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In vitro Gas Production and its Prediction on Metabolizable Energy, Organic Matter Digestibility and Short Chain Fatty Acids of Some Tropical Seeds

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Abstract: Eight tropical seeds from browse, shrubs and pulses plants were assessed for their nutritive value using *in vitro* gas production technique. Dry Matter (DM), Crude Protein (CP), crude fibre, ash, ether extract and Neutral Detergent Fibre (NDF) were analyzed. Milled seeds were incubated using 200 mg/30 ml inoculum for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h. At post incubation, the total gas volume was measured for methane using 4 ml of 10 M NaOH. Dynamics of gas production characteristics over time were described by equation Vt = Vf x [1 + exp {2-4 x S x (t-L)}] - 1. Metabolizable Energy (ME; MJ/kg DM), Organic Matter Digestibility (OMD; %) and short chain fatty acids (SCFA; µmol/200 mg DM)) were estimated. DM was lowest (88.1%) in *Leucaena leucocephala* and was the best (95.6%) in *Tephrosia bracteolata* seeds. CP ranged from 25-38.9% being the least (25.0%) for *Lablab purpureus* and the highest (38.9%) for *Tehprosia candida*. NDF of the seeds varied from 27.1% in *Tephrosia bracteolata* to 49.1% in *Leucaena leucocephala*. The volume of gas produced by the seeds consistently increased ($p<0.05$) and was significantly ($p<0.05$) highest in pulse legumes. Potential extent (Vt) of gas production ranged from 36.8-53.6 and that of fractional rate of gas production from 0.043-0.07. The ranged values 7.5-10.4, 50.7-70.4 and 0.751-1.185 for ME, OMD and SCFA respectively were significantly ($p<0.05$) highest in *Tephrosia bracteolata* seeds. The CH_4 production varied from 148 µml in *Albizia lebbeck* to 300 µml in *Carnavalia ensiformis*. The result showed that the seeds were high in nutrients, digestible and metabolizable energy with relatively low methane production and therefore could be used for ruminants as feedstuffs.

Key words: Shrubs, pulses, browse plants, gas production

INTRODUCTION

In the traditional animal husbandry, ruminants in the tropics, especially in Nigeria, are mainly fed with grasses. Improved livestock production is likely unattainable and unsustainable by grass forage alone. In Nigeria, ruminants slowly gain weight in the rainy season and rapidly loose it in the dry season (Babayemi and Bamikole, 2006a). The grasses, roughages and by-products, which constitute above 75% ration for ruminants are seasonal and of low nutritive value. The leaf of browse trees show potentials to augment nutrients, being high in protein, vitamins and minerals but also contain antinutrients (Babayemi, 2006). Grass and browse leaf alone may not earn the livestock farmers the expected rapid growth of the animals. Forage seeds, being high in crude protein are suitable as protein concentrate (Babayemi *et al*., 2004a,b) and are nutritional promising for optimum performance in grazing goats (Babayemi and Bamikole, 2006a). Leaf meal from browse plants is bulky and may have low shelf-life. The conventional seed protein sources such as groundnut and soyabean are prohibitive as they are scarce and expensive.

Unconventional seeds from browse trees in the tropics are abundant and available throughout the year. The seeds are high in protein but may also possess secondary metabolites thereby limiting their utilization by ruminants, suggesting *in vitro* gas production for evaluation. In vitro gas production is quick and less expensive means of determining the nutritive value of feeds for ruminants (Babayemi *et al*., 2004b; Babayemi and Bamikole, 2006b). Total gas production can predict methane (CH_4), Volatile Fatty Acids (VFA) and the individual molar VFA (Fievez *et al*., 2005). In the first objective (Babayemi *et al*., 2004a), the presence of secondary metabolites in eight tropical browse trees was reported. The objective of the present study was to determine the in vitro dietary evaluation of forage seeds of *Albizia saman*, *Albizia lebbeck*, *Albizia rhizonse*, *Tephrosia candida*, *Tephrosia bracteolata*, *Leucaena leucocephala*, *Lablab purpureus* and *Canavalia ensiformis* as unconventional protein sources for ruminants.

MATERIALS AND METHODS

Seed collection: Pods of *Albizia saman* and *Albizia lebbeck* were obtained from the Teaching and Research Farm, University of Ibadan, Ibadan and that of *Albizia rhizonse* from NAGRAB, Ibadan. Those pods from *Tephrosia candida*, *Tephrosia bracteolata*, *Leucaena leucocephala*, *Lablab purpureus* and *Canavalia ensiformis* were obtained from International

Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The pods were sun-dried for six days on different platforms made of concrete. Seeds were evacuated from the pod by bitten the pod with stick-club and then picked the seeds manually. Dry matter of the seeds was determined by oven drying at 105°C until a constant weight was ensured.

***In vitro* gas production:** Ammonia exhausted rumen fluid was collected from two cannulated sheep before morning feed into a thermoflask. In order to minimize the amount of ammonia in the fluid, concentrate diet was withdrawn from the sheep in the previous day. The procedure for the *in vitro* gas production was as established by Menke and Steingass (1988). The rumen liquor collected was properly mixed and filtered through a metallic sieve (1 mm sieve). A stream of carbon dioxide (CO_2) was made to pass through the sieved rumen liquor until the completion of inoculation. The liquor was then diluted four-fold with buffer without ammonium chloride (NH_4Cl). Incubation was conducted using two replicate per treatment per batch in three consecutive batches. 100 ml capacity graduated plastic syringe was used and the piston of the syringe was lubricated with vaseline. The piston was withrawn and later inserted after the introduction of the substrate. The inoculum of 30 ml was introduced through the silicon tube fitted into the tip of the syringe containing 200 mg substrate, i.e., the test samples. The content was agitated while the piston of the syringe was pushed up to eliminate the air bubbles and after which the silicon tube was tightened with metal clip, leaving about 2 cm length of the silicon tube above the clip. The prepared syringes were placed in the incubator maintained at ± 39 °C. Gas volume was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h. The content was repeatedly agitated at each time of reading.

Methane measurement: After 24 h incubation, 4 ml of 10 molar NaOH was introduced through the silicon tube, for the measurement of methane. The tip of 5 ml syringe capacity that contained the 4 ml NaOH was introduced into incubated syringe through the silicon tube just above the metal clip. The clip was carefully unscrewed and then introduced the reagent. A pop sound was heard given rise to the jacking upward of the piston of syringe, which suggested the absorption of CO_2 by the sodium hydroxide. The syringe was then turned upside down for the reading of CH_4 level.

Chemical analysis: Crude protein, crude fibre, ash and ether extract were determined following procedure of AOAC (1990). Neutral detergent fibre and acid detergent fibre were analyzed according to Van Soest *et al.* (1991).

Mathematical and statistical analysis: Dynamics of gas production characteristics over time were described by a modified Ørskov and McDonald (1979) equation Vt = Vf x [1 + exp {2-4 x S x (t-L)}] -1, where Vt = cumulative gas production (ml), Vf = maximum gas production (ml), S = rate of gas production (h^{-1}) and L = lag time (h). Graph was plotted to show the trend of the incubation. Using the procedure of Menke and Steingass (1988), Metabolizable Energy (ME in MJ = 2.20 + 0.136GV + 0.057 CP + 0.0029 CF), organic matter digestibility (OMD in % = 14.88 + 0.889 GV + 0.45 CP + 0.651 X) and short chain fatty acids (SCFA = 0.0239 GV-0.0601 ml/200 mg DM) were estimated, where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively. CH_4 was calculated in µ using Charles's law as reported (Fievez *et al.*, 2005). Data generated were subjected to analysis of variance using SAS (1988) procedure. Significant means were separated using Duncan multiple range F-test.

RESULTS AND DISCUSSION

Due to the exhorbitant prices of the protein source ingredients for livestock feedstuffs in the developing nations of the world, there is urgent needs to explore the less human demanding sources. Tropical pods are acceptable by livestock and were reported to be scavenged by animals on range (Janzen, 1981). The chemical composition of the seeds of tropical browse plants is in Table 1. Seeds were quite high in all the nutrient contents. Dry matter was lowest (88.1%) in *Leucaena leucocephala* and had the best (95.6%) in *Tephrosia bracteolata* seeds. Organic matter content ranged from 93.8% in *Tephrosia bracteolata* to 97.6% in *Canavalia ensiformis*. Crude protein ranged from 25-38.9% having the least (25.0%) from *Lablab purpureus* and the highest (38.9%) for *Tehprosia candida*. Fat content was most for *Tephrosia bracteolata* and smallest for *Lablab purpureus*. Neutral detergent fibre of the seeds varied from 27.1% in *Tephrosia bracteolata* to 49.1% in *Leucaena leucocephala*. The seeds have potential to supply the deficient crude protein, which is

Table 1: Chemical compositions (g/100 g DM) of some forage seeds as unconventional protein sources for ruminants in the tropics (n = 9)

	Composition[1]				
Forage seed	DM	OM	CP	EE	NDF
Browse trees					
Albizia saman	92.2	96.4	29.0	9.9	30.6
Albizia lebbeck	89.1	94.8	33.1	9.6	29.1
Albizia rhizonse	90.1	94.6	33.7	6.0	32.5
Browse shrubs					
Leucaena leucocephala	88.1	95.1	27.8	7.9	49.1
Tephrosia candida	92.8	94.3	38.9	14.8	31.0
Tephrosia bracteolata	95.6	93.8	38.4	16.8	27.1
Pulse legumes					
Lablab purpureus	89.8	95.9	25.0	3.1	51.4
Canavalia ensiformis	89.4	97.6	25.7	3.9	44.2

[1]DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre

Table 2: *In vitro* gas production (ml/200 mg DM) of some tropical browse seeds incubated for twenty-four hours (n = 9)

	Incubation period (Hour)						
Forage seed	2	4	6	8	10	12	24
Browse trees							
Albizia saman	2.6^{e}	7.0^{c}	11.6^{d}	14.3^{e}	19.6^{d}	23.6^{d}	39.0^{e}
Albizia lebbeck	3.6^{ed}	7.3^{c}	12.0^{cd}	14.0^{e}	17.0^{e}	20.3^{e}	33.3^{f}
Albizia rhizonse	5.3^{b}	10.6^{b}	13.0^{cd}	15.0^{e}	19.6^{d}	23.0^{d}	41.3^{d}
Browse shrubs							
Tephrosia candida	4.0^{cd}	7.6^{c}	13.6^{cd}	17.6^{c}	21.0^{cd}	27.6^{c}	45.3^{c}
Tephrosia bracteolata	5.6^{b}	13.3^{a}	19.6^{a}	25.0^{a}	31.3^{a}	38.3^{a}	55.3^{a}
Leucaena leucocephala	2.6^{e}	8.0^{c}	13.0^{cd}	15.3^{de}	20.0^{d}	23.6^{d}	43.0^{d}
Pulse legumes							
Lablab purpureus	7.6^{a}	10.3^{b}	16.6^{b}	20.0^{b}	24.6^{b}	31.3^{b}	51.0^{b}
Canavalia ensiformis	5.0^{bc}	10.0^{b}	14.0^{c}	17.0^{cd}	22.6^{c}	30.3^{b}	45.3^{c}
SEM	0.204	0.304	0.326	0.300	0.346	0.360	0.402

a,b,c,d,e,f = Means in the same column with different superscripts are significantly different ($p < 0.05$). SEM = standard error of mean

Table 3: *In vitro* gas production characteristics of some tropical browse seeds at 24 h post incubation (n = 9)

	Gas production parameters			
Treatment	V_f	S	L	R^2
Browse trees				
Albizia saman	40.4 ± 0.7^{c}	0.063 ± 0.002^{b}	3.2 ± 0.09^{ab}	0.979
Albizia lebeck	36.8 ± 2.4^{d}	0.048 ± 0.006^{c}	1.6 ± 0.06^{c}	0.974
Albizia rhizonse	40.3 ± 2.7^{c}	0.043 ± 0.002^{d}	0.41 ± 0.002^{e}	0.893
Browse shrubs				
Leucaena leucocephala	46.2 ± 1.6^{b}	0.053 ± 0.001^{bc}	3.6 ± 0.14^{a}	0.913
Tephrosia candida	44.9 ± 2.8^{b}	0.058 ± 0.004^{b}	2.8 ±0.01^{b}	0.960
Tephrosia bracteolata	53.6 ± 3.1^{a}	0.059 ± 0.003^{b}	1.16 ± 0.01^{d}	0.910
Pulse legumes				
Lablab purpureus	40.5 ± 1.9^{c}	0.070 ± 0.001^{a}	3.3 ± 0.17^{ab}	0.982
Canavalia ensiformis	42.9 ± 2.0^{c}	0.065 ± 0,002^{b}	3.6 ± 0.11^{a}	0.926

a,b,c,d,e,f = Means in the same column with different superscripts are significantly different ($p < 0.05$).

limiting in many tropical grasses (Babayemi and Bamikole, 2006a,b) and crop residues (Babayemi *et al*., 2008). Albizia species are in abundance in the growing areas of the tropics and more importantly in the rain forest zones. Jolaosho *et al*. (2006) reported that cattle relish the pods and above average of the embedded seeds are hydrolyzable.

Presented in Table 2 is the *in vitro* gas production of the seeds. In all the seeds, the volume of gas produced consistently increased ($p<0.05$) from the beginning at 2h of the incubation to the end at 24 h. Gas production in pulse legumes seemed to be pronounced while the least amount was obtained from the seeds of browse trees. Figure 2 clearly presents trend for the *in vitro* gas production characteristics of the tropical browse seeds. The cumulative volume of the gas production by the seeds increased with increasing hour of the *in vitro* incubation. The gas produced significantly ($p<0.05$) differed at all stages of incubation. The unprecedented gas production from the seeds was expected. Fodder trees and shrubs contain protein, minerals and vitamins essential for growth of rumen micro organisms that degrade feedstuff prior to gastric and intestinal digestion by the host animal (Reed *et al*., 1990). The relatively high gas production observed in the present study for all the seeds incubated was expected since the seeds were

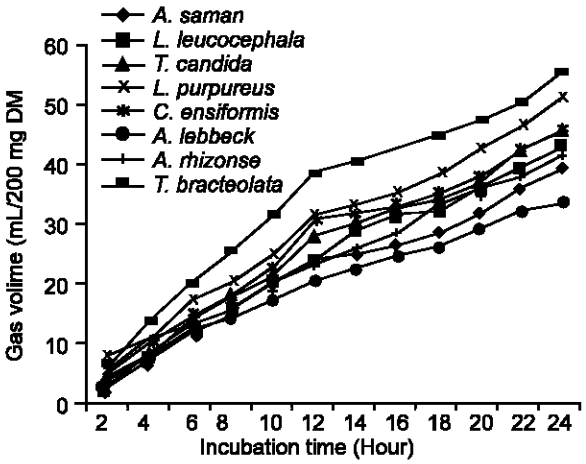

Fig. 1: Rumen *in-vitro* gas production of tropical forage seeds

high in crude protein. Hillman *et al*. (1993) reported that gas production is positively related to microbial protein synthesis. Although gas production is a nutritional wasteful product (Mauricco *et al*., 1990) but it provides a useful basis from which metabolizable energy, organic matter digestibility and short chain fatty acids may be predicted. Table 3 is the gas production characteristics

Table 4: Metabolizable energy (ME; MJ/kg DM), organic matter digestibility (OMD; %), short chain fatty acids (SCFA; µmol/200 mg DM) of some tropical browse seeds at 24 h post incubation (n = 9)

	In vitro parameters		
Forage seeds	ME	OMD	SCFA
Browse trees			
Albizia saman	8.1^{bc}	54.7^{f}	0.88^{cd}
Albizia lebeck	7.5^{c}	50.7^{g}	0.751^{cd}
Albizia rhizonse	7.9^{bc}	58.3^{de}	0.877^{cd}
Browse shrubs			
Leucaena leucocephala	8.5^{b}	57.2^{ef}	0.951^{bc}
Tephrosia candida	8.7^{b}	60.6^{cd}	1.008^{bc}
Tephrosia bracteolata	10.4^{a}	70.4^{a}	1.185^{a}
Pulse legumes			
Lablab purpureus	8.6^{b}	65.8^{b}	1.035^{b}
Canavalia ensiformis	8.7^{b}	61.6^{c}	0.988^{bc}
Standard error of mean (SEM)	0.178	0.475	0.027

a,b,c,d,e,f = Means in the same column with different superscripts are significantly different ($p < 0.05$).

of the incubated seeds. There were significant differences among the seeds incubated. The high potential extent of gas production (range 36.8 -53.6) and fractional rate of gas production (range 0.043-0.07) may be attributed to the high content of neutral detergent soluble fibre fraction in the pulse legume seeds (Hall, 2000) and followed by those of the browse tree seeds. Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA) of the seeds are shown in Table 4. The values ranged between 7.5 in *Albiza lebbeck* and 10.4 in *Tephrosia bracteolata* for ME. Similar trend was observed for OMD (range 50.7 – 70.4) and SCFA (0.751-1.185). In all the parameters, *Tephrosia bracteolata* was significantly ($p<0.05$) higher than those of other seeds. The ME, OMD and SCFA in the present study were higher than those reported for spent tea leaf (Babayemi *et al*., 2006b) and for Guinea grass/*Tephrosia candida* foliage mixtures (Babayemi *et al*., 2006c). Being seeds, they are high in crude protein and fibre as energy sources and the results are comparable to the values for ME, OMD and SCFA of the pod and seeds of *Enterolobium cyclocarpum* (Babayemi, 2006). The high fractional rate of gas production and those of ME, OMD and SCFA generally observed among the seeds could translate to higher dry matter intake in ruminants for an improved performance. Figure 2 indicates the level of methane production during incubation of various multipurpose tree seeds. Methane is a dietary energy loss and is an important greenhouse gas contributing to global warming (Johnson and Johnson, 1995) by trapping outgoing terrestrial impaired radiation 20 tons more effectively than CO_2. The IPCC (2001) reported domestic livestock as one of the largest single sources of methane with 80 to 115 million tones per year equivalent to 0.15-0.20 of total anthropogenic methane. The amount of CH_4 in the present study varied from 148 µmL in *Albizia lebbeck* to 300 µmL in *Carnavalia ensiformis*. These wide variations of CH_4 among the seeds might be due to the

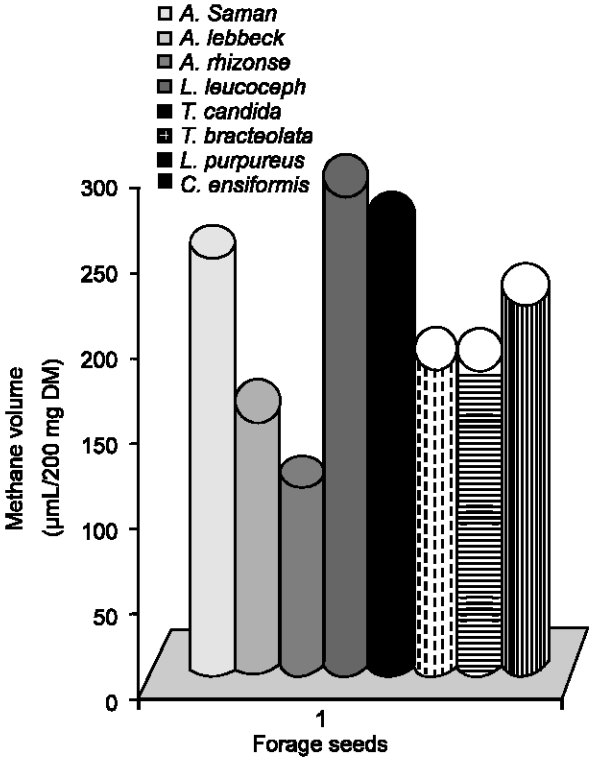

Fig. 2: *In vitro* methane production of tropical forage seeds

differing presence of the inherent secondary metabolites. Babayemi *et al*. (2004a,b) found that seeds of *Albizia lebbeck* and *Albizia rhizonse* had the potential to slightly depress methanogenesis due to the presence of saponins in these seeds, which is known to suppress protozoa, the main butyrate producers in the rumen. The effect of tannin in suppression of methane production has also been reported by Hess *et al*. (2004) who observed *in vitro* that the inclusion of the tropical legume *Calliandra calothyrsus* (270 g of condensed tannin/kg DM) in grass-based diet suppressed methane production relative to DM degraded by over 30 % and that this was probably due to tannin in the legume. Ulyatt (1966), however observed that intake (amount of feed consumed) by animal may be controlled to prevent methane rising rather than reducing animal numbers and at the same time increase productivity.

Estimation of methane during incubation is essential because of the role it plays in global warming and for proper feed formulation. Hess *et al*. (2003) observed that supplementing legumes to N-limited diet might enhance total ruminal methane synthesis and therefore suggested dietary supplementation of saponin-rich fruits of the tropical multipurpose tree *Sapindus saponaris* which were known to reduce methane emission from ruminal fermentation *in vitro* by proportionately 0.1-0.2 without negatively affecting organic matter degradation.

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