IN VITRO SCREENING OF SELECTED WILD LEGUME SEEDS FOR ENTERIC METHANE MITIGATION POTENTIAL

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Abstract
The study was conducted to evaluate the chemical composition, in vitro gas production and methanogenic property of five tropical wild legume seeds (*Luffa cylindrica*, *Piliostigma thonningii*, *Detarium microcarpum*, *Daniellia oliveri* and *Afzelia africana*). Chemical compositions were analysed, while total gas volume was measured and methane estimated by using 4 ml of 10 M NaOH. All samples (% DM) had high crude protein (22.05 –39.9) and moderate fibre concentrations (NDF, 37–49.33; ADF, 20 –22.33 and NFC, 16.78 – 30.39). There were variations ($P<0.05$) in the dry matter (DM), crude protein (CP), non-fibre carbohydrates (NFC), neutral detergent fibre (NDF) and anti-nutritional contents of the selected tropical wild legume seeds. Total gas volume produced by the seed meals steadily increased and was most pronounced ($P<0.05$) in *Afzelia africana* seed meal (60.67 ml/200 mg DM), while the least gas volume was recorded against *Piliostigma thonningii* (37.83 mL/200 mg DM). Methane mitigation potential ranged from 0.15- 0.20 mL/200 mg DM with *Afzelia africana* having the highest and *Piliostigma thonningii* the least values. Results show that all the selected wild legume seeds have good nutrient profile, moderate and safe levels of anti-nutritional factors, relatively high in vitro degradability and methane mitigation potential which qualify them as suitable feed supplements to low quality basal diets for ruminants. However, *Afzelia africana* had the best methane mitigation potential and can, therefore, be regarded as the best suitable alternative plant protein source for improved and environmentally friendly ruminant production.

Keywords: In vitro, anti-nutritional factors, methane mitigation, legume seeds, ruminants

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 produced by domesticated ruminants contributes 0.18 of total GHG emissions (FAOSTAT, 2009). Methane production by ruminants also results in a loss of energy intake of up to 0.12 (Johnson and Johnson, 1995). Therefore, reduced CH_4 production by ruminants has been recognized as an important goal because it reduces the 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). Relatively recent ruminant methane reduction strategies have included the introduction of methane inhibitors, both biological and chemical, in the animal feed, to kill off or at least reduce the activity of the methanogenic microorganisms in the gut. Such mitigation options include the use of plant secondary compounds (Soliva

et al., 2004). Many studies reported that tannins and saponins rich plants appeared to be useful in suppressing methane release by reducing the activity of rumen ciliate protozoa and methanogens (Anantasook *et al*., 2014). Therefore, the objective of this study was to determine the *in vitro* gas production and anti-methanogenic property of different tropical wild legume seeds (*Afzelia africana, Daniellia oliveri*, *Etanda africana, Piliostigma thonningii and Detarium macrocarpum*) that can be used as alternative plant protein supplements for ruminants.

Materials and Methods

This study was carried out at the Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. Five tropical wild legume seeds (*Luffa cylindrical*, *Afzelia africana*, *Daniellia oliveri*, *Piliostigma thonningii and Detarium microcarpum*) milled samples were analysed for their proximate constituents according to AOAC (2005). The seed samples were analysed for neutral detergent fibre (NDF) according to Van Soest *et al*. (1991). Non-fibre carbohydrates (NFC) were calculated and total condensed tannins and saponins were determined by the methods of Babayemi *et al*. (2004). The *in vitro* gas production was determined according to Menke and Steingass (1988). 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) sodium hydroxide (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). The actual methanogenic property was estimated as proportion of methane-to-total gas ratios. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced from sample.

Results and Discussion

Chemical composition of the legume seeds is shown in Table 1. Crude protein (CP) content was highest ($P < 0.05$) in *Afzelia africana* (39.90 g/100 g DM) and least in *Luffa cylindrica* (22.05 g/100 g DM). Concentration of NFC was highest ($P < 0.05$) in *Afzelia africana* (30.39 g/100 g DM). There were variations ($P < 0.05$) in the NDF and ADF contents of the wild legume seeds. Concentration of NDF ranged from 36 g/100 g DM in *Daniellia oliveri* seed to 45.59 g/100 g DM in *Piliostigma thonningii*. Higher concentration of tannins (0.83 g/100g DM) and saponins (4.03 g/100g DM) were recorded for *Piliostigma thonningii*. The range of the CP content of the wild legume seeds is in agreement with previous reports (Bouazza *et al*., 2012). The lowest value of CP (22.05 g/100 g DM) for *Luffa cylindrica* is well above the range of 7.0–8.0 g/100 g DM suggested as critical limit below which intake of forages by ruminants and rumen microbial activity are adversely affected (Van Soest, 1994). This justifies their use as supplements to poor quality natural pastures and crop residues. 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 fibre fraction contents of the wild legume seeds were generally moderate and within the limits established by NRC (1978) for ruminant animals for ensuring proper digestion and rumination. The range value of 30.33 – 45.59 g/100g of NDF was low to moderate when compared with low quality roughages, which ruminants can effectively degrade. The low to moderate fibre contents of the tree seeds indicate their high nutritive value since fibre plays a significant role in voluntary intake and digestibility. The gas produced significantly ($p<0.05$) differed at all stages of incubation. The highest gas production (GP) (60.16 mL/200 mg DM) and methane (9.06 mL/200 mg DM) after 24 h of incubation were recorded for *Afzelia qfricana* seed, while the lowest GP and CH_4 were found for *Piliostigma thonningii* seed (Table 2). Methane:total gas production ratio which is regarded as methane mitigation potential in this study ranged from 0.13 in *Afzelia africana* to 0.21 in *Piliostigma thonningii* (Fig. 1).

Table 1: Chemical compositions of selected tropical wild legume seeds (g/100 g DM)

Legume seed	DM	CP	EE	NFC	NDF	Tannins	Saponins
Luffa cylindrica	93.2^{ab}	22.05^{c}	5.58	26.78^{a}	37.0^{c}	0.69^{b}	3.05^{b}
Afzelia africana	94.57^{a}	36.90^{a}	8.00^{a}	24.79^{b}	30.33^{d}	0.30^{d}	1.38^{c}
Detarium microcarpum	91.34^{bc}	30.19^{b}	8.36^{c}	20.45^{a}	41.00^{b}	0.36^{d}	2.45^{b}
Piliostigma thonningii	92.92^{ab}	32.34^{ab}	9.33^{b}	22.32^{c}	45.59^{a}	0.83^{a}	4.03^{a}
Daniellia oliveri	89.42^{c}	28.70^{b}	8.36^{c}	25.94^{a}	36.00^{c}	0.50^{c}	3.06^{b}
SEM	0.8	2.50	0.4	0.5	1.3	0.05	0.28

Means in the same column with different superscripts differ significantly ($P<0.05$)

All the incubated seeds had relatively high gas production, since the seeds were high in fermentable carbohydrate content (Babayemi *et al*., 2004). Many factors such as the level of crude protein, nature and level of fibre, level of soluble or readily fermentable carbohydrates, presence of secondary metabolites and potency of the rumen liquor for incubation have been reported to determine the amount of gas produced during fermentation. In the current study, it appears the secondary metabolites more than fibre content influence *in vitro* gas production and hence degradability. The low gas production of *Piliostigma thonningii* could be attributed to its high amount of tannins (Babayemi *et al*., 2004) and saponins (Soliva *et al*., 2004) respectively.

Table 2: *In vitro* gas production (mL/200 mg DM) of some tropical tree legume seeds incubated for twenty-four hours

Legume seed	Incubation period (hour)								CH_4
	3	6	9	12	15	18	21	24	
Luffa cylindrica	2.43^{b}	8.33^{c}	10.68^{e}	20.50^{b}	24.66^{c}	28.45^{c}	36.67^{d}	39.83^{d}	6.76^{c}
Afzelia africana	4.50^{a}	11.70^{a}	14.30^{a}	22.52^{a}	33.30^{a}	40.18^{a}	53.66^{a}	64.71^{a}	9.06^{a}
Detarium microcarpum	2.67^{b}	8.33^{c}	12.46^{d}	20.62^{b}	28.22^{b}	29.11^{c}	30.46^{e}	47.65^{c}	7.66^{bc}
Piliostigma thonningii	3.00^{b}	8.00^{c}	13.18^{c}	21.00^{b}	30.00^{b}	36.16^{b}	43.33^{c}	37.83^{d}	7.33^{bc}
Daniellia oliveri	4.00^{a}	10.35^{b}	13.56^{b}	22.28^{a}	32.83^{a}	41.58^{a}	48.00^{b}	54.98^{b}	8.06^{b}
SEM	0.25	0.30	0.16	0.29	0.98	1.35	1.46	1.38	0.42

Means in the same column with different superscripts differ significantly ($P<0.05$)

Although high methane (CH_4) production implies an energy loss to the animal, forage with a higher degradability leads to a more intensive fermentation in the rumen (Rinne *et al*., 1997) and thus increase CH_4 production. This possibly explained why *Afzelia africana* had the higher CH_4 production compared with other seeds. However, better CH_4 mitigation potential of *Afzelia africana* (production of smallest volume of CH_4 per total gas volume produced) compared to other seeds could most likely be the consequence of its tannins. Bodas *et al*. (2012) reported the potency of tannins to mitigate enteric CH_4

production by decreasing archaea activity and growth, or and indirectly lower H_2 availability as consequence of decreased protozoa population. Likewise, the highest generation of CH_4 per total gas volume by *Piliostigma thonningii* seed implies lowest enteric methane mitigation effect compared with other tree legume seeds

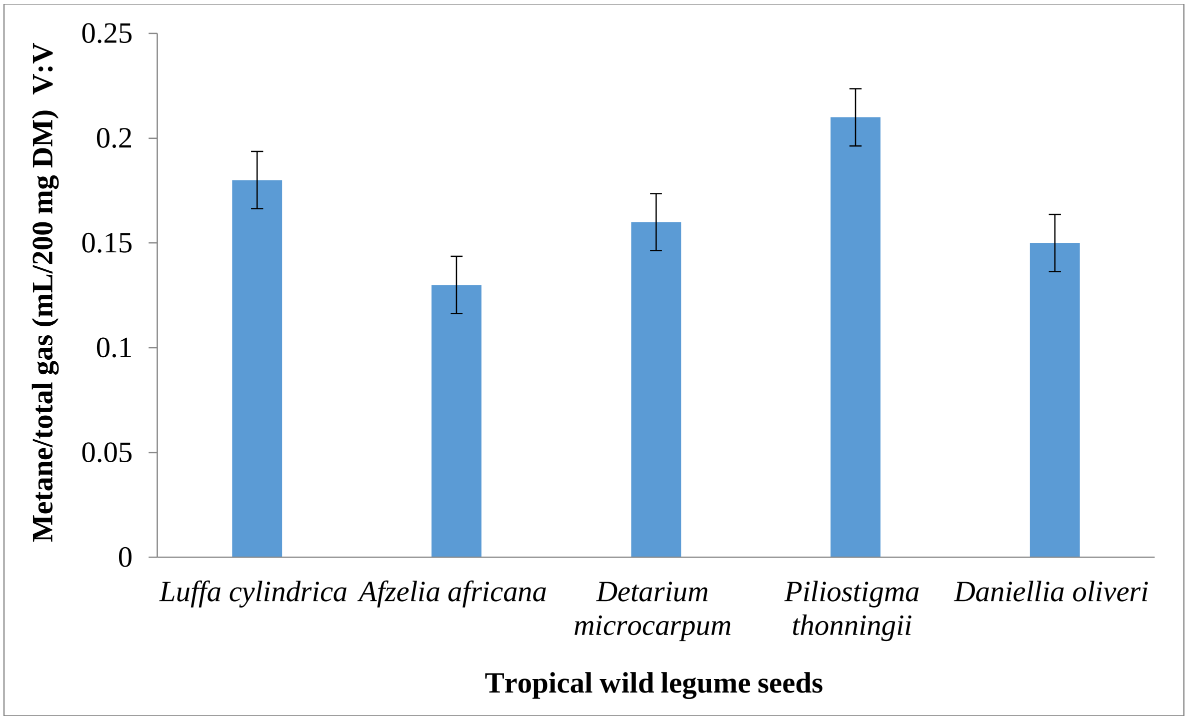

Fig. 1: In vitro methane mitigation potential of some selected tropical tree legume seeds

Conclusion

All the studied tropical legume seeds have good nutrient profile, moderate and safe levels of anti-nutritional factors and relatively high degradability and methane mitigation potential, which qualify them as suitable feed supplements to low quality basal diets for ruminants. However, *Afzelia africana* demonstrated superiority over other tropical seeds in terms of nutrient profile, *in vitro* digestibility as well as enteric methane mitigation potential because it produces the lowest methane per total gas volume. *A. africana* is, therefore, considered the best suitable alternative plant protein source for improved and environmentally friendly livestock production.

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