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), Pilostigma thonningii (PT), Detarium microcarpum (DM), Daniellia oliveri (DO) and Afzelia 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), Pilostigma thonningii (PT), Detarium microcarpum (DM), Daniellia oliveri (DO) y Afzelia 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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**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 (CH$_4$), which has a heat-trapping potential 23 times that of CO$_2$ (IPCC 2001). Increasing atmospheric concentrations of methane have led scientists to examine its sources of origin. On a global scale, enteric CH$_4$ 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 CH$_4$ 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 CH$_4$ 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 (Afzelia africana, Daniellia oliveri, Luffa cylindrica, Pilostigma thomningii and Detarium microcarpum) carried out by using rumen liquor from 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 (Na$_2$HPO$_4$ + 1.30 2KH$_2$PO$_4$ + 0.105 MgCl$_2$6H$_2$O + 1.407 NH$_3$HCO$_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. 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):

\[
\text{Methane concentration (MC \%) = \frac{\text{Net methane production/ Net gas production}}{100}}
\]

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:

\[
\text{MRP} = \frac{\text{Net methane in control − Net methane in the test}}{\text{Net methane} \times 100}
\]

**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 \( \text{CH}_4 \) 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 \( \text{CH}_4 \) concentration production of any feed is obtained when \( \text{CH}_4 \) 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 GP and MRP (\( r = -0.947 \); \( r = -0.885 \); \( p < 0.001 \)) respectively at 24 hr incubation periods, while there are strong positive correlations (\( r = 0.874 \); \( r = 885 \); \( p < 0.001 \)) 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 GP 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  |
|------------------------|-----|-----|-----|-----|------|------|-----|-----|
| *Luffa cylindrica*     | 93.20<sup>b</sup> | 22.05<sup>c</sup> | 5.58<sup>c</sup> | 20.43<sup>a</sup> | 45.59<sup>a</sup> | 22.98<sup>a</sup> | 5.30<sup>a</sup> | 0.43<sup>a</sup> |
| *Afzelia Africana*     | 94.57<sup>a</sup> | 36.90<sup>a</sup> | 9.33<sup>a</sup> | 26.78<sup>a</sup> | 30.33<sup>e</sup> | 12.00<sup>d</sup> | 4.00<sup>b</sup> | 0.32<sup>ab</sup> |
| *Detarium microcarpum* | 91.34<sup>c</sup> | 30.42<sup>c</sup> | 8.06<sup>b</sup> | 22.32<sup>d</sup> | 37.00<sup>c</sup> | 18.00<sup>c</sup> | 4.90<sup>a</sup> | 0.31<sup>ab</sup> |
| *Piliostigma thonningii* | 92.92<sup>b</sup> | 32.34<sup>b</sup> | 9.36<sup>a</sup> | 24.76<sup>d</sup> | 41.00<sup>d</sup> | 22.00<sup>a</sup> | 3.63<sup>b</sup> | 3.00<sup>c</sup> |
| *Daniellia oliveri*    | 89.42<sup>d</sup> | 28.70<sup>d</sup> | 9.36<sup>b</sup> | 22.32<sup>d</sup> | 37.00<sup>c</sup> | 18.00<sup>c</sup> | 4.90<sup>a</sup> | 0.31<sup>ab</sup> |
| 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 |

<sup>abcde</sup> 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) | CH₄  |
|------------------------|--------------------------|------|
|                        | 3           | 6   | 9   | 12  | 15  | 18  | 21  | 24  |
| *Luffa cylindrica*     | 2.43<sup>d</sup> | 8.33<sup>c</sup> | 10.68<sup>d</sup> | 20.50<sup>b</sup> | 24.66<sup>d</sup> | 28.45<sup>d</sup> | 30.46<sup>c</sup> | 37.83<sup>e</sup> | 9.06<sup>e</sup> |
| *Afzelia africana*     | 4.50<sup>a</sup> | 11.70<sup>a</sup> | 14.30<sup>a</sup> | 22.52<sup>c</sup> | 33.30<sup>a</sup> | 40.18<sup>b</sup> | 53.66<sup>a</sup> | 64.71<sup>a</sup> | 6.42<sup>c</sup> |
| *Detarium microcarpum* | 3.00<sup>bc</sup> | 8.00<sup>d</sup> | 13.18<sup>b</sup> | 21.00<sup>b</sup> | 30.00<sup>b</sup> | 36.16<sup>c</sup> | 43.33<sup>c</sup> | 47.65<sup>c</sup> | 7.66<sup>b</sup> |
| *Piliostigma thonningii* | 2.67<sup>cd</sup> | 8.33<sup>c</sup> | 12.46<sup>d</sup> | 20.62<sup>b</sup> | 28.22<sup>c</sup> | 29.11<sup>d</sup> | 36.67<sup>d</sup> | 39.83<sup>d</sup> | 7.33<sup>b</sup> |
| *Daniellia oliveri*    | 4.00<sup>ab</sup> | 10.35<sup>b</sup> | 13.56<sup>ab</sup> | 22.28<sup>a</sup> | 32.83<sup>a</sup> | 41.58<sup>a</sup> | 48.00<sup>b</sup> | 54.98<sup>b</sup> | 7.61<sup>b</sup> |
| SEM                    | 0.22        | 0.08 | 0.21 | 0.24 | 0.39 | 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 |

<sup>abcde</sup> 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.

The quantity and rate of fermentability of NFC affect ruminal pH, volatile fatty acids production and incorporation of the ruminal NH$_3$-N into microbial protein. The NFC contents of the selected seeds are adequate to stimulate NH$_3$-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 NH$_3$-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 using 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 3. Methane concentration and methane production reduction potential.

| Legume Seeds       | MC (%) | MRP (%) |
|--------------------|--------|---------|
| Luffa cylindrica   | 23.94a | 75.85a  |
| Afzelia africana   | 9.90c  | 82.89c  |
| Detarium microcarpum| 16.08c | 79.58c  |
| Pilostigma thonningii| 20.23b| 77.93c  |
| Daniellia oliveri | 13.33d | 80.46b  |
| P. value           | 0.01   | 0.01    |
| SEM                | 0.10   | 0.32    |

$abcd$ 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.

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

| Predictor       | Correlation | GP24 (mL/200 mg DM) | MC (%) | MRP (%) |
|-----------------|-------------|---------------------|--------|---------|
| CP              | r           | 0.685               | -0.915 | 0.948   |
|                 | p-value     | < 0.005             | < 0.001| < 0.001|
| EE              | r           | -0.562              | -0.722 | 0.851   |
|                 | p-value     | < 0.029             | < 0.001| < 0.001|
| NFC             | r           | 0.763               | -0.830 | 0.852   |
|                 | p-value     | < 0.001             | < 0.001| < 0.001|
| NDF             | r           | -0.927              | 0.978  | -0.947  |
|                 | p-value     | < 0.001             | < 0.001| < 0.001|
| ADF             | r           | -0.870              | 0.874  | -0.885  |
|                 | p-value     | < 0.001             | < 0.001| < 0.001|
| Tannins         | r           | -0.426              | -0.429 | 0.401   |
|                 | p-value     | 0.114               | 0.580  | 0.320   |
| Saponnin        | r           | -0.071              | 0.262  | 0.314   |
|                 | p-value     | 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 Kafilzadeh, 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, saponins 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. Parallell 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 Kafilzadeh (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 methanogenic 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 (Kamalak 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, *Afzelia 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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**Data availability.** Data are available with the first author upon reasonable request

**Author contribution statement (CRediT).** S.A. Okunade - Conceptualization, Methodology, Data curation., O.A. Olafadehan - Supervision, Investigation., M.O Umunna – Project Administration., O.J Makinde - Project administration.
REFERENCES

Addisu, S.H., 2016. Effect of dietary tannin source feeds on ruminal fermentation and production of cattle: a review. Online Journal of Animal and Feed Research, 6(2), pp. 45-56. Available at: http://www.science-line.com/index; http://www.0jafr.ir

Adubario, H.O., Oloafe, O. and Akintayo, E.T., 2011. Chemical composition, calcium, zinc and phytate inter-relationships in Albizia lebbeck and Daniellia oliveri seeds. Oriental Journal of Chemistry, 27, pp. 33 – 40. Available at http://www.orientjchem.org

Agrawal, A.R., Karim, S.A., Kumar, R., Sahoo, A. and John, P. J., 2014. Sheep and goat production: basic differences, impact on climate and molecular tools for rumen microbiome study. International Journal of Current Microbiology and Applied Sciences, 3(1), pp. 684-706. Available at: http://www.ijcmas.com

Anantasook, N., Wanapat, M. and Cherdthong, A., 2014. Manipulation of ruminal fermentation and methane production by supplementation of rain tree pod meal containing tannins and saponins in growing dairy steers. Journal of Animal Physiology and Animal Nutrition, 98, pp. 50-55. doi: 10.1111/jpn.12029. Epub 2013 Jan 7.

AOAC., 2005. Official method of analysis of AOAC International 18th ed. association of official analytical chemists; 2005 Washington D.C., USA.

Babayemi, O.J., Demeyer, D. and Fieve, V., 2004. In vitro fermentation of tropical browse seeds in relation to their content of secondary metabolites. Journal of Animal and Feed Sciences, 13, pp. 31–34. doi:10.22358/JAFS/70754/2004 Corpus ID: 55276811

Beauchemin, K.A., McGinn, S.M., Martinez, T.F. and McAllister, T.A., 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions. Journal of Animal Science, 85, pp. 1990–1996. doi: 10.2527/jas.2006-686.

Belete, S.G. and Abubeker, H., 2018. The potential of tropical tannin rich browse in reduction of enteric methane. Dairy and Veterinary Sciences, 2(3), pp. 154-162. doi: 10.31031/APDV.2018.02.000538

Black, John L., Thomas M. Davison, and Iliana Box., 2021. Methane emissions from ruminants in Australia: mitigation potential and applicability of mitigation strategies.

Elghandour, M.M.Y., Kholif, A.E. Marquez-Molina O, Vazquez-Armijo F, Punia A.K. and Salem A.Z.M., 2015. Influence of individual or mixed cellulose and xylanase mixture on in vitro rumen gas production kinetics of total mixed rations with different maize silage and concentrate ratios. Turkish Journal of Veterinary and Animal Science, 39, pp. 435-442. doi:10.3906/VET-1410-26

Elghandour, M.M.Y., Vázquez, J.C., Salem, A.Z.M., Kholif, A.E., Cipriano, M.M., Camacho, L.M. and Márquez, O., 2017. In vitro gas and methane production of two mixed rations influenced by three different cultures of Saccharomycyes cerevisiae, Journal of Applied Animal Research, 45(1) pp. 389-395. doi:10.1080/09712119.2016.1204304

FASOSTAT., 2009. Online statistical service food and agriculture organization of the united nations, Rome.

Fasoyiro, S.B., Yudi, W. and Kehinde, A.T., 2012. Processing and utilization of legumes in the tropics. In: A. Amer Eissa, ed. Trends in Vital Food and Control Engineering, Rijeka, Croatia: InTech. pp. 70-74. doi:10.5772/36496

Delgado, D. C., González, R., Galindo, J., Cairo, J. and Almeida, M., 2007. Potential of Trichantera gigantea and Morus alba to reduce in vitro rumen methane production. Cuban Journal of Agricultural Science, 41(4) pp. 319-322.

Dey, A., Paul, S. S., Dahiya, S. S. and Punia, B. S., 2018. Effects of vegetable oils supplementation on in vitro rumen fermentation, volatile fatty acid composition and methane production in buffaloes. Buffalo Bulletin, 37(1), 37-44.

Dong, R.L. and Zhao, G.Y., 2013. Relationship between the methane production and the CNCPs carbohydrate fractions of rations with various concentrate/rougahage ratios evaluated using in vitro incubation technique. Asian-Australasian Journal of Animal Science, 26, pp. 1708 – 1716. doi:10.5713/ajas.2013.133245

Ebert, P.J., Bailey, E.A., Shreck, A.L., Jennings, J.S. and Cole, N.A., 2017. Effect of condensed tannin extract supplementation on growth performance, nitrogen balance, gas emissions, and energetic losses of beef steers. Journal of Animal Science, 95, pp. 1345–1355. doi:10.2527/jas.2016.0341
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, 123-124, pp. 197-210. doi:10.1016/j.anifeedsci.2005.05.001

Fluck, A.C., Kozlowski, G.V., Martins, A.A., Mezzomo, M.P., Zanferari, F. and Stefanello, S., 2013. Relationship between chemical components, bacterial adherence and in vitro fermentation of tropical forage legumes. Ciência e Agrotecnologia, 37(5), pp. 457-463. doi:10.1590/S1413-70542013000500010

Goel, G. and Makkar, H.P.S., 2012. Methane mitigation from ruminants using tannins and saponins. Tropical Animal Health and Production, 44, pp.729–739. doi: 10.1007/s11250-011-9966-2

Harper, K.J. and McNeill, D.M., 2015. The role indf in the regulation of feed intake and the importance of its assessment in subtropical ruminant systems (the role of indf in the regulation of forage intake. Agriculture, 5, pp.778–790. doi:10.3390/agriculture5030778

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A., Yang, W., Lee C., Gerber, P.J., Henderson, B. and Tricarico, J., 2013. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. Journal of Animal Science, 91, pp. 5045-5069. doi 10.2527/jas.2013-6583

Hu, W.L., Liu, J.X., Wu, Y.M., Guo, Y.Q. and Ye, J. A., 2006. Effects of tea saponins on in vitro ruminal fermentation and growth performance in growing Boer goat. Archives of Animal Nutrition, 60, pp. 89–97. https://doi.org/10.1080/17450390500353119

IPCC., 2001. (Intergovernmental Panel on Climate Change). Climate Change 2001: The scientific basis contribution of working group I to the Third assessment report of the intergovernmental panel on climate change. 2001. Available at: http://www.ipcc.ch

Isah, O.A., Okunade, S.A., Aderinboye, R.Y. and Olafadehan, O.A., 2015. Effect of browse foliage supplementation on the performance of buckling goats fed sorghum thresher top basal diet. Tropical Animal Health and Production, 47(6), pp.1027-1032.

Jayanegara, A., Togtokhbayar, N., Makkar, H.P.S. and Becker. K., 2009. Tannins determined by various methods as predictors of methane production reduction potential of plants by in vitro rumen fermentation system. Animal Feed Science and Technology, 150(3), pp.:230-237. doi:10.1016/j.anifeedsci.2008.10.011

Jayanegara, A., Wina, E. and Soliva, C. R., Marquardt, S., Kreuzer, M. and Leiber, F., 2011. Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Animal Feed Science Technology, 163, pp. 231-243. https://doi.org/10.1016/j.anifeedsci.2010.11.009

Johnson, K.A. and Johnson, D.E., 1995. Methane emissions from cattle. Journal of Animal Science, 73, pp. 2483–2492. https://doi.org/10.2527/1995.7382483x

Kafizadeh, F. and Heidary, N., 2013. Chemical composition, in vitro digestibilityand kinetics of fermentation of whole-crop forage from 18 different varieties of oat (Avena sativa L). Journal of Applied Animal Research, 41(1), pp. 61-68. https://doi.org/10.1080/0970121912.739084

Kamalak, A., Canbolat, O., Erol, A., Kilinc, C., Kizilsimsek, M., Ozkan, C. O. and Ozkose E., 2020. Effect of variety on chemical composition, in vitro gas production, metabolizable energy and organic matter digestibility of alfalfa hays. Livestock Research for Rural Development, 17, 77 Retrieved May 15, 2020, from http://www.lrrd.org/lrrd17/7/kama17077.htm

Karabulut, A., Canbolat, O., Ozkan, C.O. and Kamalak, A., 2007. Determination of nutritive value of citrus tree leaves for sheep using in vitro gas production technique. Asian Australasian Journal of Animal Sciences, 20, pp. 529-535. https://doi.org/10.5713/ajas.2007.529

Keay, R.W.J., 1989. Trees of Nigeria. Oxford, New York, Oxford University Press,

Kennedy, P. M. and Charmley, E., 2012. Methane yields from Brahman cattle fed tropical grasses and legumes. Animal Production Science. 52, pp. 225-239. doi:10.1071/AN11103

Kulivand, M., Kafizadeh, F., 2015. Correlation between chemical composition, kinetics of fermentation and methane production of eight pasture grasses. Acta Scientiarum
Liu, Y., Ma, T., Chen, D., Zhang, N., Si, B., Deng, K., Tu, Y. and Diao, Q., 2019. Effects of teaponin supplementation on nutrient digestibility, methanogenesis, and ruminal microbial flora in dorper crossbred ewe. *Animals*, 9(1): 29. https://doi.org/10.3390/ani9010029

Menke, K.H. and Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*, 28, pp. 7-55.

Ningrat, R.W. Zain, S., Erpomen, M. and Suryani, H., 2017. Effects of doses and different sources of tannins on in vitro ruminal methane, volatile fatty acids production and on bacteria and protozoa populations. *Asian Journal of Animal Sciences*, 11, pp. 47-53. doi: 10.3923/ajas.2017.47.53

Njidda, A.A. and Nasiru, A., 2010. *In vitro* gas production and dry mater digestibility of tannin-containing forages of semi-arid region of north-eastern Nigeria. *Pakistan Journal of Nutrition*, 9, pp. 60-66. doi: 10.3923/pjn.2010.60.66

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*. Gutteridge RG, Shelton HM. (eds). Wallinford: CAB International. Pp. 177-191.

Okunade, S.A., Olafadehan, O.A. and Isah, O.A., 2014. Fodder potential and acceptability of selected treelaves by goats. *Animal Nutrition and Feed Technology*, 14, pp. 489-498. doi: 10.5958/0974-181X.2014.01351.1

Okunade, S.A., Isah, O.A., Oyekunle, M.A., Olafadehan, O.A., and Makinde, O.J., 2016. Effects of Supplementation of Threshed Sorghum top with Selected Browse Plant Foliage on Haematology and Serum Biochemical Parameters of Red Sokoto goats. *Tropical Animal Health and Production*, 48(5), pp. 979–984. doi:10.1007/s11250-016-1045-2

Okunade, S.A. and Olafadehan, O.A., 2019. Rolfe (*Daniellia oliveri*) as a protein source in locally produced concentrates for lambs fed low quality basal diet. *Journal of the Saudi Society of Agricultural Sciences*, 18, pp. 83-88. doi:10.1016/j.jssas.2017.02.001

Olafadehan, O. A., Adewumi, M. K. and Okunade, S. A., 2014. Effects of feeding tannin-containing forage in varying proportion with concentrate on the voluntary intake, haematological and biochemical indices of goats. *Trakia Journal of Sciences*, 12(1), pp. 73-81. Available online at: http://www.uni-sz.bg

Olafadehan, O. A., Njidda, A. A., Okunade, S. A., Adewumi, M. K., Awosanmi, K. J., Ijanmi, T. and Raymond, A., 2016. Effects of feeding *Ficus poli* foliage based complete rations with varying forage/concentrate ratio on performance and ruminal fermentation in growing goats. *Animal Nutrition and Feed Technology*, 16, pp. 373-382. doi:10.5958/0974-181X.2016.0033.0

Olafadehan, O.A., Okunade, S.A., Njidda, A.A., Kholif, A.E., Kolo, S.G. and Alagbe J.O., 2020. Concentrate replacement with *Daniellia oliveri* foliage in goat diets. *Tropical Animal Health and Production*, 52, pp. 227-233. doi:10.1007/s11250-019-02002-0

Oliveira, S. G., Berchielli, T. T., Pedreira, M. S., Primavesi, O., Frighetto, R. and Lima M. A., 2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. *Animal Feed Science and Technology*, 135, pp. 236–248.

Onwuka, C.F.I., 1983. Nutritional Evaluation of Some Nigerian Browse Plants in the Humid Tropics. Ph.D. Thesis. University of Ibadan: Ibadan, Nigeria.

Parissi, Z.M., Papachristou, T.G. and Nastis, A.S., 2005. Effect of drying method on estimated nutritive value of browse species using an *in vitro* gas production technique. *Animal Feed Science Technology*, 30, pp. 119-128. doi:10.1016/j.anifeedsci.2005.04.046

Patra, A. K., 2013. The effect of dietary fats on methane emissions, and its other effects on digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis. *Livestock Science*, 155, pp. 244–54. doi:10.1016/j.livsci.2013.05.023

Patra, A. K., 2014. A meta-analysis of the effect of dietary fat on enteric methane production, digestibility and rumen fermentation in sheep, and a comparison of these responses between cattle and sheep. *Livestock Science*, 162, pp. 97–103. doi:10.1016/j.livsci.2014.01.007

Patra, A.K. and Yu, Z., 2015. Effects of adaptation of in vitro rumen culture to garlic oil, nitrate, and saponin and their combinations on methanogenesis, fermentation, and
abundances and diversity of microbial populations. *Frontiers in Microbiology*, 6, pp. 1434. doi:10.3389/fmicb.2015.01434

Pilajun, R., and Wanapat, M., 2018. Chemical composition and in vitro gas production of fermented cassava pulp with different types of supplements, *Journal of Applied Animal Research*, 46, pp. 81-86. doi:10.1080/09712119.2016.1261029

Ramírez-Restrepo, C.A., Tan, C., O’Neill, C.J., López-Villalobos, N., Padmanabha, J., Wang, J.K. and McSweeney, C.S., 2016. Methane production, fermentation characteristics, and microbial profiles in the rumen of tropical cattle fed tea seed saponin supplementation. *Animal Feed Science and Technology*, 216, pp. 58-67. https://doi.org/10.1016/j.anifeedsct.2016.03.005

SAS., 2012. Statistical Analytical Systems, Users Guide Version 6. SAS Institute Inc., Cary, North Carolina, USA 2012.

Seidavati, J., Taghizadeh, A., Jannmohammadi, H., Seyd, A. R. and Sadegh, A., 2012. In vivo digestibility and in vitro intestinal digestibility of three legume seeds. *International Journal of Agriculture Research and Review*, 2, pp. 630-638.

Singh, S., Kushwaha, B.P., Mishra, A.K., Nag, S.K., Anele, U.Y., Singh, A., Bhattacharya, S., Gupta, P. K., and Jayashankar, J., 2016. Nutritive value and methane production potential of energy and protein rich feedstuffs fed to livestock in India. *Indian Journal of Animal Sciences*, 86, pp. 581–588. Available at https://www.researchgate.net/publication/303755358

Soliva, C.R., Meile, L., Cieslak, A., Kreuzer, M. and Machmuller, A., 2004. Rumen simulation technique study on the interactions of dietary lauric and myristic acid supplementation in suppressing ruminal methanogenesis. *British Journal of Nutrition*, 92, pp. 689-700. doi: 10.1079/bjn20041250

Tylutki, T.P., Fox, D.G., Durbal, V.M., Tedeschi, L.O. and Russell, J.B., 2008. Cornell net carbohydrate and protein system: A Model for Precision Feeding of Dairy Cattle. *Animal Feed Science and Technology*, 143, pp. 174-174. doi:10.1016/j.anifeedsct.2007.05.010

Uslu, O. S., Kurt, O., Kaya, E. and Kamalak, A., 2018. Effect of species on chemical composition, metabolizable energy, organic matter digestibility and methane production of some legume plants grown in Turkey. *Journal of Applied Animal Research*, 46, pp. 1158-1161. https://doi.org/10.1080/09712119.2018.1480485

Van Soest, P. J. 1994, Nutritional Ecology of the Ruminant, 2nd ed. Ithaca, NY, Comstock publishing Associates/Cornell University Press.

Waghorn, G. C. and Clark, D. A., 2016. Feeding value of pastures for ruminants. *New Zealand Veterinary Journal*, 52, (6), pp. 320-331. doi:10.1080/00480169.2004.36448.

Yisehak, K., Kibreab, Y., Taye, T., Lourenço, M.R.A. and Janssens, G.P., 2016. Response to dietary tannin challenges in view of the browser/grazer dichotomy in an Ethiopian setting: Bonga sheep versus Kaffa goats. *Tropical Animal Health and Production*, 48, pp. 125–131. doi: 10.1007/s11250-015-0931-3

Yusuf, A.M., Olafadehan, O.A., Obun, C.O., Inuwa, M., Garba, M.H. and Shagwa, S.M., 2009. Nutritional evaluation of shea butter fat in fattening of Yankasa sheep. *Pakistan Journal of Nutrition*, 8(7), pp. 1062-1067. doi:10.3923/pjn.2009.1062.1067