steingass, 1988), short chain fatty acid (Makkar, 2005) and kinetics of fermentation (McDonald, 2011) and methane reduction potential. Moreover, the in vitro gas production method can be used to examine animal waste components that impact the environment and develop appropriate mitigations. Thus, this experiment was intended to evaluate the relationship between chemical composition and in vitro fermentation parameters of grass based diet supplemented with two different tree legume forages in ruminants.

Materials and Methods

Experimental Site

The study was carried out at New Bussa located at longitude 9�.81′ 95″ N and 9�. 49 ′ 10″ N and latitude 4�.58′ 05″ N and 4�.34′ 49″ N in the Guinea savanna areas of Niger Basin, North Central Zone of Nigeria. In vitro study was carried out at the Department of Animal Production, University of Ibadan laboratory.

Forage Collection

About 6 weeks old P. maximum samples were harvested at 15 cm above ground from the wetland areas located within the New Bussa and Daniellia oleiveri and Afzelia africana were also harvested from the Campus of Federal College of Wildlife New Bussa respectively. The grass samples were chopped into pieces of 2-3 cm in length, wilted for 4 hours to reduce their moisture contents before in vitro analysis.

Experimental Diets and Design

The experiment was conducted in a completely randomized design with four treatments and three replications as outlined below: T1= Sole Panicum maximum, T2=70% Panicum maximum + 30% Daniellia oliveri foliage, T3=70% Panicum maximum + 30% Afzellia africana foliage, T4 =70% Panicum maximum + 15% Daniellia oliveri foliage +15% Afzelia africana foliage

Chemical Analyses and Calculations

Proximate analysis of milled samples of P. maximum, D.oliveri, A. africana and their mixture with P. maximum was according to AOAC (2005) and neutral detergent fibre (NDF) and acid detergent fibre according to Van Soest *et al*. (1994). Condensed tannins (CTs) and saponins (SAP) were determined by the methods of Babayemi *et al*. (2004). Non-fibre carbohydrates (NFC) were calculated as (100−CP−NDF−EE−ash)

In vitro Gas and Methane Determination

In vitro GP total gas (GP_{24}) and methane gas (CH_4) determination from the incubation of P. maximum, P. maximum with browse legume mixture were carried out by using rumen liquor from three Yankasa rams fed a mixed diet of roughage (70% DM) and concentrates (30% DM). The animals had free access to water and mineral. Rumen fluid was collected from the rams with the use of suction tube prior to morning feeding into a pre-warmed steel Thermos flask and immediately brought to the laboratory for analysis. The collected rumen liquor was strained through four layers of cheese cloth and kept at 39°C. All laboratory handling of rumen fluid was carried out under a continuous flow of carbon dioxide (Okunade *et al.,* 2014). The in vitro GP was determined according to Babayemi *et al*. (2004). Samples (200 mg) of the oven-dried and milled grass-legume mixture diets were accurately weighed into 100 ml glass syringes fitted with plungers. In vitro incubation of the samples was conducted in triplicates. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution (g/liter of 1.985(Na_2) HPO_4 + 1.30 2KH_2PO_4 + 0.105 $MgCl_2.6H_2O$+ 1.407 NH_2HCO_3 + 5.418 $NaHCO_3$ + 0.390 Cysteine HCl + 0.100 NaOH) and three blank samples containing 30 ml of medium (inoculums and buffer) only were incubated at the same time to serve as control. The syringes were placed in a rotor inside the incubator (39°C) with about one rotation per min. The gas production was recorded at 3, 6, 9, 12, 18 and 24 h. At post-incubation period, 4 mL of 10 M (NaOH) was dispensed into each of the incubated sample. Sodium hydroxide was added to absorb carbon dioxide that was produced during the process of fermentation and the remaining volume of gas was recorded as methane (Okunade *et al*., 2014).

Methane Reduction Potential Determination

Methane concentration (MC) was determined according to Jayanegara *et al*. (2009): Methane concentration (MC %) = Net methane production/ Net gas production× 100. Methane production reduction potential (MRP) was calculated by taking the highest % net methane values as 100 %: MRP = %Net methane in control − %Net methane in the test / %Net methane × 100.

Data Collected on Fermentation Parameters

The metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA), total digestible nutrients (TDN), digestible dry matter digestibility (DDM) and dry matter intake (DMI) values of experimental feedstuffs and feed mixtures were calculated as follow: ME =2.20 + 0.136GV + 0.057CP + 0.0029CF, OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash (Menke and Steingass, 1988), SCFA (μmol) =0.0222GV (at 24 hr) – 0.00425 (Makkar, 2005), TDN (% DM) = 87.84 − 0.70ADF (Schmid *et al*., 1976), DDM (% DM) = 88.9− 0.779 ADF (NRC, 2001), DMI (% of BW) = 120/NDF (NRC, 2001)

Statistical Analysis

Data was subjected to a one way ANOVA using version 9.1 of SAS software (SAS Institute, 2003). Significant differences between individual means were separated by Duncan's procedure.

Results and Discussion

Chemical Composition of Panicum Maximum and Browse Legume Foliage Supplements

Table 1 reveals the chemical composition of P. maximum, browse legume foliages and. The crude protein (9.04 % DM) in Panicum was lower relative to browse legume foliages. Among the browse legume foliages, organic matter (84.17, 83.68 % DM) and crude protein (14.71, 15.00 % DM) were similar (P>0.05). The highest NDF and ADF mean values were recorded for P. maximum but lower values (P<0.05) were recorded for the browse legume foliages. None fibre carbohydrate (NFC) was highest in Daniellia oliveri (33.23 % DM). The condensed tannins (CTs) ranged from 0.59 % DM in Panicum maximum to 0.95% DM in Daniellia oliveri which is similar (P>0.05) to that of Afzelia africana (92% DM). The saponin content were significantly (P>0.05) different among all the feedstuffs. Saponin content was lowest (P>0.05) in Panicum maximum but higher values were obtained in the browse legume foliages with Afzelia Africana (0.51% DM) having the highest value. Crude proteins content ranged from 9.04 % DM in T1 to 15.41 % DM in T2 respectively. There was similarity (P>0.05) in NDF and ADF among T2 and T4 respectively, but significantly (P<0.05) differed to T2 among the supplemented diets. NFC (10.04% DM) was highest (P<0.05) in T2, while the lowest values were obtained for T3 and T4 respectively among the supplemented diets. The same trend was recorded for CTs and SAP values among the supplemented diets. Chemical composition of Panicum maximum was lower (P<0.05) than that of supplemented diets (T2 and T3). The CP, NDF, ADF and NFC contents of the Panicum maximum grass reported in this study were within the range reported in literature (Idowu *et al*., 2020) , while lower values in the same area may be due to increased lignification associated with maturity and drought are common (Alasa, 2021). However, the CP was close to the threshold of 8%, below which optimal rumen function may be hindered if fed alone (Ikhimioya, 2008). Chemical composition is one of the factors influencing the nutritional quality of pastures. Panicum maximum used in this study is characterized with minimum CP, NFC and high NDF and ADF which may not meet the nutrient requirement of growing or dairy ruminant animals. The NDF and ADF values obtained for the grass-forage legumes mixture were within the range of 24 – 61% DM reported for tropical forage (N.R.C, 2001). The CP, NDF, ADF and NFC, CTs and saponin observed in this study meet the nutrient requirement for growing and dairy ruminant animals (NRC, 2001). Supplementation of sole Panicum with browse legume foliages in this study improved the chemical composition of the grass. This is corroborated with the report of Niderkorn *et al*. (2011).

Table 1: Chemical compositions (% DM) of experimental feed ingredients and feed mixtures

Feed ingredient	DM	OM	CP	NDF	ADF	NFC	CTs	SAP
P. maximum	92.23[a]	76.41[b]	9.04[b]	70.00[a]	54.23[a]	2.45[c]	0.59[b]	0.21[c]
D. oliveri	91.69[a]	84.17[a]	14.71[a]	48.52[b]	26.39[b]	33.23[a]	0.95[a]	0.41[b]
A. africana	90.68[b]	83.68[a]	15.00[a]	37.36[c]	24.65[b]	27.04[b]	0.92[a]	0.51[a]
SEM	0.27	0.47	0.34	0.74	2.29	0.49	0.25	0.26
P-value	0.03	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Forage mixture								
T1	92.23	76.41	9.04[b]	70.00[a]	54.23[a]	2.45[c]	0.59[c]	0.21[c]
T2	92.71	74.14	15.41[a]	55.30[c]	35.13[c]	10.04[a]	0.68[a]	0.34[a]
T3	92.72	76.37	14.02[a]	58.70[b]	47.13[b]	7.66[b]	0.67[ab]	0.33[ab]
T4	92.39	75.40	14.77[a]	57.53[b]	48.58[b]	7.64[b]	0.66[bc]	0.28[b]
SEM	0.22	0.23	0.93	0.94	2.14	1.16	0.01	0.03
P- value	0.140	0.200	0.001	0.001	0.001	0.001	0.004	0.001

abc Different superscripts in the same column are significantly different at P value 0.05.
DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, NFC: non fibre carbohydrate, CTs: Condensed Tannin content, SAP: saponins content, SEM: standard error mean

In vitro Gas, Methane and Carbon IV oxide Concentration Production

There was significant ($p<0.05$) difference among all the treatments regarding in vitro gas production at all period of incubation except for 9, 12 and 15 h of incubation (Table 2). The greatest volume (24.30 mL/200 mg DM) of net gas generated at 24h of incubation was demonstrated by T2, and lower volume (18.00 mL/200 mg DM) was found in T1 ($p < 0.05$). The reverse was observed for generation of methane concentration among the experimental diets at 24 h, with greater concentration in T1 ($p<0.05$). Lower methane concentration was demonstrated by T2 ($p <0.05$). Digestibility has been reported to be synonymous to in vitro gas production (Fievez *et al*., 2005) so that the higher the gas production the higher the digestibility. The differences in nutritional value will result in different fermentation characteristics. This corroborates the results on in vitro gas production observed in this study. The higher cumulative gas productions of supplemented grass relative to sole Panicum grass observed at 24 h incubation time may be due to differences in chemical composition. Supplemented diets had improved chemical composition (especially CP, NDF and ADF) relative to sole Panicum, thus higher gas production. Treatment two (T2) possessed lower NDF and ADF and low CTs and SAP contents, which are negatively related to gas production (De Boever *et al*., 2005) and fermentation parameters. The low content of fibre can facilitate the utilization of feed by ruminal microbes, which in turn might induce higher fermentation rates (Van Soest, 1994). Deaville and Given (2001) reported that kinetics of gas production could be affected by carbohydrate fraction. Treatment two (T2) had low level of fibre contents, indicating higher level of soluble carbohydrate, which gave the higher gas production. Furthermore, T2 had higher NFC compared to other diets. None fibre carbohydrate digests faster than the structural carbohydrates (Okunade *et al*., 2022). Many studies have compared gas production between gramineous and leguminous forages but results in these studies varied with the forage species, maturity stages and even environment (Ammar *et al*. 2010).

Table 2: In vitro gas production pattern (mL/200 mg DM) of P. maximum supplemented with legume foliage mixture

Forage mixture	Hour of Incubation								
	3hr	6hr	9hr	12hr	15hr	18hr	21hr	24hr	CH_4	CO_2
T1	1.00[b]	4.00[ab]	6.33	9.66	11.0[b]	13.6[b]	16.3[b]	18.0[c]	45.0[a]	62.3
T2	2.00[a]	5.00[a]	7.67	11.33	15.0[a]	19.3[a]	22.3[a]	24.3[a]	39.0[c]	60.1
T3	1.67[a]	3.67[b]	6.67	8.67	12.0[ab]	14.7[b]	18.2[ab]	20.3[b]	41.0[b]	60.3
T4	1.00[b]	4.33[a]	7.00	10.00	12.0[ab]	14.6[b]	18.6[ab]	20.0[b]	41.0[b]	61.5
SEM	0.24	0.53	1.00	1.35	1.41	1.55	1.70	1.45	2.56	3.39
P value	0.006	0.050	0.600	0.330	0.095	0.025	0.045	0.014	0.029	0.902

abc: Different superscripts in the same column are significantly different at P value 0.05.

The results indicated that gas production in Panicum supplemented with browse legume diets (i.e. P70 + D30, P70 + A30 and P70 + D15 + A15) was higher than that in P100 (sole Panicum) with T2 having the highest gas production. This result may indicate that total gas production positively correlated with NFC and fibre fraction contents of forages and not necessarily correlated with CP content. Methane concentration (MC) production after 24 h of anaerobic fermentation can be assessed to rank the feedstuffs in terms of anti-methanogenic potential (Li, *et al*., 2019). The lowest MC and consequently the highest percentage MRP observed for T2 and other supplemented diets may be as a result of its lower fibre fractions and higher NFC levels compared to sole Panicum. Studies have shown that low NDF and ADF and high NFC (easily fermentable carbohydrates) produce low MC and consequently high MRP (Okunade, *et al*., 2022). The levels of CTs recorded in this study are much below the range of 60 to 100 g/kg DM (6.0 to 10.0% DM), considered to depress feed intake and growth (Mbomi *et al*., 2011). Therefore, the browse species contained CTs at levels beneficial to ruminants because CTs at low level produce mild or low protein binding effect (Olafadehan, 2020). Similarly, CTcontaining forage minimizes methane emission by ruminants (methane mitigation), in addition to other benefits, when not included at a high proportion of the diet (Bodas *et al*., 2012). Saponin levels in all the samples were lower than the tolerable level of 15-20 g/kg DM reported for goats (Onwuka, 1983), which suggests the levels reported herein are not likely to affect nutritional potentials of the browses to ruminants. Feedstuffs containing saponin have been shown to act as defaunating agents (Teferedegne, 2000) and capable of reducing methane production. This implies that CTs and SAP content in all the diets may not have much effect on net gas and methane concentration production, because their values were very minimal in this study. The results suggest that among the browse legume foliage supplemented diets, T2 was the best potential feed source that could be used in ruminant diets to reduce loss of dietary energy through methane production and consequently mitigate enteric methane production from livestock to global warming.

Nutritive Values Experimental Diets

The nutritive values of experimental feedstuffs and feed mixtures are presented in Table 3. The highest ($p<0.05$) ME, OMD, SCFA, TDN, DDM and DMI values were observed in T2 , followed by T3 and T4 which were significantly ($p<0.05$) different from each other, while lower values were observed for T1.

Table 3: Nutritive value of Pannicum maxmum supplemented with legume browse foliage

Diet	ME (MJ/KgDM)	OMD (%)	SCFA (%)	TDN (%)	DDM (%)	DM1 (% BW)
T1	5.16^{c}	46.05^{c}	0.40^{b}	49.88^{c}	46.65^{c}	1.72^{c}
T2	6.39^{a}	64.78^{a}	0.54^{a}	63.25^{a}	61.53^{a}	2.17^{a}
T3	5.76^{b}	60.44^{b}	0.45^{b}	54.85^{b}	52.18^{b}	2.05^{b}
T4	5.75^{b}	60.53^{b}	0.44^{b}	53.83^{b}	51.05^{b}	2.09^{b}
SEM	0.21	1.41	0.04	1.50	1.67	0.04
P-value	0.003	0.002	0.012	0.001	0.001	0.001

a, b, c Different superscripts in the same column are significantly different at P value 0.05
ME: metabolizable energy, OMD: organic matter digestibility, SCFA: short chain fatty acid, TDN: total digestible nitrogen, DDM: digestible dry matter, DMI: dry matter intake, BW: body weight, SEM: standard error mean.

According to Abegunde *et al*. (2011), the rate at which feed substrate is degraded in the rumen is as important as the extent of digestion. This study showed that supplementing either Daniellia oliveri or Afzelia africana or their mixture to Panicum grass improved the feeding value (i.e. ME, OMD, SCFA, TDN, DDM and DMI) of the grass. Treatment two (T2) seem to have the greatest impact on in vitro ruminal fermentation. This could be associated with improved gas production as observed in Table 2. However, the ME values recorded in this study were below the value of 8.4 MJ/kg DM, which is recommended for growing ruminants (NRC, 2007), except the ME for the accessions. Short chain fatty acids are the most important end-products of carbohydrate digestion in ruminants (Scott *et al*. 2008) since SCFA provide energy for the various activities in animals. The short chain fatty acid recorded in this study particularly for supplemented grass showed improved energy contents in relation to sole grass. Other studies have suggested that the concentration of SCFA is positively correlated with the amount of substrate fermentation in the rumen (McDonald *et al*., 2011). In this study, it was observed that supplementation of Panicum grass with browse legume foliages increased the proportion of OMD, the DDM and DMI, which in turn resulted in an increase in the concentration of SCFA. The higher nutritive values (TDN and DDM) of legume forage supplemented diets as compared to the sole grass in this experiment may be attributed to improvement of CP induced by supplementation. This explains the potential of legumes supplemented grass to be digested easily more than sole grass. Moreover, the low content of NDF and ADF induced by supplementation can facilitate the utilization of feed by ruminal microbes, which in turn might induce higher fermentation rates, therefore improving digestibility (Van Soest, 1994). Among experimental diets, the lowest NDF and ADF content were observed in T2, which lead to the higher DMI than other diets.

Correlation Study

Relationship between chemical composition and in vitro GP at 24 h, MRP and nutritive values are presented in Table 4. The CP content of the experimental diets was positively correlated ($r = 0.637$; $p = 0.026$) with net gas volume production at 24 hour (NGV_{24}), SCFA and total digestible nutrient (TDN) respectively. Crude protein positively correlated with MRP, ME, OMD and DMI respectively. Positive correlation was observed between NFC content, NGV and MRP at 24 hr incubation periods NFC had strong negative relationship ($r = -0.830$; $p < 0.001$) with MC. NDF and ADF had high negative correlation ($r = -0.927$; $r = -0.870$; $p < 0.001$) with in vitro NGV_{24} and MRP respectively. In like manner NDF and ADF had strong negative correlation with all the nutritive value parameters ($p = 0.05$) except for DDM. Positive relationship was observed between CTs and in vitro NGP_{24} and MRP. Condensed tannins (CTs) also shown slight positive correlation ($r = 0.645$, $r = 0.652$, $r = 0.586$; $p < 0.05$) with ME, OMD and SCFA respectively, while strong positive relationship ($r = 0.747$; $p = 0.005$, $r = 0.718$; $p < 0.009$) existed between CTs, TDN and DMI respectively. There was a high correlation ($r = 0.05$) between SAP, NGV_{24}, MRP and all the nutritive value parameters except for DDM. Reports have shown that the volume of gas produced during anaerobic fermentation is related to differences in chemical compositions in the feeds and the availability of those components for rumen microorganisms. The strong positive correlation between CP content of the experimental diets and total GP may be attributed to the generally high CP of all the diets. Normal rumen microbial activities get hampered when dietary CP is below the threshold of 8 % DM which is the minimum level required for optimal ruminal microbial function (Norton, 2003). The higher CP of the browse legume foliages supplemented diets than sole Panicum grass possibly increased the microbial multiplication activities and fermentation, resulting in enhanced in vitro GP. Parallel results were obtained in previous studies (Okunade *et al*., 2022). The significant negative relationship between CP and MC and strong positive correlation of CP with MRP are desirable as is indicative of decrease

Table 4: Correlation (r) between chemical compositions (% DM), total gas production, methane reduction potential and nutritive value of Pannicum maximum supplemented with legume foliage

Predictor	R	GP_{24} (mL/200 mg DM)	MRP (%)	ME (MJ/Kg DM)	OMD (%)	SCFA (%)	TDN (%)	DDM (%)	DM1 (%BW)
CP	R	0.637^{*}	0.829^{**}	0.808^{**}	0.829^{**}	0.632^{*}	0.628^{*}	0.228	0.935^{**}

	p-value	0.026	0.001	0.001	0.001	0.027	0.029	0.476	0.000
EE	R	0.069	0.369	0.256	0.282	0.054	0.177	0.366	0.535
	p-value	0.832	0.238	0.422	0.375	0.867	0.583	0.243	0.073
NDF	R	- 0.626*	-0.891**	- 0.780**	- 0.801**	- 0.626*	-0.758**	0.022	- 0.999*
	p-value	0.029	0.000	0.003	0.002	0.029	0.004	0.946	0.000
ADF	R	- 0.730**	- 0.819**	- 0.761**	- 0.767**	-0.738**	-1.000**	0.084	- 0.775**
	p-value	0.007	0.001	0.004	0.004	0.006	0.000	0.795	0.003
NFC	R	0.621*	0.882**	0.716**	0.729**	0.626*	0.831**	0.163	0.926**
	p-value	0.031	0.000	0.009	0.007	0.029	0.001	0.613	0.000
CTs	R	-0.581*	0.654*	-0.645*	-0.652*	0.586*	0.747**	0.014	-0.718**
	p-value	0.047	0.021	0.024	0.022	0.045	0.005	0.967	0.009
SAP	R	-0.710**	0.844**	-0.805**	-0.815**	-0.708**	-0.750**	0.205	-0.868**
	p-value	0.10	0.001	0.002	0.001	0.010	0.005	0.522	0.000

GP_{24}: Total gas volume production after 24 hr post incubation, MRP: Methane reduction potential. SEM: standard error of mean. ** p < 0.01 * p < 0.05. CP: Crude protein, EE: Ether extracts, NFC: non fibre carbohydrate, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CTs: tannins, SAP: saponnins

MC and increase MRP. Several reports on correlations between CP and GP, MP and MRP are not consistent. Kalivand and Kafizadeh (2015) obtained a positive but insignificant correlation between CP and MC and a negative insignificant relationship between CP and MRP. Inconsistence between results may be due to variations in substrate, chemical composition and the diets of the animals from which inoculums were collected.

The negative correlation between NDF and ADF and GP may be a result of bulkiness of structural carbohydrates (Okunade *et al*., 2014; Olafadehan *et al*., 2014) which affects ruminal fermentation due to reduced microbial activity and hence GP (Isah *et al*., 2015, Kulivand and Kafilzadeh, 2015). Therefore, fibrous feeds would contribute more to anthropogenic methane emissions from livestock. The result is further buttressed by the positive correlation between fibre fraction and MC, implying that fibrous feeds could increase methane production. Ruminal fermentation of structural carbohydrates favours the synthesis of acetic acid and production of Hydrogen ion which is used to reduce CO_2 to CH_4 (Kennedy and Charmley, 2012). Some previous reports contradict positive correlation between CT and MC and MRP (Beauchemin *et al*., 2007). The discrepancies in the effect of tannins on methane reduction potential may be as result of doses, types, molecular weight, sources of tannins and quality of diets (Belete and Abubeker, 2018). Likewise, previous studies (Liu *et al*., 2019) suggest that saponin supplementation in the diet of ruminant animals could reduce methane emission by inhibiting the growth of ruminal methanogens and protozoa, and may have different effects on cellulolytic bacteria. Weak relationship between saponins and NGV24, MC and MRP we observed in this study could be as a result of low concentration and source of saponins present in the seed meals.

Conclusion

There was significant difference between chemical compositions of P. maximum and the Daniellia oliveri and Afzelia africana legume foliage. P. maximum with lower CP, higher fibre fractions (NDF and ADF) with lower tannins and saponins content relative to browse legume foliage is typical succulent low quality roughage. There was similarity between chemical compositions of Daniellia oliveri and Afzelia africana legume foliage. Browse legume foliage had moderately low tannins and saponin. Browse legume foliage supplementation of P. maximum at 70% to 30% of either Daniellia or Afzelia or their mixture improved the chemical composition of the grass which is low quality roughage. Supplementation of P. maximum with legume browse foliage improved in vitro fermentation parameters (ME, OMD, DDM, DMI (% BW) and SCFAs) of the grass. There was strong positive correlation between CP, NFC and ME, OMD, DDM, DMI (% BW) and SCFAs, MRP of the diets, while negative relationship existed between NDF, ADF, TAN and SAP and ME, OMD, DDM, DMI (% BW) and SCFAs, MRP. Feeding sheep with P. maximum alone depresses ME, OMD, DDM, DMI (% BW) and SCFAs, of the animals. Supplementation of P. maximum with legume browse foliage such as D. oliveri and A. africana improved ME, OMD, DDM, DMI (% BW) and SCFAs, of the animals and reduce enteric methane and CO_2 gas production in ruminant animals and be recommended for a further systematic evaluation to determine its effect on feed use efficiency of low-quality forage should be conducted.

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