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CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION OF FRUITS OF FOUR TROPICAL FORAGE SPECIES

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ABSTRACT

Chemical composition and *in vitro* gas production of *Canavalia ensiformis*, *Tephrosia bracteolata*, *Enterolobium cyclocarpum* and *Moringa oleifera* fruits (pods with seeds) were evaluated for their potentials as feeds or supplements for ruminants in a completely randomized design replicated four times. The results revealed that the crude protein content was highest ($P<0.05$) (196.27 g kg^{-1}) in *T. bracteolata* while the least value (147.45 g kg^{-1}) was in *E. cyclocarpum*. The values for neutral detergent fibre was least (410.00 g kg^{-1}) in the pods of *C. ensiformis* while the highest was in *E. cyclocarpum* (740.00 g kg^{-1}). *Canavalia ensiformis* consistently had significant ($P<0.05$) highest values in the calculated dry matter intake, dry matter digestibility and relative feed value above other plant pods. Highest gas was from *M. oleifera* (30.00 ml/200 mg DM) during the *in vitro* gas production. Methane production was significantly ($P<0.05$) highest in *T. bracteolata* and *M. oleifera* (0.21 mM) followed by *C. ensiformis* (0.20 mM) and the least methane was produced by *E. cyclocarpum* (0.18 mM). In conclusion, plant pods evaluated possessed many desirable nutritive potentials with good biological values and their use as supplements to matured and senescent grasses as well as crop residues especially in the dry season will help to reduce the cost of feeding animals as well as improve their performance.

Key words: forage plants, *in vitro*, methane, pods, tannin

INTRODUCTION

Forage plants (grasses, legumes and browse plants) are the cheapest major nutritional components in the diets of ruminants, particularly in the rural and sub-urban areas of the tropics (Akinsoyinu and Onwuka, 1988). Productive livestock enterprise is likely unattainable and unsustainable by these forages alone throughout the year, due to seasonal effects on the plants which makes them lignified, low in quality and quantity (Ojo *et al*., 2016). Since protein is the most limiting part of the nutrients from forages during the dry season, there is need to find alternative sources that will be used to enhance the nutritional value of poor quality roughages that is normally available for ruminants during the season. This has to be relatively cheap and easily available, so that the feed cost for the ruminants can be significantly reduced (Sarnklong *et al*., 2010).

However, forage legumes do not feature prominently in the existing farming systems, despite their potential to improve soil fertility, check soil erosion and contribute to the livestock feed resource base. Thairu and Tessema (1987) reported that productive legumes are absent from pastures in the semi-arid regions for various reasons such as failure to tolerate heavy grazing, drought susceptibility, non-availability and high cost of seeds.

Due to their limited availability, the nutritive evaluation of alternative protein sources is a necessary area of research. The promising examples includes pods from *Canavalia ensiformis, Tephrosia bracteolata*, *Enterolobium cyclocarpum* and *Moringa oleifera* which can serve as good protein supplements to poor quality roughages for ruminants during the season.

Akinlade *et al*. (2007) reported *Canavalia ensiformis* (Jack bean) to be of high yielding nutritious pods and as well as a good green manure forage. *Tephrosia bracteolata* a leguminous shrub is very rich in protein and grows to about 2 m in height (Ayoade *et al.*, 1998). *Enterolobium cyclocarpum* are evergreen forage that can produce very high protein content (Arigbede *et al.*, 2008). The tree is a legume tree which belongs to the family *Mimosadeae* (Idowu *et al.*, 2013) and has been used in intensive feed garden. As a leguminous multipurpose plant, it has the potential of fixing atmospheric nitrogen into the soil and can also be exploited for feeding of ruminants. *Moringa oleifera* is a multipurpose tree that is cultivated both for human food and animal feed. Reports have shown that *Moringa oleifera* leaves have potential prebiotic effects due to its content of antioxidant phytochemicals, such as chlorogenic acid and caffeic acid (Siddhuraju and Becker, 2003). Also, the leaf meal which is widely available in many tropical countries, is also a good source of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Teixeira *et al*., 2014), with high amounts of vitamins, minerals and amino acids which can make it a potential ingredient for livestock nutrition.

Several tropical browse and shrub legumes which are seed and fruit bearing have been reported with lush crude protein and other nutrients (Babayemi *et al*., 2004a). The fruits from forage plants in the tropics are abundant and are of no nutritional significance to humans and at the same time constitute nuisance to the environments. The report of Janzen (1981) on the consumption of fruits of plants by free-ranging horses presents the potential utilization of tropical pods as acceptable feed resource that could be scavenged, and used as ingredient for formulation of diets for livestock feeding.

Babayemi and Bamikole (2006) had advocated for evaluation of any unconventional feeds with *in vitro* gas production method since the plants to evaluate may possess secondary metabolites thereby limiting their utilization by ruminants. *In vitro* gas production is a quick and less expensive means of determining the nutritive value of feeds for ruminants (Babayemi *et al*., 2004b). Total gas production can be used to predict methane (CH_4), volatile fatty acids (VFA) and the individual molar VFA (Fievez *et al*., 2005). The objective of the present study was to determine the chemical composition, *in vitro* evaluation and methane production of *Canavalia ensiformis, Tephrosia bracteolata*, *Enterolobium cyclocarpum* and *Moringa oleifera* pods as supplements in ruminant feeds.

MATERIALS AND METHODS

Experimental Sites Description

The field experiment was carried out at the Department of Pasture and Range Management farm, Federal University of Agriculture, Abeokuta, Nigeria. The area lies within the savannah agro-ecological zone of Western Nigeria (Latitude 7^o 13 49.46 N, longitude 3^o 26 11.98 E, average annual rainfall of 1037 mm). Temperatures are fairly uniform with daytime values of 28– 30 ^{o}C during the rainy season and 30–34 ^{o}C during the dry season with the lowest night temperature of around 24 ^{o}C during the harmattan period between December and February. The laboratory analyses were

conducted at the National Animal Production Research Institute at Shika, Zaria, Kaduna State, Nigeria located within the grid (11°–11°13 'N, 6°55 ' –7°33 'E) in the sub-humid zone of Nigeria. Mean annual rainfall varies from 700–1300 mm with a long-term average of 1050 mm. Approximately 95 percent of the rainfall occurs during May–October. The highest mean maximum temperature of 36 °C is recorded in April while the lowest mean minimum temperature of about 11.5 °C occurs in December–January.

Collection of Forage Pods

Matured pods of *C. ensiformis, T. bracteolata, E. cyclocarpum* and *M. oleifera* were collected manually from the Pasture Introduction Section.

Experimental Design

The design of the experiment was a Completely Randomized Design which was replicated four times.

Chemical Composition Analyses

Five hundred (500) grammes each of the pods from the four forage plants were oven-dried at 65 ^{o}C constant weight and were later milled in a Thomas-Wiley mill (1 mm sieve) and analysed for proximate composition (AOAC, 2010) and fibre analysis (Van Soest *et al.*, 1991). Non-fibre carbohydrate was calculated as NFC = 100 – (CP + Ash + EE + NDF) (Sniffen *et al.*, 1992). Organic Matter as 100-Ash (Lanyasunya, *et al.*, 2007). Tannin contents were determined according to the procedures of Jaffe (2003).

Calculated Parameters

CHO = OM content – (CP + EE) (Arieli *et al.*, 1999), where CHO = Total carbohydrate, OM = Organic Matter content, CP = crude protein content and EE = ether extract.

DMD = Dry Matter Digestibility is an estimate of digestible fibre in the forage.

DMD (%) = 88.9 - 0.779ADF (% of DM).

DMI = Dry Matter Intake.

DMI (% of body weight) =120/NDF (% of DM). (Agric - facts, 2006)

RFV= (PDMD × PDMI)/1.29, where RFV = Relative Feed Value

The *in vitro* gas production was determined following the procedure of Menke and Steingass (1988) by using 100 ml glass syringes fitted with plungers for 24-hour with 200 mg of each samples replicated six times.

Fifty (50) ml of the supernatants of *in vitro* rumen liquor after the incubation period were decanted into plastic bottles. The liquor were used for the determination of acetic, propionic and butyric acids following Samuel *et al*. (1997) procedures. Methane gas production was calculated from the volatile fatty acids proportions using the formula prepared by Ørskov *et al*. (1968). The formula is expressed as: Methane = 0.5 (Acetate) – 0.25 (Propionate) + 0.5 (Butyrate).

All data collected were subjected to one-way Analysis of Variance and treatment means separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The chemical composition of *C. ensiformis, T. bracteolata, E. cyclocarpum* and *M. oleifera* dried pods is presented in Table 1. *Tephrosia bracteolata* pods had highest (P<0.05) significant values of 886.67 g kg^{-1} dry matter (DM), 196.27 g kg^{-1} crude protein (CP) and 92.77 g kg^{-1} organic matter content. *Moringa oleifera, C. ensiformis* and *E. cyclocarpum* pods had CP contents of 175.13, 170.03 and 147.45 g kg^{-1} respectively. The highest tannin content was in *E. cyclocarpum* (1.99 mg kg^{-1}) and the least was in *C. ensiformis* (0.91 mg kg^{-1}). The CP contents in the pods of the forage plants in this study fell within and even above values reported by Tanner *et aI.* (1990), for different Acacia species (*Acacia albida* (143 g kg^{-1} DM), *Acacia nilotica* (130 g kg^{-1} DM), *Acacia sieberiana* (127 g kg^{-1} DM) and *Acacia tortilis* (136 g kg^{-1} DM)). Similar value for CP in *M. oleifera* (172.7 g kg^{-1} DM) in this study was reported by Aderinboye *et al*. (2011). Moreover, The CP content in *E. cyclocarpum* in

this study was close to the value (15.7 g/100 g DM) reported by Babayemi (2006) for the same sample. The high CP content contained in the pods of forage plants in this study suggests that the forages are potential sources of protein for ruminants. These values were higher than the recommended range of 7 – 14 % for ruminant performance (NRC, 1981). Janzen, (1981) reported that tropical pods of plants are acceptable by livestock and were seen to be scavenged by animals on range. Higher CP contents recorded in the pods could have been responsible for this.

The range of CP in this study would provide adequate nitrogen requirement by rumen microorganisms to maximally digest the main components of dietary fibre leading to the production of volatile fatty acid (Lamidi and Ogunkunle, 2016).

Values of ash obtained for plants in this study were higher than what was reported by Arigbede *et al*. (2012) (59 – 107 g kg^{-1}) for some browse trees. The ether extract content in *M. oleifera* parts in this study was lower than the value (19.44 %) reported by Aderinboye *et al*. (2011) for pods of *M. oleifera*.

Highest value (1.99 mg kg^{-1}) of tannin was recorded for *E. cyclocarpum* pods compared to other plants. Tannin concentration in a plant have been implicated for availability of CP in a diet to ruminants feeding on them (Onwuka, 1992). Higher anti nutritional factors (ANFs) have been implicated as antimicrobial, low gas production and hence low methane production (Goel *et al*., 2008). Higher tannin contents have been reported to lead to lower nutritive value in plants (Gebeyew *et al*., 2015).

Table 1. Proximate composition of some forage plant pods

Forage pods	DM	CP	EE	Ash	OM	NFC	Tannin
	← g kg^{-1} DM →						mg kg^{-1}
Canavalia ensiformis	843.34^{b}	170.03^{c}	93.34^{b}	103.55^{b}	89.43^{b}	633.09^{a}	0.91^{d}
Tephrosia bracteolata	886.67^{a}	196.27^{a}	123.34^{a}	70.21^{c}	92.77^{a}	610.19^{b}	1.37^{b}
Enterolobium cyclocarpum	880.00^{a}	147.45^{d}	103.34^{b}	136.87^{a}	86.12^{c}	612.34^{ab}	1.99^{a}
Moringa oleifera	853.34^{b}	175.13^{b}	123.34^{a}	140.22^{a}	85.78^{c}	561.31^{c}	1.25^{c}
SEM	3.33	0.18	5.00	2.66	0.43	5.98	0.003
P-value	0.0002	<0.0001	0.0083	<0.0001	<0.0001	0.0004	<0.0001

$^{a, b, c, d:}$ *Means within the same column with different superscripts are significantly different (p<0.05)*
SEM: Standard Error of Means; DM: Dry matter content; CP: Crude protein; EE: Ether extract; OM: Organic matter content; NFC: Non fibre carbohydrate

The values obtained for neutral detergent fibre (NDF) in this study ranged significantly (P<0.05) from 410.00 g kg^{-1} DM in *C. ensiformis* to 740.00 g kg^{-1} DM in *E. cyclocarpum* while acid detergent lignin (ADL) ranged from 24.81 g kg^{-1} DM to 34.52 g kg^{-1} DM in *E. cyclocarpum* and *C. ensiformis* respectively (Table 2). Singh and Oosting (1992) reported that plants with NDF content of 45 – 65 % and below 45 % are generally considered as medium and high quality feeds respectively. The NDF values of plant pods recorded in this study were below the 65 g kg^{-1}

DM (except in *E. cyclocarpum*) suggested as the limit above which intake of tropical feeds by ruminants would be limited (Eastridge, 2006). Ruminants will require adequate fibre in their diets so as to aid rumen microorganisms to perform optimally. This is necessary to lead to the production of volatile fatty acid (Trevaskis *et al*., 2001; Lamidi and Ogunkunle, 2016) which in turn facilitates microbial protein synthesis (Lamidi and Aina, 2013). The NDF values observed were considered adequate for efficient rumen function.

The highest NDF value in *E. cyclocarpum* above 65 g kg^{-1} DM could be due to increased secondary thickening in cells associated with plant support and water transport (Buxton, 1989).

The chemical composition in the pods of plants under this study revealed that they have potentials to fill in the gap in ruminant nutrition especially crude protein content, which is limited in many tropical grasses most especially during dry season. Basal diets available for livestock feeding during the dry season mainly comprises of matured and senescent grasses, and crop residues which are both low in protein, energy and minerals. This problem of inadequate nutrients of ruminants can be solved by supplementing the basal diets with plants pods that will help boost the nutrient density of feeds during dry season most importantly.

Table 2. Fibre composition of some forage plant pods

Forage pods	NDF	ADF	ADL	Hemicellulose	Cellulose
	← g kg^{-1} DM →				
Canavalia ensiformis	410.00[b]	158.82	34.52[a]	251.18[ab]	124.30
Tephrosia bracteolata	450.00[b]	280.30	27.08[ab]	169.70[b]	253.23
Enterolobium cyclocarpum	740.00[a]	336.60	24.81[b]	403.40[a]	311.79
Moringa oleifera	510.00[b]	247.47	32.57[a]	262.53[ab]	214.90
SEM	47.63	48.85	1.67	54.21	48.47
P-value	0.0061	0.3541	0.0480	0.0152	0.3199

[a, b:] *Means within the same column with different superscripts are significantly different (p<0.05)*
SEM: Standard Error of Means; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin

Table 3 showed a significant (P<0.05) difference in all the calculated parameters of the plant pods investigated in this study except in dry matter digestibility (DMD) (P>0.05). *Canavalia ensiformis* significantly (P<0.05) had highest value of 3.03 % for the dry matter intake (DMI) while least value of 1.63 % was in *E. cyclocarpum* pods. *Enterolobium cyclocarpum* pod significantly (P<0.05) had lowest value of 80.98 for relative feed value (RFV) in the plant pods while highest value of 180.61 was in *C. ensiformis*. The range of DMI values (1.63 – 3.03 %) obtained for pods in this present study was similar to the value of 2.80 % required by growing kids (NRC, 1981). The higher CP contents of the pods in this study could have led to higher DMI results. Higher intake of CP encourages higher proliferation of microbes in the rumen. The higher growth of the microbes enhances efficient degradation, high passage rate and thus more feed intake (Akinlade *et al*., 2007).

The RFV of plant pods in this study compare relatively well with values of 151 – 125 DM

required for grass and legume hays to be considered as having a quality status of first grade (Kazemi *et al*., 2012). However, *E. cyclocarpum* had values slightly below the starting point according to the RFV scale. In RFV scale, RFV below 100 is considered lower than basic starting point, which is RFV 100.

Higher RFV values indicate higher forage quality. If a forage has an RFV value greater than 100, it should result in greater intake of digestible dry matter by cattle (Ralph Ward, 2008). The RFV is a relative measurement to help farmers compare feeds. High-quality alfalfa and corn typically have RFV values greater than 130 and may reach over 160. High producing dairy cows require a feed with RFV above 130.

Isah *et al*. (2012) reported increase in predicted dry matter intake (PDMI) value, as NDF for browses investigated declines. Similar result was observed in this study. The implication of this is that, as the percentage of NDF increases in forage, animals will consume less (Schroeder, 1994). This study revealed that the various plant pods investigated had competitive nutrient levels. *Canavalia ensiformis* with the highest values for DMI, DMD and RFV, showed an inherent potential that can improve the performance characteristics of ruminants.

All the plant pods investigated in this study may be regarded as having high relative nutritive value for ruminants in Nigeria based on the results.

Table 3. Estimated parameters of some forage plant pods

Forage pods	DMD (%)	DMI (%)	RFV (%)	CHO (g kg^{-1} DM)
Canavalia ensiformis	76.53	3.03[a]	180.61[a]	63.09[a]
Tephrosia bracteolata	67.06	2.78[ab]	143.02[ab]	60.81[a]
Enterolobium cyclocarpum	62.68	1.63[b]	80.98[b]	61.04[a]
Moringa oleifera	69.62	2.37[ab]	128.05[ab]	55.93[b]
SEM	3.81	0.260	17.59	0.68
P-value	0.3542	0.445	0.0336	0.0009

[a, b, c:] Means within the same column with different superscripts are significantly different ($p<0.05$)

SEM: Standard Error of Means; DMI: Dry matter intake (% of body weight); DMD: Dry matter digestibility; RFV: Relative feed value; CHO: Carbohydrate content

Sommart *et al*. (2000) reported that gas volume is a good parameter to predict digestibility, volatile fatty acids production and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. The sequential *in vitro* gas production of the pods increased significantly ($P<0.05$) from the 3 hours of incubation throughout the incubation period (Table 4). Menke *et al*. (1979) reported that gas volume at 24 hours after incubation had a direct relationship with metabolizable energy of the feed stuffs, feed intake (Blummel and Berker, 1997) and growth rate of calves (Blummel and Orskov, 1993). Gas production tests are used routinely in feed research as gas volumes are related to both extent and rate of substrate degradation (Blummel and Berker, 1997). Mebrahtu and Tenaye (1997) reported that the amount of gas released when feeds are incubated *in vitro* were closely related to digestibility of feed for ruminants. Higher gas production value of *Moringa oleifera* could be as a result of its high CP content which have supplied essential nutrients especially nitrogen for microbial

utilization to enhance higher digestibility of the pods (Reed *et al*., 1990).

Higher *in vitro* gas production denote that there could be parts that contained high amounts of soluble fermentable nutrients apart from high CP as judged by their relatively high gas production (Babayemi *et al*., 2009). Variations in gas production of plant pods as observed in this study may be attributed to compositional differences of the chemical constituents in the plant parts especially fibre fractions and ANFs, which also contribute to the dynamics of substrate degradation.

Table 4. *In vitro* gas production (ml/200 mg DM) of some forage plant pods

Forage pods	3	9	12	15	18	24
	← Incubation period (Hour) →					
Canavalia ensiformis	1.00	3.00^{c}	5.00^{b}	6.00^{c}	7.00^{b}	8.00^{b}
Tephrosia bracteolata	2.00	6.00^{bc}	7.00^{b}	10.00^{bc}	13.00^{ab}	19.00^{ab}
Enterolobium cyclocarpum	2.00	12.00^{ab}	16.00^{a}	19.00^{ab}	24.00^{a}	28.00^{a}
Moringa oleifera	5.00	15.00^{a}	17.00^{a}	22.00^{a}	27.00^{a}	30.00^{a}
SEM	0.58	1.44	1.88	2.74	3.61	3.90
P-value	0.0654	0.0070	0.0190	0.0364	0.0417	0.0512

$^{a, b, c:}$ *Means within the same column with different superscripts are significantly different (p<0.05)*

SEM: Standard Error of Means

Table 5 showed the acetic, propionic and butyric acids as well as methane production after incubating of pods for 24 hours in *in vitro* gas production. Significant (P<0.05) differences were recorded for all the parameters investigated. *Tephrosia bracteolata* and control had significantly (P<0.05) highest values for acetic, propionic and butyric acids above all other plants pods.

Lower volatile acids proportions in the inoculums with plant pods recorded after *in vitro* gas production compared with inoculums without pods could be due to the elevated content of condensed tannin in them (Getachew and Makkar, 2002). Moreover, presence of tannin in the plant pods could have led to alterations in rumen fermentation. Although *T. bracteolata* had higher tannin content compared with other plants, highest fermentation values could be adduced to more CP contents it possessed above others. According to Mbugua *et al*. (2008), tannins and alkaloids in forages can adversely affect their fermentation characteristics and thereby their nutritive values.

The levels of VFA proportions reported in the current study showed that the level of tannins in the pods were within the range that does not reduce the bioavailability and utilization of nutrients present in them.

The lower methane production in this study could have been due to the presence of tannin in the plant pods, which would have formed complexes with dietary proteins in the rumen, protecting the protein from microbial attack, hence preventing the fermentation in the rumen. Adelusi *et al*. (2015) reported that if fermentation of organic matter in the rumen is reduced, there will be a shifting of the site of digestion from the rumen to

the intestines. This act will divert hydrogen away from methane production during ruminal

fermentation, inhibiting methanogenesis by ruminal bacteria or by optimizing the rumen fermentation and thereby decreasing methane emission per unit of organic matter digested. Influence of tannin in the plant pods could have affected methanogenesis, hence leading to reduced methane production. Goel and Makkar (2012) reported that tannin suppresses methanogenesis by reducing the methanogenic population directly or by reducing the protozoa population.

Table 5: *In vitro r*umen fermentation parameters of some forage plant pods

Forage pods	Acetic Acid	Propionic acid	Butyric acid	Methane
	← (%) →			mM
Control	0.815	0.544	0.015	
Canavalia ensiformis	0.587[c]	0.393[c]	0.011[b]	0.20[b]
Tephrosia bracteolata	0.743[a]	0.497[a]	0.013[a]	0.21[a]
Enterolobium cyclocarpum	0.623[b]	0.417[b]	0.011[b]	0.18[c]
Moringa oleifera	0.563[d]	0.377[d]	0.010[c]	0.21[a]
SEM	0.003	0.003	0.000	0.002
P-value	<0.0001	<0.0001	<0.0001	<0.0001

[a, b, c, d:] *Means within the same column with different superscripts are significantly different (p<0.05)*
SEM: Standard Error of Means

CONCLUSION

The chemical composition, *in vitro* gas production as well as the methane contents of the pods of the different forage plants evaluated revealed that they have potential to serve as supplements to matured and senescent grasses as well as crop residues especially in the dry season. This will help to reduce the cost of feeding animals as well as improve their performance**.**

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