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Antinutrtional Factors, Nutritive Value and *in vitro* Gas Production of Foliage and Fruit of *Enterolobium cyclocarpum*

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Abstract: *Enterolobium cyclocarpum* foliage is yet to be accepted by ruminants in Nigeria as recommended for intensive garden use. In a comparative study, the nutritive value of the leaf, seed, pod and fruit was investigated by proximate, secondary metabolites and *in vitro* gas production methods. Proximate composition showed that all the samples were quite high in crude protein (10.4-21.7%), ether extract (10.0-11.2%) and low in crude fibre (39.8-63.5%). All sample contained saponin but a medium in the leaf and negligible in others. Phenol was not detected but all had steroids. The browse parts had sufficient composition of phosphorus, calcium, magnesium, potassium, iron and copper to meet the requirements for ruminants. The constituent of manganese, zinc and sodium were less than recommended levels required for growth, reproduction and milk. The net gas volume (ml), metabolisable energy (MJ/kg DM), organic matter digestibility (%) and short chain fatty acids (μmol) ranged from 31.35-73.0, 7.66-13.55, 51.42-89.82 and 0.69-1.68, respectively, being highest in the seed and lowest in the leaf. The studies showed that the seeds, pod and whole fruit had nutritive value and therefore, may serve as potential supplements for ruminants in Nigeria.

Key words: *Enterolobium cyclocarpum* · secondary metabolites · gas production · metabolisable energy · organic matter digestibility

INTRODUCTION

The prohibitive cost of concentrate diets for ruminants in the tropics during the dry season necessitates continuous search for less expensive and high nutritive feedstuffs. Browse plants are available in the off season but most of them are less beneficial to livestock as they contain antinutritional factors. For example, full utilization of *Leucaena leucocephala* and *Gliricidia sepium* are always hampered by the presence of mimosine and coumarin respectively in them (Babayemi *et al*., 2006).

Enterolobium cyclocarpum is a native Neotropical mimosaceous legume tree, found mainly in the deciduous lowlands forests (Janzen, 1981). The legume is easily established and fast growing to maturity over a short period of time than the most common legume plants in Nigeria. In a preliminary study, Ezenwa (1998) recommended the legume to be suitable for use in intensive feed garden in Southwestern Nigeria, being fast and luxurious in growth. In the southwest of Nigeria, *Enterolobium cyclocarpum* foliage has not been accepted by sheep, goats and cattle (Unpublished) possibly due to the presence of antinutrional factors. Higher percentage of the tropical browse and shrub legumes are seed and fruit bearing and had been reported to be high in crude protein and other nutrients (Babayemi *et al*., 2004a). In another study, the seeds showed beneficial effects when degraded by the rumen microbial organisms for the production of volatile fatty acids (Babayemi *et al*., 2004b), showing the potential of browse tree seeds in livestock production.

E. cyclocarpum has large, indehiscent, dry and sweet fruits that drop to the ground when ripe. The mesocarp is soft while the seed is relatively hard. According to Janzen (1981), its fruits are avidly eaten by free-ranging horses, suggesting its utilisation for livestock. Spot test of determining secondary metabolites (Babayemi *et al*., 2004a) and *in vitro* fermentation technique are rapid and cost effective methods of assessing the nutritive value of feedstuffs. The present study was undertaken to determine the toxic factors, nutrient composition and the *in vitro* digestibility of the leaf, seed and whole fruit of *Enterolobium cyclocarpum*.

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MATERIALS AND METHODS

Foliage and fruit collection: In January 2006, the foliage and fruits were collected from Enterolobium cyclocarpum plot that was established in 1995, from the Teaching and Research Farm, University of Ibadan, Ibadan. The location is 7° 27'N and 3° 45'E at altitude 200-300 m above sea level; mean temperature of 25-29°C and the average annual rainfall of about 1250 mm. The leaves were obtained after felling the tree while fruits were hand picked from the ground. Dry matter of the leaves and the fruits were immediately determined at 105°C.

Proximate composition: Crude protein, crude fibre, ether extract and ash contents of the leaf, seeds, pod and the whole fruits were determined according to AOAC (1990). Crude Protein (CP) analysis was by the process of Kjeldahl. It was effected through the breaking down of 2 g sample (n = 2) in 25 ml concentrated tetraoxo sulphate VI acids plus selenium, using Gerhardt Kjeldahtherm (Gerdart GmbH + Co. kg Fabrik fur Laborgerate Postfach 1628 D53006 Bonn) until an opaque colour was obtained. The digested sample was rested for 12 h, diluted with distilled water and make up to the mark in 250 ml volumetric flask. Five mililiter of the digest was pipette and distilled with 40% sodium hydroxide and the ionised ammonium was trapped by boric acid. The distillate was immediately titrated (n = 3) with 0.01 N hydrogen chloride. In order to obtain the percentage CP, the amount of nitrogen was multiplied by a factor 6.25. Calcium, phosphorus, magnesium, manganese, iron, copper and zinc were digested in 1:4 perchloric acid and concentrated *trioxo nitrate* V. The minerals in the digested sample were determined using atomic absorption spectrophotometer (GBC 908AA, GBC Australia) but sodium and potassium in flame photometer (Corning Flame Photometer 410).

Qualitative determination of saponin, phenols and steroids: Saponin, phenols and steroids were determined as reported by Babayemi and Bamikole (2006). Briefly, 2 g each of the leaf, seeds, pod and whole fruit of *Enterolobium cyclocarpum* were extracted with 30 ml of Petroleum Ether (PE) and 25 ml of methanol water (MW, 9/1, v/v). The mixture was shaken at 250 revolutions per minute for 1.5 h, filtered and separated by a funnel. The lower (MW) and upper layers were emptied into 50 ml volumetric flasks. From the MW fraction, 1.67 ml was dispensed in 9 ml distilled water, filtered and out of it; 1 ml was taken into a test tube. The test tube was shaken for 30 sec and left to stand for 15 min. Saponin content was evaluated from the height of the foam layer as negative (< 5 mm), low (5-9 mm), medium (10-14 mm) and high (>15 mm). For phenol analysis, 1 ml from the MW fraction was dispensed into five bottles with 1% iron III chloride (w/v) added at different levels (0.2, 0.4, 0.6, 0.8 and 1 ml, respectively). Phenols form complexes with ferric iron, resulting in a blue solution and hence, their presence was scored as: no phenols (no colour change), hydrolysable (dark-blue) and condensed tannins (dark-green). For steroids, 10 ml from PE fraction was evaporated in a water bath at 45ºC and 0.5 ml chloroform; 0.25 ml acetic anhydride and 0.125 ml concentrated tetraoxo sulphate IV were added. The mixture was agitated briefly and the colour reaction was accessed being steroids (blue or green), triterpenoids (read, pink or purple) or saturated steroids (light yellow).

***In vitro* gas production:** Rumen fluid was obtained from 3 WAD female goats through suction tube before morning feed, normally fed with concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was as reported (Fievez *et al*., 2005) using 120 ml calibrated syringes in three batch incubation at 39ºC. Into 200 mg sample (n = 3) in the syringe was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$ + Na_2HPO_4 + KCl + NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$) (1:4, v/v) under continuous flushing with CO_2. The gas production was measured at 6, 12, 18, 24 and 30 h and after 30 h post incubation, 4 ml of sodium hydroxide (10 M) was introduced to estimate methane production. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Statistical analysis: Metabolisable Energy (ME) was calculated as ME = 2.20 + 0.136GV + 0.057 CP + 0.0029 CF (Menke and Steingass, 1988). Organic matter digestibility (OMD%) was assesses as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988). Short Chain Fatty Acids (SCFA) as 0.0239 GV-0.0601 (Getachew *et al*., 1999) was also obtained, where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively. Data obtained were subjected to analysis of variance. Where significant differences occurred, the means were separated using Duncan multiple range F-test of the SAS (Statistical Analysis System Institute Inc., 1988) options.

RESULTS AND DISCUSSION

In Table 1 is the proximate composition of the leaf, seed, pod and whole fruit of *E. cyclocarpum*. The leaf was lowest (39.08%) and highest (87.34%) in seed for DM matter content. All the parts are relatively high in protein, ranging from 10.04 in pod to 21.7% in the seed. The materials were also high in crude fibre (range 39.8-63.5%). Ether extract was almost the same for all the parts of the legume ranging between 10.0% in the seed and 11.2% in the pod. The samples were low in ash content. The advantage of the fruit over the leaf of the Enterolobium is obvious, as the fruit was high in DM, CP and CF. The foliage of the legume is yet to be relished by livestock in Nigeria, but the fruits are seen to be scavenged by ranging animals elsewhere (Janzen, 1981).

Table 1: Proximate composition (g/100 g DM) of the leaf, seed, pod and whole fruit of *Enterolobium cyclocarpum*

	Nutrient composition				
Parameters	Dry matter	Crude protein	Crude fibre	Ether extract	Ash
Leaf	39.08	18.6	48.2	11.0	4.9
Seed	87.34	21.7	63.5	10.0	4.2
Pod	83.23	10.4	39.8	11.2	4.6
Whole fruit	82.39	15.7	46.1	10.7	4.9

Table 2: Quality of saponin, phenols and steroids in leaf, seed, pod and whole fruit of *Enterolobium cyclocarpum*

	Saponin			Steroids	
E. cyclocarpum	Foam height (mm)	Comment	Phenols	Colour	Comment
Leaf	6.0	Medium	None	Green	steroids
Seed	1.0	Negligible	None	Deep green	steroids
Pod	2.5	Negligible	None	Light green	steroids
Whole fruit	1.5	Negligible	None	Light green	steroids

Table 3: Some major (g/100 g DM) and trace (ppm) mineral compositions of the leaf, seeds, pod and whole fruit of *Enterolobium cyclocarpum*

	Enterolobium parts			
Minerals	Leaf	Seed	Pod	Whole fruit
Major minerals				
Calcium	0.95^{a}±0.08	0.68^{b}±0.110	0.89^{a}±0.09	0.71^{b}±0.190
Phosphorus	0.41^{b}±0.06	0.28^{c}±0.070	0.54^{a}±0.15	0.39^{b}±0.060
Magnesium	0.38^{a}±0.03	0.33^{b}±0.010	0.39^{a}±0.03	0.31^{b}±0.050
Sodium	0.13^{a}±0.05	0.05^{b}±0.001	0.09^{a}±0.02	0.06^{b}±0.003
Potassium	1.83^{a}±0.17	1.36^{c}±0.22 0	1.68^{b}±0.14	0.88^{d}±0.120
Trace minerals				
Iron	210.44^{b}±7.18	203.15^{c}±3.110	219.70^{a}±5.13	213.51^{a}±1.660
Copper	19.15^{a}±1.04	10.09^{d}±0.920	14.25^{b}±0.16	12.45^{c}±1.170
Zinc	57.13^{a}±2.11	46.21^{c}±1.140	48.19^{b}±0.35	41.37^{d}±0.860
Manganese	113.17^{a}±3.10	98.43^{b}±0.590	71.13^{d}±2.14	84.66^{c}±1.970

$^{a\text{-}c}$ Means along the same column with different superscripts are significantly different ($p<0.05$)

Table 2 presents the saponin, phenols and steroids determined qualitatively in the samples. All the parts of the Enterolobium contained steroids. Steroid is always present in plants but the quantity may differ, depending on the nature of the plant (Babayemi *et al*., 2004a). The leaf contained saponin, an important anitnutritional factor. Neither hydrolysable nor condensed tannin was present in the leaf, seed and whole fruit.

Figure 1 shows the *in vitro* gas production of the leaf, seed, pod and whole fruit of *Enterolobium cyclocarpum.* In all the parts of the legume, there was a steady increase in the gas production for over a period of 30 h. The gas production was in the order of seed>whole fruit>pod>leaf. The reason for the low gas production in the leaf could be attributed to the high amount of saponin (Table 2). Saponin is known to deter the activities of bacteria in the rumen (Babayemi *et al*., 2004b). A high saponin in the leaf and the low ones in the seed, pod and whole fruits is an important factor as it determines the extent of their fermentation in the rumen. A non availability of tannin in the samples suggests that some of them be valuable protein supplements in ruminant diets (Aganga and Mosase, 2001). This was further manifested in the methane production (Fig. 2) where methane preponderance showed inhibitory features. This is nutritionally meaningful to the ruminants. When dry matter degradation occurs in the rumen by the action of micro organisms, there is production of gas which mainly constitutes hydrogen, carbondioxide and methane.

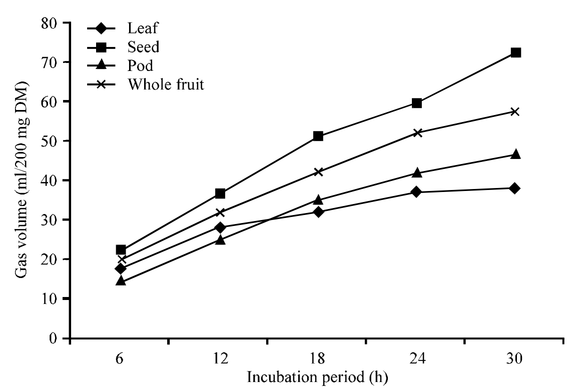

Fig. 1: *In vitro* gas production of the leaf, seed, pod and whole fruit of *Enterolobium cyclocarpum*

Table 4: Net gas volume (NGV = ml/200 mg DM), Metabolizable energy (ME = MJ/kg DM), organic matter digestibility (OMD=%), short chain fatty acids (μ mol) of the different parts of *Enterolobium cyclocarpum*

	Gas production parameters			
Forage parts	NGV	ME	OMD	SCFA
Leaf	31.33[d]	7.66[d]	51.42[d]	0.69[d]
Seed	73.00[a]	13.55[a]	89.82[a]	1.68[a]
Pod	42.00[c]	8.62[c]	57.20[c]	0.94[c]
Whole fruit	52.33[b]	10.35[b]	68.9[b]	1.19[b]
SEM	0.700	0.075	0.622	0.013

[a-d] = Means on the same column with different superscripts are significantly different ($p<0.05$)

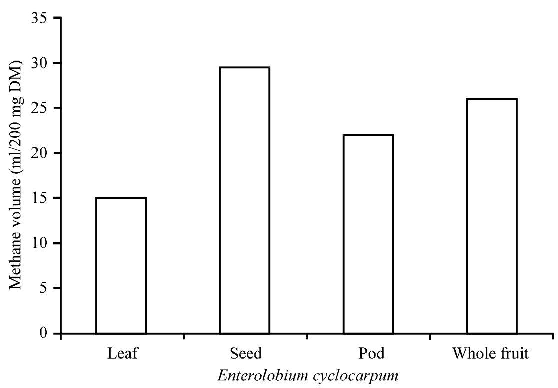

Fig. 2: Methane production of the leaf, seed, pod and whole fruit of *Enterolobium cyclocarpum*

Methane production has negative effects on the animals in one hand as it is an energy loss to the animal and on the other hand, when accumulates in the rumen, it results in bloat. Saponin in some tropical fruits was also observed as an active compound responsible for the suppression of methanogenesis in faunated and defaunated rumen fluid (Hess *et al*., 2003).

Table 3 presents the mineral composition of the Enterolobium parts assessed. The calcium constituent ranged between 0.65% in seed and 0.95% in leaf. Phosphorus content ranged from 0.28% in seed to 0.54% in pod. Magnesium content ranged from 0.31% in whole fruit to 0.39% in pod. Sodium content was generally low and it ranged from 0.05% in seed to 0.13% in leaf. Potassium content ranged from 0.88% in whole fruit to 1.68% in leaf. The content of iron ranged from 203.15 ppm in seed to 219.7 ppm in pod. Copper was also lowest (10.09 ppm) in seed and highest (19.15 ppm) in leaf. Zinc content ranged between 41.37 ppm in whole fruit and 57.13 ppm in leaf. A wider range was observed for manganese as it ranged from 71.13 ppm in pod and 113.17 ppm in leaf. Generally, the major minerals except sodium, were within the range values previously reported (McDowell, 1985). The values are adequate to meet the requirement for growth, reproduction and milk in West African dwarf sheep and goats. The calcium and phosphorus ratio were within the approved 1:1 to 2:1 range recommended (McDowell, 1985). Similar trend was also reported for some tropical browse plants (Aganga and Mosase, 2001). Zinc, manganese, copper and sodium contents in the present study were extremely deficient in the Enterolobium parts. This therefore connotes that the feed may be fortified with the minerals in form of either salt lick or diet inclusions.

Data presented in Table 4 are Metabolisable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA). The ME (MJ) ranged from 7.66 in leaf to 13.55 in seed. Organic matter digestibility (%) ranged between 51.42 in leaf and 89.82 in seed. Short chain fatty acids content followed the same trend being highest (1.68) in seed and lowest in leaf (0.69). There were significant differences in all these parameters among the leaf, seeds, pod and whole fruit. Metabolisable energy was highest ($p<0.05$) in the seed and significantly lowest ($p>0.05$) in the leaf. Similar trends were observed for OMD and SCFA productions. A mutual relationship exists between total gas production and ME, OMD and SCFA (Aganga and Mosase, 2001).

CONCLUSIONS

The different parts of the *Enterolobium cyclocarpum* have nutrient potentials to be utilized by ruminants. The leaf contains more saponin than other parts, leading to its low *in vitro* gas production parameters, suggesting its less acceptability by livestock. The seed and the whole fruit can be used as supplement, being high in net gas production, metabolisable energy, organic matter digestibility and short chain fatty acids.

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