



Nutritive assessment of selected browse forage species from Semi-arid environment of Nigeria using *in vitro* gas production technique

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Abstract

The chemical composition and *in vitro* gas production of some browse species were evaluated. Crude protein (CP) content was highest ($P < 0.05$) in *Ceiba pentandra*. Neutral detergent fibre, acid detergent fibre and acid detergent lignin were generally high ranging from 412.10 – 688.10; 211.60 – 265.60 and 88.30 – 121.30 g kg⁻¹ DM respectively. The total condensed tannins (TCT) was highest ($P < 0.05$) in *Celtis integrifolia*. The values for phenolics, saponin and phytate were highest in *Ceiba pentandra*, while *Batryospermum paradoxum* has the highest value for oxalate. *In vitro* gas production (GP) was lowest and highest in *Anaëolus leocarpus* (3.66 ml/200 mg DM) and *Adansonia digitata* (28.33 ml/200 mg DM) respectively. The GP from the insoluble but degradable fraction (b), potential gas production (a+b) and volume of gas produced (ml) at time t (Y) were highest in *Adansonia digitata*. The CP of browses weakly and positively correlated with incubation period at all hours and fermentation characteristics (a, b, a+b, c, t and Y). All the fibre fractions were negatively correlated with incubation periods and the fermentation characteristics. The secondary metabolites showed a weakly but negative correlation with fermentation characteristics. It can be concluded that the browse forages have nutritive potential as fodders for ruminants in tropical environment.

Key words: Browses, Nutritive assessment, gas production, *in vitro*

Introduction

Dry season feeding of ruminants in most tropical areas has always been a problem for farmers since inadequate good pasture exists during this period leading to poor performances of these animals. One possible way to alleviate this problem and maintain production in the tropics is to feed them with crop residues and browse plants. These feed resources are not consumed by man but can be converted by ruminants into animal products suitable as human food. This therefore reduces total cost of animal production without a decrease in productivity and also maintains efficient feed utilization.

Browse plant play a significant role in nutrition of ruminant livestock in the tropical region. Browse species, because of their resistance to heat, drought, salinity,

alkalinity, drifting sand, grazing and repeated cutting, are the major feed resources during the dry season (Fagg and Stewart, 1994). Some parts of browse species can be found during the dry season including pods, fruits and leaves. Most trees/shrubs produce their leaves during wet season, thus browse is more available during the spring (August to May) (Palgrave, 1983). The nutritional importance of browse plants is especially significant for free ranging animals in extensive communal system of production.

Browse trees and shrubs could be considered as a feed supplement in areas with a long period of dry season (e.g. semi-arid) since they provide forage for grazing ruminants throughout the year (e.g. evergreen species) or at specific critical periods of the year (i.e., deciduous species),

particularly after herbage senescence when the quantity and quality of herbaceous species are limited (Devendra, 1990). Browse species that have shown remarkable promise in Nigeria are *Leucaena leucocephala*, *Gliricidia sepium* and *Gmelina arborea*. Much pressure has been placed on these fodders such that they become inadequate to sustain ruminants during the usually prolonged dry season period (Babayemi, 2007).

Several chemical and biological procedures have been used to evaluate the nutritional quality of ligneous species foliage (Dowman and Collins, 1982). Though most of these methods are based on gravimetric measurements (Getachew *et al.*, 1998), yielding results of various precision. In contrast, the *in vitro* gas technique of Menke and Steingass (1988) has been suggested as a precise technique to measure both rate and extent of fermentation, in addition to the residue of fermentation, at various times of incubation (Blümmel and Ørskov, 1993).

The objective of this study was to determine the nutritive value and anti-nutritive substances of some browsable trees used as feed for ruminant livestock in the semi-arid region of Nigeria and also examine their relationships with *in vitro* gas production parameters.

Materials and Methods

Selection of Forage Samples

Ten browse species were selected after an initial field survey to evaluate the utilization of multipurpose tree and shrub forages as livestock feed in North-eastern region of Nigeria. The leaf browses were harvested by hand clipping leaves from five branches on ten mature trees in Maiduguri metropolitan council area of Borno State, Nigeria in the dry season. The forage species were as follows: *Adansonia digitata*, *Anageisus celecarpus*, *Anageisus leocarpus*, *Batryospermum paradoxum*, *Buahinea nufescens*, *Ceiba pentandra*, *Celtis integrifolia*, *Khaya senegalensis*, *Kigalia africana*, *Poupartia sirrea*. Only the leaves

and leaf petioles were harvested in order to mimic the parts browsed by ruminants.

Collection and processing of the browse leaves

The leaves were collected in gunny bags and dried in a shade for 6 days. The leaf browse was then milled through a 2.0mm screen for *in-vivo* digestibility, and a sub sample taken and milled further through 1.0 mm screen for use in gas production trial and proximate analysis.

In vitro gas production

Rumen fluid was obtained from 3 West African dwarf female goats through suction tube before morning feeding. The goats were 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 in addition 40% Guinea grass; *Panicum maximum*). Incubation was as reported by some studies using 120 mL calibrated syringes in three batch incubation at 39 °C. Into 200 mg sample (n=12) in the syringe was introduced 30 mL inoculums containing cheese cloth strained rumen liquor and buffer (NaHCO₃+3Na₂HPO₄+KCl+NaCl+MgSO₄.7H₂O+CaCl₂.2H₂) (1:4, v/v) under continuous flushing with CO₂. The gas production was measured at 3, 6, 12, 24, 48, 72 and 96 h. The net gas volumes data was then fitted in the equation (Menke and Steingass, 1988):

$$y = a+b (1-e^{-ct})$$

Where

a = the gas production from the immediately soluble fraction (ml)

b = the gas production from the insoluble fraction (ml)

c = the gas production rate constant for the insoluble fraction b (h)

a+b = the potential gas production (ml)

t = incubation time (h)

y = gas produced at the time "t"

Results

The chemical composition of the browse plants are presented in Table 1. The browse forages generally have high CP content with *Ceiba pentandra* (CP) having the highest (173.90g kg⁻¹ DM while *Poupartia sirrea* (PS) has the lowest value of 132.20g kg⁻¹ DM. All the browse forage were significantly different (P<0.05) for CP. The forage have relatively high ether extract and organic matter ranging from 30.30 in *Khaya Senegalensis* to 51.60 g kg⁻¹ DM in *Analgeosus leocarpus* and *Poupartia sirrea* and 742.60 in *Poupartia sirrea* to 868.70g kg⁻¹ DM in *Khaya senegalensis*. Generally the fibre fraction (NDF, ADF and ADL) are generally high with *Kigalia africana* (Ka) having the highest value (688.10g kg⁻¹ DM). There were significant differences (P<0.05) for the fibre fractions among the forages. Ka had the highest NDF and ADF content compared to other forages. Browse forages had relatively low anti nutritive factors. The tannin content change from 0.11 in *Bauhenia nufesence* to 0.28 mg g⁻¹ in *Ceiba pentandra*. The browse forages had undetectable phenolics ranging from 0.31 in *Poupartia sirrea* to 0.71 mg g⁻¹ in *Ceiba pentandra*. There were significant (P<0.05) differences for Saponin contents among the forages. The oxalate content ranged from 4.62 in *Adansonia digitata* to 8.00 mg g⁻¹ in *Batryospermum paradoxum*. The phytate contents of the forages was highest in *Ceiba pentandra* (7.33 mg g⁻¹) and lowest (2.22 mg g⁻¹) in *Kigalia Africana*. Anti-nutritional factors showed significant (P<0.05) difference for all the browse forages.

***In vitro* gas Production**

Average gas production from incubation of the browse forages is shown in Fig 1. At any stage of gas production, *Adansonia digitata* produced more (P<0.05) gas than the other forage samples. The values for the cumulative gas production ranged from 3.66 in *Analgeosus leocarpus* to 78.33 ml/200 mg DM in *Adansonia digitata*.

Fermentation characteristics of semi-arid browse forages

The GP from the immediately soluble fraction (*a*) as shown in Table 2, is generally low for all the browse forages, with values ranging from 1.33 in *Analgeosus leocarpus* and *Batryospermum paradoxum* to 3.67 mg/200 g DM in *Anageisus celecarpus*. The value for GP for insoluble but degradable fraction (*b*) was highest in *Adansonia digitata* (25.00 mL) and least in *Anageisus celecarpus* (2.67 mL). The potential gas production (*a+b*) was generally low for all the browse forages with the highest value (28.33 mL) in *Adansonia digitata* and the least value (4.00 mL) in *Analgeosus leocarpus*. The rate of GP (*c*) ranged from 0.028 in *Kigalia africana* to 0.057 in *Bauhenia nufescens*. The GP production (*Y*) at time 't' ranged between 3.00 in *Bauhenia nufescens* and 11.33 mL/200 mg DM in *Adansonia digitata*.

Correlation Coefficient (r) between Chemical Composition and *in vitro* GP and Fermentation Characteristics

Correlation coefficient (r) between chemical composition and *in vitro* GP and fermentation characteristics is presented in Table 3. The CP content of the browse forages showed positive but weak correlation at all incubation periods and also with fermentation characteristics. This follow similar pattern with other parameters except for OM which was observed to be strongly and positively correlated with gas production intervals 't'.

Correlation between anti-nutritive factors and *in vitro* fermentation characteristics.

The correlation between anti-nutritive factors and fermentation characteristics is shown in Table 4. There were weak and negative correlation between the anti-nutritive substances and fermentative characteristics.

Correlation between *in vitro* gas productions, fermentation characteristics and anti-nutritive factors. The gas production from immediately soluble fraction (ml) was positively but weakly

correlated at all incubation time (Table 5) while the gas production from the insoluble but degradable fraction (b) was positively and strongly correlated at all incubation periods. Similar pattern was observed for potential gas production (a+b) and volume of gas produced at time “t”, (Y) which are strongly and positively correlated with gas production and anti-nutritive substance were generally weak and positively correlated at all incubation period. TCT ($r=0.149$) at 12h to 0.354 at 3h, SAP ($r=0.18n$ at 12h to 0.283 at 3h); OXA (0.082 at 48h to 12h); PHE 0.001 at 3h to 0.095 at 48h) and PHE (0.027 at 48h to 0.200 at 3h) respectively.

Discussion

Chemical Composition of browse plants studied

The high CP content of the forages indicated the potentiality of browse fodder to ruminants fed on low quality roughage. CP content reported in this study are similar to those reported by Njidda *et al.* (2013) and Njidda (2020) for semi-arid browses. Addition of browse fodder supplements into the basal diets would overcome animal nitrogen requirements, and could enhance utilization of these feed resources. Differences in CP contents between browse forage species could be due to variations in factors controlling protein accumulation in forages during the growth process (Salem *et al.* 2007). Larbi *et al.* (2011) also reported that other source of variation could be stage of foliage growth at vegetative sampling and the proportion of foliage sampled (twigs, leaves, of soft stems). Location and climate influence on forage growth and plants nutrients accumulation could also be factors (Parissi *et al.* 2005).

The NDF and ADL content of the browse forage were generally high and are similar to the values reported by Njidda *et al.* (2017) and this can limit feed intake. The increase in value of NDF and ADL might be due to oven-drying which the forages were subjected to. This finding is similar to the report of Parissi *et al.* (2005) who reported that NDF and ADL increase due to

formation of insoluble condensed tannin-protein polymers. In addition, heat treatment causes polymerization of sugar residues with amino acids resulting in a Maillard complex with physical and chemical properties similar to lignin (i.e. artifact lignin) (Van Soest, 1994)

Tannin present in browse forages have been shown to be the major limitation to the use of the forages in livestock diets especially as protein supplements. This is because in ruminants, the polyphenolics tend to alter the composition of rumen micro-organism complex and inhibit microbial enzymes complex with the fed nutrients and their metabolic products may be absorbed from the rumen and produce toxic effects at the tissue level (Mangan, 1988). However, low levels (30-40 mg/g DM) of tannins have nutritional benefits for ruminants by protecting dietary proteins from excessive ruminal degradation without affecting forage intake or fibre digestion (Barry *et al.*, 1986). Phenolic content of the browses are lower than 7.75 mg/g DM reported by Osuga *et al.* (2006). Njidda (2010a) reported that total phenolic content seems to be highest in the dry season and is associated with reduced live weight gain in the dry season (Edward, 2000). There also appeared to be an inverse relationship between concentration of phenolic compounds and palatability. The saponin content of feedstuffs had been shown to be defaunating agents (Teferedegne, 2000) and capable of reducing methane production (Babayemi *et al.*, 2004b). Cheeke (1971) reported that saponins have effect on erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat (ruminant) inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption. Saponins have been reported to alter cell wall permeability and therefore produce some toxic effect when ingested (Belmar *et al.*, 1999). The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins. Sharma *et al.* (1969) observed that 4-7

weeks of *ad libitum* feeding of *albizzia* gave rise to toxic manifestations in sheep. Symptoms include listlessness, anorexia, weight loss and gastro-enteritis. Toxicity from saponins can be reduced by repeatedly soaking the feed in water, though the level in this present study may not pose any problem to the animals. The values for oxalate ranged from 4.62 to 8.96 mg/g DM. It has been reported that values upto 20 g/kg oxalate can be lethal to chicken (Acamovic *et al.*, 2004). However, given time to adapt, the microorganisms in the rumen can metabolize moderate amounts of oxalate. The phytin levels reported in this study are lower than the 13.80 to 25.20 mg/g reported by Okoli *et al.* (2003) for southeastern browses in Nigeria. These levels are unlikely to have any adverse effect in ruminant diets.

***In vitro* gas Production**

Gas production parameters indicate differences in nutritional value that were generally closely related to chemical composition (Njidda *et al.* 2017). The high IVGP 96 in *Adansonia digitata* suggests a high extent of fermentation throughout the incubation period compared to other leaves. Differences observed in gas production among the leaves could be due to the proportion and nature of their fibre (Salem *et al.*, 2007 and Rubanza *et al.*, 2003). Chemical composition, *in vitro* fermentation and digestibility are largely affected by plant species, plant morphological fraction, environmental factors, and stage of maturity (Mbugua *et al.* 2008). These factors influence the amount of substrate OM that is fermented and the short chain fatty acids (SCFAs) produced upon fermentation. This is because gas production results from fermentation of the feed OM and CO₂ produced from the buffering of the SCFAs by the bicarbonate buffer. Condensed Tannins (CTs) have been widely reported to decrease both rate and extent of gas production (Getaachew *et al.*, 2000 and Frutos *et al.*, 2002), with this suppressive

effect probability being a result of a reduction in the attachment of microbes to feed particles (McAllister *et al.*, 1994) and a specific incubation of ruminal degradation through an initiation of micro-organism growth and microbial enzymes activity (McSweeney *et al.*, 2001). The possible explanation to the low gas production may be due to cell wall content (NDF, ADF and ADL) which has negative correlation with gas production at all incubation times. (Njidda *et al.*, 2017). This may tend to reduce the microbial growth and enzymes activity (McSweeney *et al.*, 2002) or intestinal bacterial activity (Salem *et al.*, 2004). Cerrillo and Juarez (2004) further explain that 81% of the variation in potential gas production could be explained by the changes in ADF content.

***In vitro* Fermentation Characteristics of the Browse Forages**

Kinetics gas production obtained from the exponential model is presented in Table 3. The extent of gas production (a+b) was higher for *Adansonia digitata* compared to other browse forages in this study. Khazaaal *et al.* (1995) indicted that the intake of a feed is mostly explained by the rate of gas production (c) which affects the rate of passage of the feed through the rumen, whereas the potential gas production (a+b) is associated with the degradability of the feed. Thus the higher values obtained for the (c) and (a+b) parameters in the browse forages, may indicate a better nutrient availability for rumen micro-organisms in animals grazing such vegetative species in semi-arid areas. This could be possible due to high level of NDF and lignin in the browse which hinder rumen microbe from degrading the feed material.

Chemical composition and *in vitro* fermentation and digestibility are largely affected by plant species, plant morphological fraction, environmental factors, and stage of maturity. These factors influence the amount of substrate OM that is fermented and the short chain fatty acids (SCFAs) produced upon fermentation. This

is because gas production results from fermentation of the feed OM and CO₂ produced from the buffering of the SCFAs by the bi-carbonate buffer.

Correlation between chemical constituents and gas production at 96 hrs of the browse forages (Table 3) shows that there were both positive and negative relationships between the volume of gas produced and their chemical constituents. This is consistent with the reports of Getachew *et al.* (2003) and Arigbede *et al.* (2006). This could be as a result of the feed constituents, such as NDF and ADF, which contribute more gas if they are degradable as shown by their positive effect on gas production. The positive correlation between CP and gas production of the browse forage leaves is consistent with the report of Njidda *et al.* (2016).

Correlation between anti-nutritive substances and *in vitro* fermentation characteristics

All fermentation parameters were correlated with secondary metabolic (Table 4). The correlation between secondary metabolites and fermentation characteristics indicates a weakly and negative correlation. The result is similar to the report of Salem *et al.* (2007) and Njidda *et al.* (2016). Rubanza *et al.* (2003) who reported a negative correlation between chemical composition and phenolic compounds. The negative correlation with the estimated parameters is consistent with the findings of Frutos *et al.* (2002). Larbi *et al.* (1998) reported a weak relationship between secondary metabolites and gas production parameters. Kamalak *et al.* (2005).

Significant positive correlation between fermentation characteristics and secondary metabolites (*a*, *b*, *a+b*, *c*, *t* and *Y*) respectively could be explained by relatively less adverse effect of total phenolics and tannins on digestibility (Njidda, 2010b). Khazaal *et al.* (1994) found a positive correlation between condensed tannins and

degradability estimates, a reason that could be attributed to different nature and tannin activity. The relatively lower rate and extent of GP from browse forages leaves could also be attributed to compositional differences of the browse forages, especially CP, fibre nature and concentration of polyphenolics and may be other anti-nutritional components (Salem *et al.* 2007).

Correlation between IVGP, fermentation characteristics and anti-nutritive substances.

The rate of gas production was weak but positively correlated with the soluble fraction (*a*) (Table 5). Differences in feed degradability are largely attributed to phenolic compounds and tannin as well as fibre type and proportion in forages. All the *in vitro* fermentation characteristic parameters showed significant differences among browse forages. The extent of GP (*a+b*) was higher for *Adansonia digitata* than the other browse forages species.

Khazaal *et al.* (1995) indicated that the intake of a feed is mostly explained by the rate of gas production (*c*) which affects the rate of passage of the feed through the rumen, whereas the potential gas production (*a+b*) is associated with the degradability of the feed. Thus, the values of the studied fermentation characteristic parameters suggest that nutrient availability for rumen microbes in animals grazing such vegetative specie will be affected since higher values of such parameters (*c* and *a+b* of feed) indicate a better nutrient availability for rumen microorganism. This could be possible due to the high level of NDF and lignin in the browse which may hinder rumen microbe from degrading the feed material. Also the higher fibre content may affect the feed intake. These factors might have contributed to the lower values observed for the fermentation characteristics of the browse forages. The gas production (*a*) from quickly soluble fraction is in agreement with those reported by Kamalak *et al.* (2005) whereas gas production (*b*)

from slowly fermentable fraction and potential gas production ($a+b$), and gas production rate (c) was lower than those reported that by Filya *et al.* (2002) and Kamalak *et al.* (2005). This might be due to differences in rumen fluid content and chemical composition of the browse forages used.

Conclusion

Though the gas production and fermentation characteristics were generally low for all the browse forages, their high CP, moderate fibre levels, low condensed tannins and all year round availability underscore their nutritive potential as fodders for ruminants. They may, therefore, be used either as a sole fodder or supplementary fodder to low nutritive roughages, particularly grasses that are unavailable during the critical dry season period.

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Table 1: Chemical composition and anti-nutritive factors of experimental brose forages

	Browse species										SEM
	Ad	Ac	Al	Bp	Bn	Cp	Ci	Ks	Ka	Ps	
Chemical Composition (g kg⁻¹ DM)											
CP	160.00 ^b	149.10 ^e	150.70 ^d	145.9 ^f	114.90 ^j	173.90 ^a	153.60 ^c	139.60 ^g	134.02 ^h	132.20 ⁱ	0.88*
EE	30.60 ^f	31.60 ^e	51.60 ^a	50.00 ^b	47.30 ^c	31.60 ^e	31.00 ^e	30.30 ^f	34.60 ^d	51.60 ^a	0.23*
OM	809.70 ^g	8380 ^d	848.30 ^c	859.00 ^b	812.70 ^f	828.00 ^e	794.60 ^h	868.70 ^a	766.70 ⁱ	742.60 ^j	2.46*
NDF	412.10 ⁱ	542.80 ^e	542.10 ^e	572.30 ^d	493.10 ^g	514.60 ^f	595.90 ^b	486.20 ^h	688.10 ^a	591.20 ^c	1.33*
ADF	219.70 ^f	231.30 ^e	241.80 ^d	265.60 ^a	231.40 ^e	244.80 ^c	246.00 ^c	211.60 ^g	255.20 ^b	230.30 ^e	2.26*
ADL	116.30 ^d	88.30 ⁱ	130.00 ^b	112.60 ^f	93.70 ^h	112.40 ^f	105.60 ^e	121.00 ^c	97.00 ^g	140.30 ^a	2.12*
Anti-nutritive substances (mg g⁻¹ DM)											
TCT	0.13 ^e	0.20 ^b	0.17 ^d	0.19 ^c	0.11 ^f	0.28 ^b	0.39 ^a	0.21 ^b	0.08 ^g	0.14 ^d	0.02*
PHE	0.50 ^b	0.37 ^f	0.31 ^h	0.42 ^d	0.32 ^g	0.71 ^a	0.39 ^e	0.48 ^c	0.37 ^f	0.31 ^h	0.02*
SAP	1.91 ^h	2.16 ^f	2.49 ^c	2.36 ^e	2.90 ^b	2.99 ^a	2.40 ^d	2.02 ^g	2.02 ^g	1.08 ⁱ	0.08*
OXA	4.62 ^g	4.72 ^f	5.04 ^f	8.00 ^a	5.81 ^d	6.14 ^c	4.58 ^g	7.20 ^b	5.02 ^f	5.10 ^e	0.23*
PHY	2.95 ^h	3.95 ^d	3.15 ^g	3.40 ^f	3.51 ^e	7.33 ^a	2.85 ⁱ	5.81 ^b	2.22 ^j	3.97 ^c	0.20*

A, b, c, d= means within the same row bearing different superscript are significantly different (p<0.05); * = Significant (p<0.05); SEM= Standard error of means. Ad=Adansonia digitata, Ac=Anageisus celecarpus, Al=Analgeosus leocarpus, Bp=Batryospermum paradoxum, Bn=Buahineia nufescens, Cp=Ceiba pentendra, Ci=Celtis integrifolia, Ks=Khaya senegalensis, Ka=Kigalia africana, Ps=Poupartia sirrea; CP=Crude protein; EE=Ether Extract; OM=Organic Matter; NDF=Neutral detergent fibre; Acid detergent fibre; Acid detergent lignin; TCT=Total Condensed Tannin; SAP=Saponin; OXA=Oxalate; PHE=Phenolics; PHY=Phytate

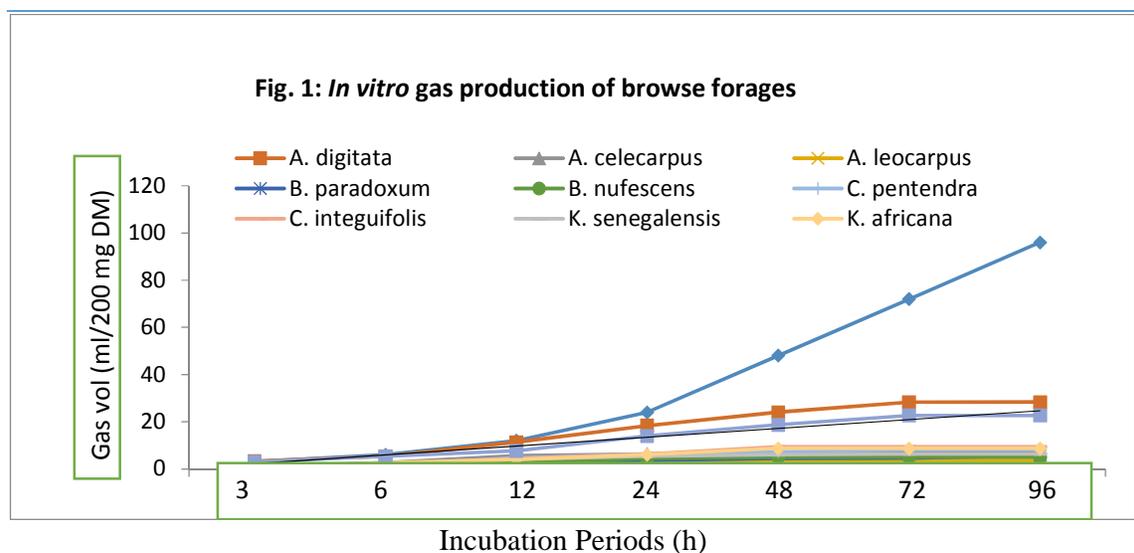


Table 2: In vitro Fermentation Characteristics

Browse Forages	A	b	a+b	c	t	y
<i>Adansonia digitata</i>	3.33	25.00	28.33	0.032	12.00	11.33
<i>Anageisus celectarpus</i>	3.67	4.33	8.00	0.046	14.00	5.00
<i>Analgeosus leocarpus</i>	1.33	2.67	4.00	0.053	16.50	3.50
<i>Batryospermum paradoxum</i>	1.33	11.00	12.33	0.046	18.00	6.67
<i>Buahinea nufescens</i>	1.00	4.67	5.67	0.057	11.00	3.00
<i>Ceiba pentendra</i>	2.00	6.33	8.33	0.05	14.00	4.67
<i>Celtis integrifolis</i>	2.33	7.00	9.33	0.034	10.00	4.33
<i>Khaya senegalensis</i>	2.33	4.00	6.33	0.042	16.00	4.00
<i>Kigalia Africana</i>	2.67	6.00	8.67	0.028	13.00	4.00
<i>Poupartia sirrea</i>	3.00	19.67	22.67	0.035	10.00	8.33
SEM	1.21	2.67	2.12	0.019	1.37	1.21

a = gas production from the immediately soluble fraction (ml); *b* = gas production from the insoluble but degradable fraction (ml); *a + b* = potential gas production (ml); *c* = constant of gas production (fraction/h); *t* = gas production intervals i.e. 3, 6, 12, 24, 36, 48, 72, 84 and 96 hours; *Y* = volume of gas produced (ml) at time *t*

Table 3: Correlation between chemical composition and *in vitro* gas production, *in vitro* fermentation characteristics

	CP	EE	OM	NDF	ADF	ADL
3	0.001	0.270	0.199	0.148	0.137	0.183
6	0.016	0.100	0.360	0.021	0.157	0.120
12	0.022	0.101	0.142	0.172	0.230	0.060
24	0.032	0.034	0.254	0.128	0.213	0.122
48	0.024	0.032	0.326	0.077	0.164	0.119
72	0.022	0.017	0.309	0.088	0.168	0.152
96	0.022	0.014	0.298	0.086	0.153	0.156
a	0.060	0.310	0.130	0.012	0.156	0.004
b	0.018	0.003	0.196	0.087	0.048	0.177
a+b	0.023	0.012	0.211	0.077	0.063	0.153
c	0.006	0.160	0.354	0.129	0.013	0.010
t	0.057	0.022	0.654**	0.005	0.066	0.009
Y	0.075	0.001	0.072	0.132	0.051	0.143

***P<0.001; **P<0.01; *P<0.05; CP=Crude protein; EE=Ether Extract; OM=Organic Matter; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent lignin; PHE=Phenolics; a = gas production from the immediately soluble fraction (ml) b = gas production from the insoluble but degradable fraction (ml); a + b = potential gas production (ml); c = constant of gas production (fraction/h); t = gas production intervals i.e. 3, 6, 12, 24, 36, 48, 72, 84 and 96 hours; Y = volume of gas produced (ml) at time t

Table 4: Correlation between anti-nutritive substances and *in vitro* fermentation characteristics

	TCT	PHE	SAP	OXY	PHY
a	0.004	-0.066	-0.130	-0.189	-0.008
b	0.273	-0.019	-0.276	-0.119	-0.109
a+b	0.251	-0.025	-0.288	-0.140	-0.091
c	0.005	-0.343	-0.103	-0.015	-0.163
t	0.044	-0.416	-0.008	-0.178	-0.127
Y	0.240	-0.002	-0.307	-0.267	-0.141

***P<0.001; **P<0.01; *P<0.05; TCT=Total Condensed Tannin; SAP=Saponin; OXA=Oxalate; PHE=Phenolics; PHY=Phytate; a = gas production from the immediately soluble fraction (ml) b = gas production from the insoluble but degradable fraction (ml); a + b = potential gas production (ml); c = constant of gas production (fraction/h); t = gas production intervals i.e. 3, 6, 12, 24, 36, 48, 72, 84 and 96 hours; Y = volume of gas produced (ml) at time t;

Table 5: Correlation between *in vitro* gas productions, fermentation characteristics and anti-nutritive substances

	3	6	12	24	48	72	96
a	0.210	0.542*	0.559*	0.429	0.417	0.385	0.373
b	0.889***	0.800***	0.765***	0.855***	0.859***	0.884***	0.906***
a+b	0.888***	0.861***	0.830***	0.894***	0.918***	0.937***	0.119
c	0.442	0.312	0.312	0.335	0.416	0.374	0.374
t	0.193	0.252	0.180	0.267	0.316	0.287	0.269
Y	0.825***	0.746***	0.808***	0.805***	0.779***	0.804***	0.829***
TCT	0.354	0.168	0.149	0.188	0.176	0.199	0.205
SAP	0.283	0.342	0.187	0.197	0.182	0.196	0.208
OXA	0.121	0.123	0.193	0.115	0.082	0.097	0.103
PHE	0.001	0.031	0.045	0.053	0.095	0.064	0.060
PHY	0.200	0.061	0.051	0.061	0.027	0.050	0.055

***P<0.001; **P<0.01; *P<0.05; a = gas production from the immediately soluble fraction (ml); b = gas production from the insoluble but degradable fraction (ml); a + b = potential gas production (ml); c = constant of gas production (fraction/h); t = gas production intervals i.e. 3, 6, 12, 24, 36, 48, 72, 84 and 96 hours; Y = volume of gas produced (ml) at time t; TCT=Total Condensed Tannin; SAP=Saponin; OXA=Oxalate; PHE=Phenolics; PHY=Phytate