



CHEMICAL COMPOSITION AND ITS RELATIONSHIP WITH IN VITRO METHANE MITIGATION POTENTIAL OF SELECTED WILD LEGUME SEEDS †

[COMPOSICIÓN QUÍMICA Y SU RELACIÓN CON EL POTENCIAL DE MITIGACIÓN IN VITRO DE METANO DE ALGUNAS SEMILLAS DE LEGUMINOSAS SILVESTRES]

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SUMMARY

Background. Increasing atmospheric concentrations of methane (CH₄) have led scientists to examine its sources of origin. Mitigation of enteric CH₄ production by ruminants has been recognized as an important goal because it reduces greenhouse gas emission and improves feed efficiency. **Objective.** The study evaluated the chemical composition and its relationship with *in vitro* total gas (GP₂₄) and *in vitro* methane (CH₄) production parameters of five tropical wild legume seeds [*Luffa cylindrica* (LC), *Piliostigma thonningii* (PT), *Detarium microcarpum* (DM), *Daniellia oliveri* (DO) and *Azelia africana* (AA)]. **Methodology.** Chemical compositions were analysed, while total GP volume was measured and CH₄ estimated after 24 h incubation. **Results.** Total GP₂₄ produced by the seeds steadily increased and was most pronounced ($P < 0.05$) in AA seed meal (64.71 mL/200 mg DM), and the least in LC (37.83 mL/200 mg DM). CH₄ concentration (MC) varied ($P < 0.05$) from 9.90 in AA to 23.93 in LC. Methane reduction potential (MRP) was higher ($P < 0.05$) for AA seed meal and lowest for LC. There were positive correlations ($r = 0.685^{**}$, $r = 0.763^{*}$ respectively) between crude protein and non-fibre carbohydrates (NFC) contents of the seeds and total gas production at 24 h incubation. Fibre fractions (NDF and ADF) were positively, ($r = 0.978$ and $r = 0.874$ respectively) correlated with MC, and negatively ($r = -0.927$ and $r = -0.870$, respectively) associated with total GP₂₄ and MRP. CP, EE and NFC had a more pronounced positive correlation ($r = 0.948^{**}$, $r = 0.851^{**}$ and $r = 0.852^{**}$ respectively) with MRP. **Implication.** Results suggest that all the selected seeds have the potential to reduce methane production and positively impact rumen fermentation. **Conclusion.** Seed containing more nutrients (CP, EE, and NFC) reduced enteric methane production more than any other of the chemical components in the study.

Key words: Wild legume seeds; chemical composition; methane reduction potential; climate change

RESUMEN

Antecedentes. El aumento de las concentraciones atmosféricas de metano (CH₄) ha llevado a los científicos a examinar sus fuentes de origen. Se ha reconocido que la mitigación de la producción de CH₄ entérico por los rumiantes es un objetivo importante porque reduce la emisión de gases de efecto invernadero y mejora la eficiencia alimentaria. **Objetivo.** El estudio evaluó la composición química y su relación con los parámetros de producción *in vitro* de gas total (GP₂₄) y metano (CH₄) *in vitro* de cinco semillas de leguminosas silvestres tropicales [*Luffa cylindrica* (LC), *Piliostigma thonningii* (PT), *Detarium microcarpum* (DM), *Daniellia oliveri* (DO) y *Azelia africana* (AA)]. **Metodología.** Se analizó la composición química, el volumen total de GP y se estimó el CH₄ después de 24 h de incubación. **Resultados.** El total de GP₂₄ producido por las semillas aumentó constantemente y fue más pronunciado ($P < 0.05$) en harina de semillas AA (64.71 mL / 200 mg MS), y la menor en LC (37.83 mL / 200 mg MS). La concentración de CH₄ (MC) varió ($P < 0.05$) de 9.90 en AA a 23.93 en LC. El potencial de reducción de metano (MRP) fue mayor ($P < 0.05$) para la harina de semillas AA y más bajo para LC. Hubo correlaciones positivas ($r = 0.685^{**}$, $r = 0.763^{*}$ respectivamente) entre la proteína cruda y los carbohidratos no fibrosos (NFC) de las semillas y producción total de gas a las 24 h de incubación. Fracciones de fibra (NDF y ADF) fueron positivamente relacionadas ($r = 0.978$ y $r = 0.874$ respectivamente) con MC, y negativamente ($r = -0.927$ y $r = -0.870$, respectivamente) con GP₂₄ y MRP totales. CP, EE y NFC tuvieron una correlación positiva más pronunciada ($r = 0.948^{**}$, $r = 0.851^{**}$ y $r = 0.852^{**}$ respectivamente) con MRP.

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Implicaciones. Los resultados sugieren que todas las semillas seleccionadas tienen el potencial de reducir la producción de metano e impactar positivamente la fermentación ruminal. **Conclusión.** Las semillas que contienen más nutrientes (CP, EE y NFC) redujeron la producción de metano entérico más que cualquier otro componente químico del estudio.

Palabras clave: Semillas de leguminosas silvestres; composición química; potencial de reducción de metano; cambio climático.

INTRODUCTION

Climate change is transforming the planet's ecosystems and threatening the well-being of current and future generations. One of the main greenhouse gases (GHG) is methane (CH₄), which has a heat-trapping potential 23 times that of CO₂ (IPCC 2001). Increasing atmospheric concentrations of methane have led scientists to examine its sources of origin. On a global scale, enteric CH₄ produced by domesticated ruminants contributes 8% of total GHG emissions (Black *et al.*, 2021)). Methane production by ruminants also results in a loss of energy intake of up to 2% to 14% of gross energy (Johnson and Johnsons, 1995). Therefore, reduced CH₄ production by ruminants has been recognized as an important goal because it reduces GHG emission and improves feed efficiency. A number of dietary and management mitigation options and policies have been advocated for lowering methane production from livestock production systems (Hristov *et al.*, 2013). Methane reduction strategies have included the introduction of methane inhibitors, both biological and chemical, in the animal feed, either to kill or at least reduce the activity of the methanogenic microbiome in the lower gut. Such mitigation options include the use of plant secondary compounds (Soliva *et al.*, 2004). Many studies reported that tannins and saponins containing plants appeared to be useful in suppressing methane release by reducing the activity of rumen ciliate protozoa and methanogens (Anantasook *et al.*, 2014). Also, the nutritive value of feed in terms of crude protein and types and concentration of carbohydrate and fibre fraction as well as their intake can influence the quantity of CH₄ generated during enteric fermentation (Singh *et al.*, 2016).

Therefore, the objective of this study was to determine the *in-vitro* gas production (GP), enteric methane reduction property as well as correlation between chemical composition and methane reduction potential of different tropical wild legume seeds: *Azelia africana*, *Daniellia oliveri*, *Luffa cylindrica*, *Piliostigma thonningii* and *Detarium microcarpum* (Keay, 1989) that can be used as alternative plant protein supplements for ruminants.

MATERIALS AND METHODS

Description of the experimental site

This study was carried out at the Federal College of Wildlife Management, New Bussa, Niger State,

Nigeria. New Bussa is 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 Savanna Areas of Niger Basin, North Central Zone of Nigeria.

Chemical analysis

Five tropical wild legume seeds (*Luffa cylindrical*, *Azelia africana*, *Daniellia oliveri*, *Piliostigma thonningii* and *Detarium microcarpum*) were collected from plant stands naturally established within the rangeland of Federal College of Wildlife Management, New Bussa, Niger state Nigeria. The seeds were dried at 60°C for 48 h and the ground using 1 mm sieve with electrically operated Wiley mill. The milled samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash according to AOAC (2005) and neutral detergent fibre (NDF) and acid detergent fibre according to Van Soest *et al.* (1994). Condensed tannins and saponins were determined by the methods of Babayemi *et al.* (2004). Non-fibre carbohydrates (NFC) were calculated as (100–CP–NDF–EE–ash).

Source of inoculums

In vitro GP (total gas and CH₄) determination from the incubation of selected wild legume seeds was carried out by using rumen liquor from three Red Sokoto goats fed a mixed diet of *Panicum maximum* (60% DM) and concentrates (40% DM). The animals had free access to water and mineral. Rumen fluid was collected from the goats with the use of suction tube prior to morning feeding in a pre-warmed steel Thermos flask and immediately brought to the laboratory for analysis. The collected rumen liquor was strained through four layers of cheesecloth and kept at 39°C. All laboratory handling of rumen fluid was carried out under a continuous flow of carbon dioxide.

In vitro gas and methane determination

The *in vitro* GP was determined according to Menke and Steingass (1988). Samples (200 mg) of the oven-dried and milled seed 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 (Na₂)HPO₄ + 1.30 2KH₂PO₄ + 0.105 MgCl₂·6H₂O + 1.40₇ NH₂HCO₃ + 5.418 NaHCO₃ + 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. 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, 24, 36 and 48 h. At post-incubation period, 4 mL of 10 M (NaOH) was dispensed into the each 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 (Fievez *et al.*, 2005).

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 for the control (*Luffa cylindrica*) as 100 %. MRP was calculated with respect to methane concentration for hay since all the samples studied can be regarded as forage:

MRP = %Net methane in control – %Net methane in the test / %Net methane × 100

Statistical analysis

Data were subjected to one-way ANOVA in a completely randomized design using version 9.1 of SAS software (SAS Institute, 2012). The significant difference between individual means was separated by the tukey test of the same software. Mean differences were considered significant at $P < 0.05$. Correlation and regression options of the SAS were used to test the relationships between the seed meals nutrients and GP at 24 h incubation, MC and MRP.

RESULTS

Chemical compositions of selected wild legume seed meals

The chemical composition of the legume seeds is shown in Table 1. There were variations ($P < 0.05$) in the chemical composition of the wild legume seeds. Crude protein (CP) was highest ($P < 0.05$) in AA and least in LC. Highest and lowest ($P < 0.05$) levels of EE were observed for PT and LC respectively. Non-fibre carbohydrate (NFC) was highest in AA and DO and lowest in LC ($P < 0.05$). Concentrations of NDF and ADF were the greatest and the lowest in AA and LC, respectively ($P < 0.05$). Higher concentrations of tannins and saponins were recorded for LC, while DM seed had the lowest values of both ($P < 0.05$).

In vitro gas and methane production of selected legume seed meals

The net gas volume and CH₄ produced increased steadily and significantly ($p < 0.05$) at all stages of incubation (Table. 2). The highest total gas volume (NGV) and lowest methane production after 24 h of incubation were produced by AA seed meal, while the lowest NGV and highest methane were produced by LC seed meal.

Methane concentration and methane reduction potential

The amounts of methane concentration (MC) and MRP produced by the different tropical wild legume seed meals are shown in the table 3. Although, methane production from feed fermentation implies an energy loss to ruminant animals, the actual CH₄ concentration production of any feed is obtained when CH₄ production is expressed as the percentage of total gas volume produced. The percentages of MC produced and MRP after 24 hours of fermentation varies between 13.86 and 23.94 mL/200 mg DM, and 75.85 and 82.89 mL/200 mg DM respectively.

Correlations coefficients

Relationship between chemical composition and *in vitro* GP at 24 h, MC and MRP are presented in Table 4. The CP content of the seed meals was negative correlated ($r = -0.915$; $p = 0.001$) with MC and strongly positively correlated ($r = 0.685$; $p = 0.005$, $r = 0.948$; $p = 0.001$) with GP and MRP respectively. The EE is negatively correlated ($r = -0.562$; $p < 0.029$, $r = -0.722$; $p < 0.001$) with NGV and MC at 24 hr incubation periods and strongly ($r = 0.851$; $p < 0.001$) correlated with MPR. Positive correlation was observed between NFC content, NGV and MRP at 24 hr incubation periods ($r = 0.763$, $r = 0.852$; $p < 0.001$). NFC had strong negative relationship ($r = -0.830$; $p < 0.001$) with MC. Fibre fractions (NDF and ADF) were strongly negatively correlated ($r = -0.927$; $r = -0.870$; $p < 0.001$) with *in vitro* GP₂₄ and MRP ($r = -0.947$; $r = -0.885$; $p < 0.001$) respectively at 24 hr incubation periods, while there are strong positive correlations ($r = 0.874$; $r = 0.885$; $p < 0.001$) between MC and NDF or ADF contents of wild legume seed species. No relationship was observed between either condensed tannins (CT) or saponins and *in vitro* GP₂₄ and methane production parameters.

DISCUSSION

Chemical compositions of selected wild legume seed meals

The chemical compositions of all the legume seed meals were comparable with earlier reports (Adubiaro *et al.*, 2011; Fasoyiro *et al.*, 2012).

Table 1. Chemical compositions of five tropical wild legume seeds (g/100 g DM).

Legume seed	DM	CP	EE	NFC	NDF	ADF	CT	SP
<i>Luffa cylindrical</i>	93.20 ^{ab}	22.05 ^e	5.58 ^c	20.43 ^e	45.59 ^a	22.98 ^a	5.30 ^a	0.43 ^a
<i>Afzelia Africana</i>	94.57 ^a	36.90 ^a	9.33 ^a	26.78 ^a	30.33 ^e	12.00 ^d	4.00 ^b	0.32 ^{ab}
<i>Detarium microcarpum</i>	91.34 ^c	30.42 ^c	8.06 ^b	22.32 ^d	37.00 ^c	18.00 ^c	4.90 ^a	0.31 ^{ab}
<i>Piliostigma thonningii</i>	92.92 ^b	32.34 ^b	9.36 ^a	24.76 ^c	41.00 ^b	22.00 ^a	3.63 ^b	0.25 ^b
<i>Daniellia oliveri</i>	89.42 ^d	28.70 ^d	8.36 ^b	25.94 ^b	36.00 ^d	20.00 ^b	3.00 ^c	0.38 ^{ab}
SEM	0.38	0.16	0.09	0.10	0.13	0.39	0.38	0.05
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.019

^{abcde} means in the same column with different superscripts differ significantly ($P < 0.05$).

SEM: Standard Error of Mean. CP: Crude protein, EE: Ether extracts, NFC: non fibre carbohydrate, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: Condensed tannins, SP: Saponin.

Table 2. In vitro gas production (mL/200 mg DM) of five tropical wild legume seeds.

Legume seed	Incubation period (hour)								CH ₄
	3	6	9	12	15	18	21	24	
<i>Luffa cylindrica</i>	2.43 ^d	8.33 ^c	10.68 ^d	20.50 ^b	24.66 ^d	28.45 ^d	30.46 ^e	37.83 ^e	9.06 ^a
<i>Afzelia africana</i>	4.50 ^a	11.70 ^a	14.30 ^a	22.52 ^a	33.30 ^a	40.18 ^b	53.66 ^a	64.71 ^a	6.42 ^c
<i>Detarium microcarpum</i>	3.00 ^{bc}	8.00 ^d	13.18 ^b	21.00 ^b	30.00 ^b	36.16 ^c	43.33 ^c	47.65 ^c	7.66 ^b
<i>Piliostigma thonningii</i>	2.67 ^{cd}	8.33 ^c	12.46 ^c	20.62 ^b	28.22 ^c	29.11 ^d	36.67 ^d	39.83 ^d	7.33 ^b
<i>Daniellia oliveri</i>	4.00 ^{ab}	10.35 ^b	13.56 ^{ab}	22.28 ^a	32.83 ^a	41.58 ^a	48.00 ^b	54.98 ^b	7.61 ^b
SEM	0.22	0.08	0.21	0.24	0.390	0.35	0.41	0.24	0.19
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

^{abcde} means in the same column with different superscripts differ significantly ($P < 0.05$). SEM: Standard Error of Mean.

However, lower or higher values might have been reported in previous literatures, chemical composition for forages is subjected to wide fluctuations depending largely on soil, parts of plant analyzed and climate characteristics. Chemical composition of the legumes seed meals indicates their nutritive potential as high quality feedstuffs in the diets of livestock. In this study, the least concentration of CP we recorded exceed 10 g/100 g DM for ruminant maintenance requirements and about 19 g/100 g DM for high-producing dairy cows or young growing stock (Waghorn and Clark 2004). This justifies the use of all the legume seeds studied as supplements to poor quality natural pastures and crop residues that are deficient in CP.

Table 3. Methane concentration and methane production reduction potential.

Legume Seeds	MC (%)	MRP (%)
<i>Luffa cylindrica</i>	23.94 ^a	75.85 ^e
<i>Azizelia africana</i>	9.90 ^e	82.89 ^a
<i>Detarium microcarpum</i>	16.08 ^c	79.58 ^d
<i>Piliostigma thonningii</i>	20.23 ^b	77.93 ^c
<i>Daniellia oliveri</i>	13.33 ^d	80.46 ^b
P. value	0.01	0.01
SEM	0.10	0.32

^{abcde} means in the same column with different superscripts differ significantly (P<0.05).

MC: Methane concentration; MRP: Methane reduction potential. SEM: standard error of mean.

The quantity and rate of fermentability of NFC affect ruminal pH, volatile fatty acids production

and incorporation of the ruminal NH₃-N into microbial protein. The NFC contents of the selected seeds are adequate to stimulate NH₃-N utilization in the rumen (Tylutki *et al.*, 2008). The optimal concentration of NFC is important in ruminant diets to avoid acidosis and other metabolic problems. The moderate NFC of the legumes seed meals is of nutritional benefits because the quantity and rate of fermentability, ruminal pH, volatile fatty acids production and incorporation of the ruminal NH₃-N into microbial protein (Olafadehan *et al.*, 2016).

The fibre fraction (NDF and ADF) contents of the wild legume seeds were generally moderate and within the limits established by Harper and McNeill (2015) for ensuring proper digestion and rumination in ruminants animals. The fibre contents of the browse seeds indicate their high nutritive value since fibre plays a significant role in voluntary intake, ruminal fermentation and digestibility (Okunade *et al.*, 2014).

The generally moderate concentration of tannins and saponins of the seed meals is indicative of the possibility of utilising the seeds meals as a feedstuff without jeopardizing the performance and health of animals consuming such feed, particularly ruminants. Sheep and cattle can tolerate 20-50 g/kg DM condensed tannins (CT), unlike goats which can tolerate up to 100 g/kg DM (Adissu 2016). Saponins levels in all the samples were lower than the tolerable level of 15-20 g/kg DM reported for goats (Onwuka 1983), which suggests that the levels reported herein are not likely to affect nutritional potentials of the seed when fed to ruminants.

Table 4. Correlation (r) between chemical compositions (g/100 g DM), total gas production and methane production parameters

Predictor	Correlation	GP ₂₄ (mL/200 mg DM)	MC (%)	MRP (%)
CP	<i>r</i>	0.685	- 0.915	0.948
	<i>p-value</i>	< 0.005	< 0.001	< 0.001
EE	<i>r</i>	- 0.562	-0.722	0.851
	<i>p-value</i>	< 0.029	< 0.001	< 0.001
NFC	<i>r</i>	0.763	-0.830	0.852
	<i>p-value</i>	< 0.001	< 0.001	< 0.001
NDF	<i>r</i>	- 0.927	0.978	- 0.947
	<i>p-value</i>	< 0.001	< 0.001	< 0.001
ADF	<i>r</i>	- 0.870	0.874	- 0.885
	<i>p-value</i>	< 0.001	< 0.001	< 0.001
Tannins	<i>r</i>	- 0.426	- 0.429	0.401
	<i>p-value</i>	0.114	0.580	0.320
Saponnin	<i>r</i>	- 0.071	0.262	0.314
	<i>p-value</i>	0.911	0.346	0.254

GP₂₄: 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

***In vitro* gas and methane production of selected legume seed meals**

The values of GP₂₄ and CH₄ production in the current study are relatively higher than those reported by Seifdavati and Taghizadeh (2012) for some legume seeds. Differences in the substrate chemical composition and management of the inoculums donors may cause the disparity between results. Higher GP₂₄ and CH₄ production of AA relative to other seed species indicates superior ruminal fermentability, in response to the higher CP, lower fibre fractions and higher NFC. Soluble carbohydrates (NFC) do not bind with tannins and are, therefore, readily fermented in the rumen to release gas. The low gas produced by LC seed meal after 24 hours may be related with its relatively high fibre fractions, CT and saponins and low level of NFC. Plants' structural carbohydrates and secondary metabolites have been reported to inhibit fermentability which affects gas and methane production (Delgado *et al.*, 2007; Okunade *et al.*, 2014). Phenolic compound such as tannins or saponins may impair microbial fermentation and gas production due to their bactericidal and bacteriostatic effects on the rumen microbes (Ebert *et al.*, 2017) and inactivation of microbial enzymes. This may be responsible for low NGV and methane generation observed in LC with higher level of tannins and saponins compared with other seed meals.

In the current study, it appears the NFC and fibre fraction influenced *in vitro* gas and CH₄ production more than any other chemical constituent. The result collaborates the reports of (Fluck, *et al.*, 2013; Kulivand and Kafizadeh, 2015). However, in the present study we opine that the amount of GP₂₄ and CH₄ produced generally could not have been as a result of phenolic compounds in the legume seed meals because the source of inoculums is from goat. Goats in the tropics can tolerate as high as 10 g/100g DM tannins content which is higher than the highest level recorded. Goats in the tropical environment have been reported to have evolutionary adaptation for high tannins rich browses (Yisehak *et al.*, 2016). Likewise, saponin concentration recorded in this study was lower than the tolerable level of 1.2 – 2.0 g/100g DM for ruminant animals (Onwuka 1983).

Methane concentration and methane reduction potential

Methane concentration production after 24 h of anaerobic fermentation can be assessed to rank the feedstuffs in terms of anti-methanogenic potential (Uslu, *et al.*, 2018). The lowest MC and consequently the highest percentage MRP observed for AA and other seed meals may be as a result of its lower fibre fractions and higher NFC levels compared to other legume seed meals. Studies have

shown that low fibre fraction (ADF and NDF) and high NFC (easily fermentable carbohydrates) produce low MC and consequently high MRP (Uslu, *et al.*, 2018). The results suggest that among the legume seed meals, Afzelia seed meal was the best potential protein source that could be used in ruminant diets to reduce loss of dietary energy through methane production and consequently mitigate enteric methane production contribution from livestock to global warming.

Correlations between chemical composition and response variables

Reports have shown that the volume of gas produced during anaerobic fermentation is related with differences in chemical compositions in the feeds and the availability of those components for rumen microorganisms (Elghandour *et al.*, 2015; Pilajun and Wanapat 2018). The strong positive correlation between CP content of the seed meals and total GP may be attributed to the generally high CP of all the legume seed meals. 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 far higher CP of the seed meals than this threshold possibly increased the microbial multiplication activities and fermentation, resulting in enhanced *in vitro* GP. Parallel results were obtained in previous studies (Karabulut *et al.*, 2007; Njidda and Nasiru 2010; Elghandour *et al.*, 2017). The significant negative relationship between CP and MC and strong positive correlation of CP with MRP are desirable as is indicative of decreases MC and increased 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 significant correlation between EE and *in vitro* GP₂₄ and methane concentration and its consequent positive correlation with MRP in our current study confirm earlier findings that dietary fat inhibits *in vitro* GP₂₄ and methane production in the rumen due to suppression of some rumen microbiota, particularly protozoa (Yusuf *et al.*, 2009), although the extent of the reduction varies depending on types of fatty acid, ruminant species used and the inclusion level, diet, (Patra 2013; Patra, 2014; Dey *et al.*, 2018).

In vitro GP₂₄ and MRP increased as NFC increased. Generally, the types of carbohydrate present in the diet to a larger extent dictate the volume of *in vitro* GP₂₄ and methane production. Readily fermentable carbohydrates (NFC) diets are fermented very rapidly by rumen microbiome with concomitant

increase in lactic acid and volatile fatty acids production, particularly propionate (Olafadehan *et al.*, 2016), resulting in increased gas production and reduced pH which inhibits protozoal and methanogen activity and hence CH₄ production. Similarly, more propionate production reduces hydrogen availability for methanogenesis (CH₄ production) by the protozoa and methanogens. The results agree with reports of Dong and Zhao (2013) and Kulivand and Kafilzadeh (2015) who reported that the carbohydrate fractions were closely correlated to the *in vitro* rumen total gas and methane production.

The negative correlation between fibre fractions (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 (Isah *et al.*, 2015) and hence GP (Kamalakar *et al.*, 2005). The negative relationship between fibre fraction and MRP indicates that a diet with a higher fibre level would reduce methane mitigating potential, in agreement with earlier finding (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 H₂ which is used to reduce CO₂ to CH₄ (Kennedy and Charmley, 2012). This result is consistent with earlier reports by Heidary and Kafilzadeh (2012).

Condensed tannins and saponins have been reported to decrease the population of protozoa, which have symbiotic relationship with methanogen (Ningrat *et al.*, 2017), resulting in reduced GP, methanogenesis and methane production. In addition, beneficial effect of moderate CT in methane abatement has been reported (Okunade and Olafadehan, 2019; Olafadehan *et al.*, 2020). Though insignificant, the negative relationships between CT or saponins and NGV₂₄, MC and weak positive relationship with MRP confirms CT and saponins to some extent reduced MC and consequently increased MRP in this study. Although, some previous reports contradict positive correlation between CT and MC and MRP (Oliveira *et al.*, 2007; Beauchemin *et al.*, 2007), the earlier reports of Jayanegara *et al.* (2009) and Piñeiro-Vázquez *et al.* (2015) agree with present study. 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; Agrawal *et al.*, 2014; Jayanegara *et al.*, 2011). Likewise, previous studies (Goel and Makkar 2012; 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. The result is in agreement with earlier reports (Patra and Yu, 2015; Ramírez-Restrepo 2016). Overall, anti-methanogenic property of any phenolic rich browse plant depends not only on the presence of one of phenolic compound, but the presence of other phenolic compounds.

CONCLUSION

All the studied tropical legume seed meals have good nutrient profile, moderate and safe levels of condensed tannins and saponins, relatively high *in vitro* gas production and methane mitigation potential, which qualify them as suitable feed supplements to low quality basal diets or as alternative protein sources in the diets of ruminants. However, *Azelia africana* demonstrated superior feeding potential over other tropical wild legume seed meals based on its nutrient profile, gas production, as well as enteric methane mitigation potential. It may, therefore, be used as alternative protein source for sustainable and environmentally friendly with livestock production. Further *in vivo* trials are, however, required to confirm its nutritive value and methane mitigation potential as a protein feed ingredient.

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