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IN VITRO GAS PRODUCTION AND DRY MATTER DIGESTIBILITY OF SEMI-ARID BROWSES OF NORTH EASTERN NIGERIA

A. A. NJIDDA[1]*, I. IKHIMIOYA[2]

[1]Bayero University, Nigeria; [2]Ambrose Alli University, Nigeria

ABSTRACT

The *in vitro* gas production of semi-arid browse species were evaluated. The relationship between *in vitro* gas measured on incubation of tannin-containing browses in buffered rumen fluid and calculated from short chain fatty acid (SCFA) production was investigated. Crude protein (CP) contents in the browses ranged from 138.50 to 166.50 g.kg^{-1} dry matter (DM). The NDF, ADF and ADL were 373.00 to 512.00, 162.00 to 419.00, and 49.00 to 127.00 g.kg^{-1} DM respectively. Total condensed tannin (TCT) ranged from 0.15 to 0.39 mg/g DM. The TCT significantly correlated with gas production (r = 0.99; P < 0.05). A strong correlation (R^2 = 0.99; P< 0.05) was observed between measured *in vitro* gas production and that calculated from SCFA. The relationship between *in vitro* gas measured on incubation of browse leaves and that calculated from SCFA allows prediction of SCFA from gas production. The studies showed that the leaves of the browse forages had nutritive value and therefore, may serve as potential supplements for ruminants in Nigeria.

Key words: *In vitro*, browse, semi-arid, digestibility, forage

INTRODUCTION

Shrub and tree leaves are an important component of diets for goats, cattle, deer, game, and sheep (Papachristou and Nastis, 1996) and play an important role in the nutrition of grazing animals in areas where few or no alternatives are available (Meuret et al., 1990). The presence of tannins and other phenolic compounds in a large number of nutritionally important shrubs and tree leaves hampers their utilization as animal feed (Tolera et al., 1997). High levels of tannins in leaves restrict the nutrient utilization and decrease voluntary food intake, nutrient digestibility and N retention (Silanikove et al., 2001).

The leaves of the evergreen tree and shrub are used as emergency food by goat and sheep in the semi-arid region of North Eastern Nigeria. However there is little information on their nutritive values. Chemical composition, in combination with in vitro digestibility and ME content can be considered useful indicators for preliminary evaluation of the potential nutritive value of previously uninvestigated shrub and tree leaves (Ammar et al., 2005). Current chemical analytical techniques do not reflect the biological effects of tannin, and therefore, the use of in vitro techniques has been proposed to supplement the chemical analysis (Nsahlai et al., 1994). The gas production technique has proved to be efficient in determining the nutritive value of feeds containing anti-nutritive factors (Siaw et al., 1993). The present study was undertaken to determine the nutritive value of semi-arid browses of North Eastern Nigeria using the in vitro gas production technique.

***Correspondence:** E-mail: ahjidda@yahoo.com
A. A. Njidda, Department of Animal Science, Bayero University,
Kano P.M.B. 3011, Kano State, Nigeria
Tel. +2348035161644 or +2348057017763

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MATERIALS AND METHODS

Forage samples

Eight indigenous browse samples (leaves) commonly consumed by ruminants were used in this study. The species were: *Ficus polita, Ficus thonningii, Batryospermum paradoxum, Kigalia africana, Celtis integuifolis, Khaya senegalensis, Leptadenia lancifolia* and *Ziziphus abyssinica.* All forages were harvested from Gwoza local government area of Borno State in Nigeria. The area is located at 11.05º North and 30.05º East and at an elevation of about 364 above sea level in the North Eastern part of Nigeria. The ambient temperature ranges between 30ºC and 42ºC being the hottest period (March to June) while it's cold between November to February with temperatures ranging between 19 - 25ºC. The browse forages were harvested from at least 10 trees per species randomly selected from four locations within the study area at the end of the season. The harvested samples were then pooled for each individual tree species and then oven dried at 105ºC for 24h to constant weight and ground to pass through a 2.0mm sieve. The samples were then sub-sampled to obtain three samples for each tree species and subsequently used for the laboratory analysis.

Chemical Analysis

Browse species were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash according to AOAC (2005). The leaf samples were analyzed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and cellulose according to Van Soest *et al.* (1991). Total condensed tannin was (Polshettiwar *et al*., 2007).

In vitro gas production

Rumen fluid was obtained from 3 WAD female sheep 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 brewer's grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was done according to Fievez et al. (2005) using 120 ml calibrated syringes in three batches at 39ºC. Into 200 mg sample (n = 3) in the syringe was introduced 30 ml inocula containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$ + 3 Na_2 HPO_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 3, 6, 9, 12, 15, 18, 21 and 24h. After 24 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.

In vitro Dry Matter Digestibility (IVDMD)

After 24h digestion, the samples were transferred into test tubes and centrifuged for 1h in order to obtain the residues which was then filtered using Whatman No. 4 filter paper by gravity and the residues placed in for drying at 65ºC for 24h. The dry residues were weighed and digestibility calculated using the equation as follows:

$$IVDMD(\%) = \frac{\text{Initial DM Input} - \text{DM residue} - \text{Blank}}{\text{Initial DM Input}} * 100$$

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 assessed 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

The result of the detailed composition of the leaves of the browse forages is presented in Table 1.

Table 1: Composition of semi arid browses [NDF, ADF, ADL ($g.kg^{-1}$ DM); TCT (mg/g DM)]

Browse Forage	DM	CP	EE	Ash	NDF	ADF	ADL	TCT	IVDMD
Ficus polita	952.00	162.10^{a}	30.00^{c}	100.00^{d}	373.00^{fg}	272.00^{d}	49.00^{d}	0.20^{b}	63^{c}
Ficus thonningii	952.00	164.70^{a}	20.00^{d}	180.00^{a}	512.00^{a}	412.00^{a}	100.00^{c}	0.24^{b}	63^{c}
B. paradoxum	958.00	146.30^{b}	50.00^{a}	80.00^{e}	476.00^{b}	321.00^{b}	116.00^{b}	0.19^{c}	64^{c}
Kigalia Africana	964.00	138.50^{c}	30.00^{c}	180.00^{a}	384.00^{f}	296.00^{c}	121.00^{a}	0.08^{d}	64^{c}
Celtis integuifolis	962.00	158.90^{a}	30.00^{c}	160.00^{b}	423.00^{d}	312.00^{b}	125.00^{a}	0.39^{a}	67^{b}
Khaya senegalensis	970.00	141.10^{b}	30.00^{c}	100.00^{d}	446.00^{c}	321.00^{b}	113.00^{b}	0.21^{b}	68^{b}
Leptadenia lancifolia	958.00	166.50^{a}	40.00^{b}	180.00^{a}	412.00^{d}	317.00^{b}	127.00^{a}	0.15^{c}	64^{c}
Ziziphus abyssinica	970.00	143.70^{b}	20.00^{d}	140.00^{c}	396.00^{e}	162.00^{e}	59.00^{d}	0.38^{a}	70^{a}
Mean	960.75	152.72	31.25	140.00	427.75	301.62	101.25	0.23	65.38
SEM	0.14^{NS}	0.18	0.12	0.69	0.88	0.82	0.74	0.01	0.42

a, b, c, means in the same column with different superscript differ significantly ($P<0.05$). DM = Dry matter; CP = Crude Protein; EE = Ether Extract; NDF = Neutral detergent fibre; ADF = Acid detergent fibre

Table 2: Net Gas Volume, Metabolizable Energy, Organic Matter Digestibility, Short Chain Fatty Acid of semi-arid browse forages

	Gas production parameter			
Browse Forage	NGV	ME	OMD	SCFA
Ficus polita	3.50[b]	3.72	32.48[c]	0.03[c]
Ficus thonningii	4.50[a]	3.84	37.94[a]	0.05[a]
Batryospermum paradoxum	1.50[d]	3.28	28.44[e]	-0.02e
Kigalia Africana	2.50[c]	3.39	34.83[b]	-0.0003[f]
Celtis integuifolis	4.50[a]	3.78	36.28[a]	0.05[a]
Khaya senegalensis	3.00[b]	3.49	30.83[d]	0.01[d]
Leptadenia lancifolia	4.00[a]	3.77	37.24a	0.04[b]
Ziziphus abyssinica	1.50[d]	3.35	31.62[c]	-0.02[e]
MEAN	3.13	3.58	33.70	0.02
SEM	0.16	0.04NS	0.42	0.002

Net Gas Volume (NGV = ml/ 200 mg DM), Metabolizable Energy (ME=MJ.Kg^{-1} DM), Organic Matter Digestibility (OMD=%), Short Chain Fatty Acids (mmol) of semi-arid browse forages

The crude protein (CP) contents of the browses studied had a similar range as those from West Africa (Rittner and Reed, 1992). All the browses used in the current study had a CP content of above 13% DM. The results of the current study indicate that most tropical browse species are high in CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in tropical regions. These findings are in agreement with those of Rittner and Reed (1992) and Makkar and Becker (1998). The tannin content of the browse were low, ranging between 0.08 to 0.38 mg/g DM. The tannin values in browse could be higher than the values obtained in this study, since a considerable amount of tannins are bound to either fibre and/or proteins and remain unextracted (Jackson et al., 1996). The beneficial effect of forages containing low levels of tannins could be due to the protection of proteins from microbial degradation by tannins, thus increasing the amount of undegraded protein entering the small intestine (Barry et al., 1986).. In addition, a higher flow of microbial protein to the intestine has been observed as a result of higher efficiency of microbial protein synthesis (Getachew et al., 2000). However, higher concentration of tannins in the diet is associated with reduction in organic matter digestibility (Silanikove et al., 1997; Waghorn and Shelton, 1997).

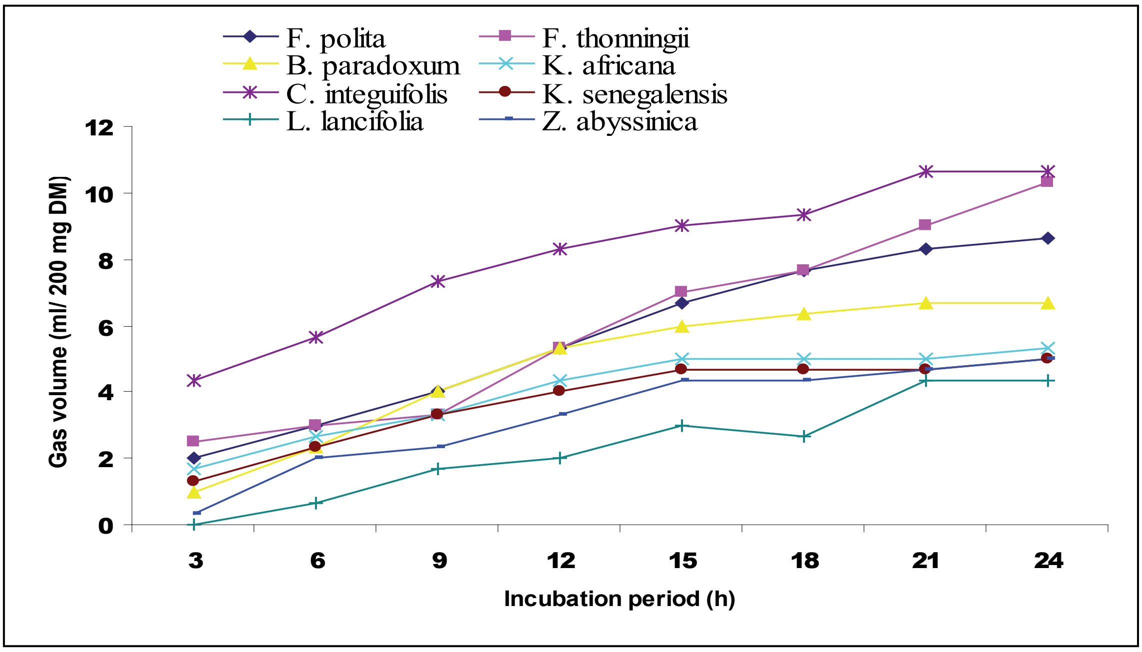

Fig. 1: ***In vitro*** **gas production by semi-arid browse forage**

Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of the browse forages are presented in Table 2. The values for the ME, OMD and SCFA ranged as follows: 3.28 in *B. paradoxum* to 3.84 in *F. thonningii,* 28.44 in *B. paradoxum* to 37.94 in *F .thonningii*, -0.0003 in *K. africana* to 0.05 *F.thonningii,* respectively. There were significant differences (P<0.05) among the forages in ME, OMD and SCFA. The values obtained in the present study were similar to those reported for tropical browses (Getachew *et al.,* 2002) but lower to those reported for forages by Babayemi (2007). Feedstuffs that are inherent in certain anti-nutritive factors had been reported to be low in metabolisable energy and organic matter digestibility (Aregheore and Abdulrazak, 2005).

Figure 1 shows the *in vitro* gas production of the browse forages. There was a steady increase in the gas production for over a period of 24h. Significant differences (P<0.05) occurred among the forages in NGV, OMD, ME and SCFA. The highest and lowest gas productions were obtained from *Ficus thonningii* and *Ziziphus abyssinica*, respectively. There are many factors that may determine the amount of gas to be produced during fermentation, depending on the nature and level of fibre, the presence of secondary metabolites (Babayemi et al., 2004) and potency of the rumen liquor for incubation. It is possible to attain potential gas production of feedstuffs if the donor animal from which rumen liquor for incubation is collected meets the nutrient requirement. Generally, gas production is a function of and a mirror of degradable carbohydrate and therefore, the amount depends on the nature of the carbohydrates (Demeyer and Van Nevel, 1975; Blummel and Becker, 1997).

The *in vitro* gas production pattern of the forages shown in Figure 1 indicated that more degradation of dry matter were still possible beyond 24 h. The situation here depicted that of typical dry season in Nigeria, when most of the forages are fibrous and therefore take longer time to degrade in the rumen. The highest gas production was obtained from *Ficus thonningii* for the reason that was not clear since the secondary metabolite is within the normal range as shown in Table 1, although high crude protein in feed enhances microbial multiplication in the rumen, which in turn determines the extent of fermentation.

The correlation between *in vitro* gas productions measured after 24 h incubation of tropical browses and that calculated from SCFA was similar to that reported for conventional feeds (Blummel *et al*., 1999). About 94% of the variation in the *in vitro* gas production on incubation of browse leaves was explained by SCFA produced, which mainly comes from carbohydrate fermentation. These results suggest that from browses with a wide range of CP contents, the SCFA production from sources other than carbohydrates is negligible.

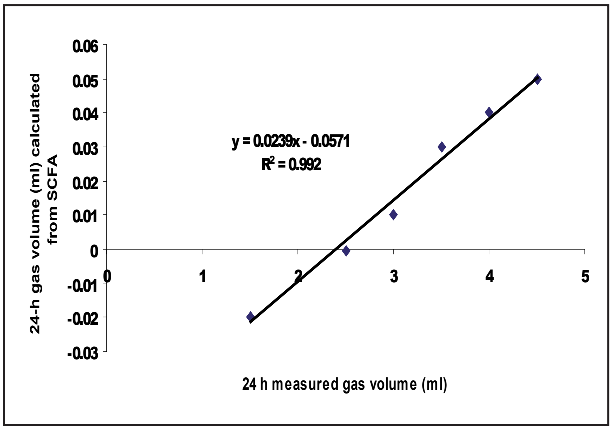

Fig. 2: Relationship between measured gas at 24 h incubation of tannin containing browses and SCFA production

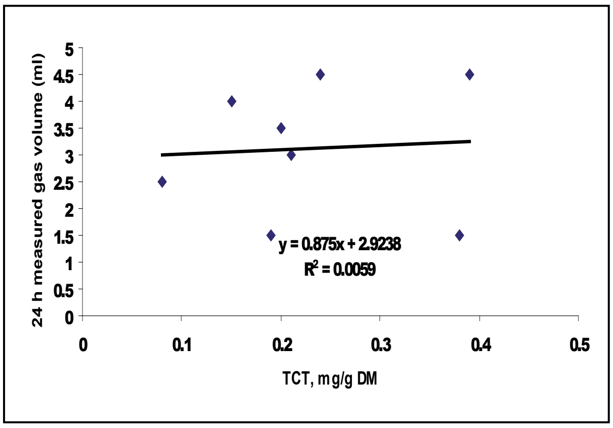

Fig. 3: Relationship between measured gas at 24 h incubation and TCT (mg/g DM)

The relatively strong correlation between CT and percentage increase in gas production (r = 0.99, n = 7) observed in the present study was consistent with those of Tolera *et al.* (1997) and Getachew *et al.* (2002).

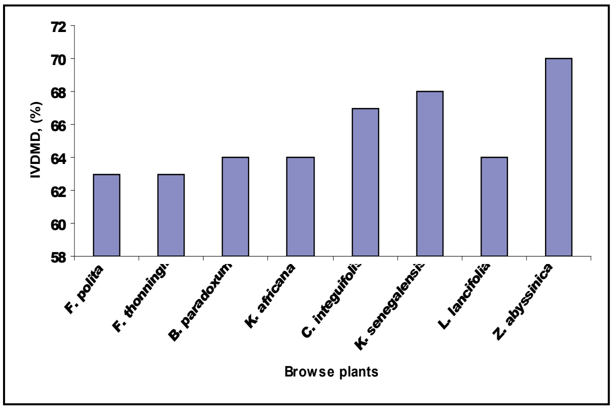

Fig. 4: Methane production of semi-arid browse forages

Methane (ml/200 mg DM) production (Figure 4) ranged from 2 to 5 among the forages, the least and the highest being from *F. thonningii* and *L. lancipholia* respectively. In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production. Methane production indicates an energy loss to ruminants and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi and Bamikole, 2006b) as an intergrated part of carbohydrate metabolism (Demeyer and Van Nevel, 1975).

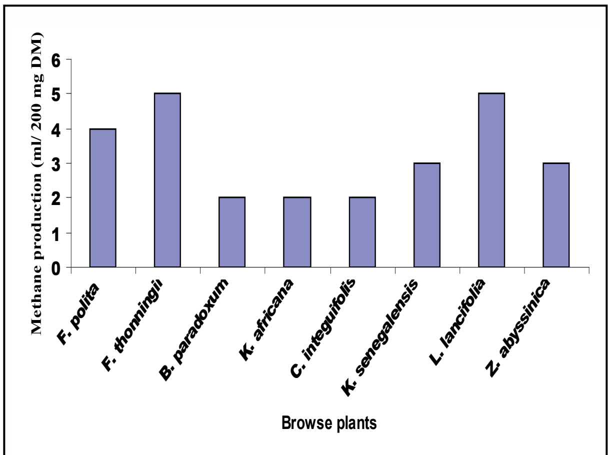

Fig. 5: IVDMD of semi-arid browse forages

The results of the IVDMD are shown in figure 5. The IVDMD was higher for all browses with *Z. abbyssinica* recording the highest value (70%). In tree leaves, tannins are present in the NDF and ADF fractions and are tightly bound to the cell wall and cell protein and seem to be involved in decreasing digestibility (Reed *et al*., 1990). The higher IVDMD observed may be due to the low level of tannins in the browse plants which suggest that it could be a valuable protein supplement in ruminant diets (Aganga and Mosase, 2001). This was further manifested in methane production (Fig. 4) where it showed inhibitory features. These suggest that feeds containing high level of CP and low levels of tannins could generate more methane in the rumen.

CONCLUSION

Chemical composition and *in vitro* digestibility can be considered as useful indicators for the preliminary evaluation of the likely nutritive value of previously uninvestigated shrubs. Semi-arid browses are forages with high protein concentrations and effective *in vitro* DM digestibility. As such, they have potential as forage for farmers during the long period of dry season when feed is scarce.

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