

## ***In-vitro* fermentation and preference of some browse plants by West African dwarf goats**

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### **ABSTRACT**

The study was carried out to investigate the suitability of utilising some selected browse plants: *Leuceana leucocephala*, *Gliricidia sepium*, *Azadiractha indica*, and *Gmelina arborea* in the feeding of ruminant livestock. The proximate composition and *in-vitro* gas production techniques were used to assess the nutritive value of the leaves as well as their forage acceptability using a cafeteria method involving eleven adult West African Dwarf goats. The crude protein (CP) ranged from 11.59 – 26.78%, the crude fiber (CF) ranged from 10.13 – 14.33%. The extent of gas production ranged from 5.99 – 7.34ml/200mg, while the rate of gas production ‘c’ ranged from 0.05 – 0.14ml/hr. The mean values for short chain fatty acid (SCFA), metabolisable energy (ME), organic matter digestibility (OMD) and Methane were (0.10 – 0.15μmol), (3.72 – 4.77MJkg/DM), (33.06 – 40.84%) and (1.50 – 2.50ml) respectively. The acceptability of the forage was best in *Leuceana leucocephala* and *Gliricidia sepium*, better in *Gmelina arborea* and poor in *Azadiractha indica*. The results showed that these selected browse plants have good level of nutrients and all except *Azadiractha indica* are well accepted by West African Dwarf goats.

**Key words:** *In-vitro*, Preference, Browse Plants

### **INTRODUCTION**

Multipurpose trees and shrubs (MPTS) commonly called browses are important components of agro-forestry systems in West Africa. In ruminant livestock production, feeding of browse forage has become an essential practice especially in the dry season when there is always scarcity of grass and herbaceous legume forages. Browse plant species have high potentials as important feed resources for ruminants during the dry season and are quite palatable. They are trees or perennial shrubs that remain green all year round, thus constituting a ready source of feed during off season (Oji *et al.*, 2007). Additionally, they have appreciable level of crude protein,

vitamins and mineral elements and may serve as valuable feed supplements to low quality diets (Ayo-Enwerem *et al.*, 2008). Moreover these trees and shrubs are sources of fuel; wood and timber, increase soil fertility, serve as wind and water erosion control and some of them are medicinal (Oji *et al.*, 2003). The *in-vitro* gas production technique is a laboratory estimation of rate and extent of nutrient disappearance in livestock feed sample for the purpose of assessing the potential nutritive value of the feed. It is also a method that is reproducible and parameters obtained correlate well with *in-vivo* trials (Fajemisin, 2002). *In-vitro* gas production technique has the advantage of not only being less expensive and less time consuming but it allows for more precision

in experimental conditions than the *in-vivo* method (Makkar, 2002). It is convenient, fast and allows a large number of samples to be handled at a time. It is based on the quantification of substrate degraded and of gas or short chain fatty acid produced in rumen fermentation system (McDonald *et al.*, 1995). Free choice intake and acceptability study is a quick assessment of physical quality of a feed. Coefficient of Preference (CoP) is a direct measure of acceptability and nutritional capability of feedstuff or forage. In recent times, cafeteria technique has been used to assess the acceptability of some forage (Bamikole *et al.*, 2004; Babayemi *et al.*, 2006 and Babayemi, 2007). The feed intake or the palatability of forage is regulated by many factors: harvesting, physical and metabolic feedback, and secondary metabolites. Voluntary intake is the overall acceptance and relish with which an animal consumes any given food stuff or diet (Marten, 1978). It is the result of a summation of many different factors sensed by the animal in the process of locating and consuming feed. This depends on number of factors such as appearance, odour, taste, texture, temperature and in some cases auditory properties of the food. These various factors are in turn, affected by the physical and chemical nature of the feed. The effect on individual animal may be modified by physiological and psychological differences. Intake therefore is an important measure of feeding value of forage. Thus this study was designed to evaluate the nutrient potential of some browse plant leaves (*Gliricidia sepium*, *Leucaena leucocephala*, *Azadirachta indica* and *Gmelina arborea* using the *in-vitro* gas production technique and to evaluate how readily these selected forages are consumed by ruminant animals with a view to making appropriate recommendation to farmers who practice cut- and – carry system of grazing.

## MATERIALS AND METHOD

### *Collection of forage samples*

The forage samples were harvested from the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan. The plants samples were air-dried at room temperature for seven days and milled to pass through 1mm mesh and stored in different air-tight labeled containers.

### *Chemical analysis*

Known weight of each air-dried sample was oven dried at 65<sup>0</sup>C to a constant weight for dry matter determination (DM). Proximate analysis; crude protein, crude fibre, ether extract and ash were analysed according to the standard methods of (AOAC, 2000).

### *In-vitro Gas Method*

The *in-vitro* study was carried out at the Animal Science Department, University of Ibadan, Nigeria. Samples were incubated *in-vitro* with rumen fluid in calibrated glass syringe following the procedure of Menke and Steingass (1988). Rumen liquor was obtained from three mature West African Dwarf goats using suction tube prior to morning feeding. Incubation procedure was as reported by (Menke and Steingass, 1988) using 100ml calibrated transparent plastic syringes with fitted silicon tube. About 200mg (0.2g, n=3) of 1mm milled samples each were weighed into 100ml calibrated glass syringes in triplicate. The syringes were filled with 30ml of inoculums (i.e. rumen liquor and buffer). Vaseline was applied to the piston to ease movement and to prevent escape of gas. The syringes were pre-warmed (39°C) for 1 hour, before addition of 30±1.0ml of rumen-buffer mixture (9.8gNaHCO<sub>3</sub>+2.77gNa<sub>2</sub>HPO<sub>4</sub>+0.57gKCl+0.47gNaCl+0.12gMgSO<sub>4</sub>.7H<sub>2</sub>O+0.16g/litre

CaCl<sub>2</sub>.H<sub>2</sub>O) (1:4, v/v) under continuous flushing with CO<sub>2</sub>. Incubation was terminated after 24h incubation and gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24. 6ml of NaOH (10 Molar) was introduced to estimate methane production as reported by (Fievez *et al.*, 2005). The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of the gas produced was plotted against the incubation time, and the gas production characteristics were estimated using the equation  $Y = a + b(1 - e^{-ct})$  described by Qrskov and McDonald (1979), where Y = volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), b = gas produced from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time. The post incubation parameters such as Metabolisable energy (ME (MJ/Kg DM)) and Organic matter digestibility (OMD %) were estimated as established by (Menke and Steingass, 1988), and short chain fatty acids (SCFA) was calculated as reported by (Getachew *et al.*, 1999).

$ME = 2.20 + 0.136G_v + 0.057CP + 0.029CF$  (Menke and Steingass, 1988).

$OMD = 14.88 + 0.889G_v + 0.45CP + 0.651XA$  (Menke and Steingass, 1988).

$SCFA = 0.0239G_v - 0.0601$ ; (Getachew *et al.*, 2000).

Where G<sub>v</sub>, CP, CF, and XA are net gas production (ml/200mg DM), crude protein, crude fibre and ash of the incubated samples respectively.

Samples of *Gmelina arborea*, *Leucaena leucocephala*, *Azadirachta indica*, and *Gliricidia sepium* were oven dried at 65°C to a constant weight for dry matter determination, crude protein, crude fibre,

ether extract and ash were analysed according to the standard methods of (AOAC, 2000).

### Acceptability study

The acceptability study was carried out at goats unit of the Federal College of Animal Health and Production Technology, Ibadan. Four different browse plants namely *Leucaena leucocephala*, *Gliricidia sepium*, *Azadirachta indica* and *Gmelina arborea* were used for the study. Eleven adult West African Dwarf goats housed in a group pen were used in the cafeteria feed preference study that lasted for two weeks, including one week of adaptation. The forages were harvested fresh, 4 kg each forage were introduced on cafeteria basis to the animals in four different plastic feeders, and thus each animal had free access to each of the forage in the feeders. The positioning of the forages was changed daily to prevent bias by the animals taking a particular part of the pen as the position for a particular type of forage. The amount consumed was monitored for six hours per day and quantity consumed was recorded. After then, the animals were released to go for grazing. Feed preference was determined from the coefficient of preference (CoP) value calculated from the ratio between the intakes of each individual feed sample divided by the average intake of the nine feed samples (Karbo *et al.*, 1993, Bamikole *et al.*, 2004). On the basis, a feed was taken to be relatively preferred if the CoP value is greater than unity.

$CoP = \frac{\text{intake of individual feed offered}}{\text{mean intake of all the feed offered}}$

### Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the procedure of SAS (1999). Significant means were

separated using the New Duncan's Multiple Range Tests.

## RESULTS

### Proximate composition

The proximate composition of the browse plants is shown in Table 1. Dry matter ranged from 27.07% in *G. sepium* to 39.26% in *A. indica*, while the crude protein ranged from 11.59 to 26.78%. The highest was observed in *L. leucocephala* while *G.*

*arborea* recorded the lowest. Crude fiber ranged from 10.13% in *A. indica* to 14.33% in *L. leucocephala*. The highest ash content was observed in *A. indica* (14.72%) while the lowest (9.09%) was observed in *G. arborea*. Ether extract ranged from 11.18% to 15.35%. *L. leucocephala* recorded the highest (15.35%). *G. arborea* had the highest value (58.15%) of NFE content while lowest value (27.48%) was observed in *G. sepium*.

**Table 1: Proximate composition (%) of the browse plants**

Samples	Dry matter	Crude protein	Crude fibre	Ash	Ether-extract	NFE
<i>Leuceana leucocephala</i>	36.43	26.78	14.33 <sup>a</sup>	9.85	15.35	37.72
<i>Gliricidia sepium</i>	27.07	25.50	13.08 <sup>ab</sup>	11.10	13.83	27.48
<i>Azadiractha indica</i>	39.26	15.30	10.13	14.72	11.18	48.66 <sup>ab</sup>
<i>Gmelina arborea</i>	35.58	11.59	11.90	9.09	11.27	58.15 <sup>a</sup>
SEM		0.003	0.000	0.000	0.015	0.000

Means in the same column with the same superscripts are not significantly different (p<0.05)

### In-vitro gas production

Presented in Tables 2 and 3 are the *in-vitro* gas production characteristics and parameters. The immediate soluble 'a' ranged from 3.33 to 4.67ml/200mg/DM. *A. indica* recorded the lowest while *G. sepium* had the highest. The extent of gas production 'a+b' ranged from 5.99 in *A. indica* to 7.33 ml/200mg/DM in *L. leucocephala*. A similar trend was also observed in insoluble but degradable fraction 'b' *A. indica* recording the lowest

value (2.33ml/200mg/DM) while *L. leucocephala* had the highest value (3.00 ml/200mg/DM). The rate of gas production 'c' ranged from 0.05 to 0.14 ml/hr.

OMD% ranged from 33.06 to 40.84%, while SCFA ranged from 0.10 to 0.15 µmol. ME ranged from 3.72 to 4.77 MJ/kg/DM and methane ranged from 1.50 to 2.50ml. The lowest values of all the parameters were observed in *Azadiractha indica* while the highest were observed in *Leuceana leucocephala* and *Gliricidia sepium*.

**Table 2: *In-vitro* gas production (ml/200mg/DM) characteristics of the selected browse plants**

Samples	A	a+b	B	C
<i>Leuceana leucocephala</i>	4.33 <sup>ab</sup>	7.33 <sup>a</sup>	3.00 <sup>a</sup>	0.14 <sup>a</sup>
<i>Gliricidia sepium</i>	4.67 <sup>a</sup>	7.34 <sup>a</sup>	2.67 <sup>ab</sup>	0.07 <sup>b</sup>
<i>Azadiractha indica</i>	3.33 <sup>c</sup>	5.99 <sup>c</sup>	2.33 <sup>b</sup>	0.05 <sup>b</sup>
<i>Gmelina aborea</i>	4.00 <sup>b</sup>	6.67 <sup>b</sup>	2.67 <sup>ab</sup>	0.08 <sup>c</sup>
SEM	0.87	1.44	0.79	0.03

Means in the same column with the same superscripts are not significantly different (p<0.05)

**Table 3: *in-vitro* gas production (ml/200mg/DM) parameters of the selected browse plants**

Samples	OMD (%)	SCFA(μmol)	ME(MJkg/DM)	Methane(ml)
<i>Leuceana leucocephala</i>	39.39 <sup>a</sup>	0.15	4.67 <sup>a</sup>	2.50
<i>Gliricidia sepium</i>	40.84 <sup>a</sup>	0.15	4.77 <sup>a</sup>	2.50
<i>Azadiractha indica</i>	33.06 <sup>c</sup>	0.10	3.72 <sup>c</sup>	1.50
<i>Gmelina aborea</i>	35.94 <sup>b</sup>	0.12	4.16 <sup>b</sup>	2.50
SEM	1.28	0.03	0.20	1.00

Means in the same column with the same superscripts are not significantly different (p<0.05)

Table 4 shows the mean daily intake and CoP of WAD goats fed the selected browse plants. The mean daily intake ranged from 0.60kg/DM in *A. indica* to 5.99kg/DM in *L.*

*leucocephala*, similar trend was observed in CoP, *A. indica* recording the lowest value (0.16) while *L. leucocephala* had the highest value (1.56).

**Table 4: Coefficient of Preference of some browse plants by WAD goats**

Browse plants	Mean daily intake (kg)	Coefficient of Preference (CoP)	Ranking of forage preference
<i>Leuceana leucocephala</i>	5.99	1.56	1
<i>Gliricidia sepium</i>	5.54	1.44	2
<i>Azadiractha indica</i>	0.60	0.16	4
<i>Gmelina aborea</i>	3.24	0.84	3

## DISCUSSION

The variations in DM contents of the MPTS could be attributed to stage of growth (Oji, *et al.*, 2003). Crude protein ranged from 11.59% in *Gmelina arborea* to 26.78% in *Leuceana leucocephala*. The crude protein content of the browse plants exceeds the minimum recommended range of 7.0 - 8.0% for efficient functioning of rumen microorganisms (Van Soest, 1994) and higher than the range of 11.0 - 13.0% known to be capable of supplying adequate protein for maintenance and moderate growth in goats (NRC, 1981). Browse plants have been reported to have high CP of high digestibility, and are also high in vitamins and minerals (Odeyinka, 2001; Asaolu *et al.*, 2011). Crude fiber ranged from 10.13% in *Azadiractha indica* to 14.33% in *Leuceana luecocephala*. High levels of fibre have been acknowledged to be inversely related to feed digestibility and nutrient availability (McDonald *et al.*, 1995). The intercept value 'a' at 24 hours ranged from 3.33 ml/200mgDM in *A. indica* to 4.67 ml/200mgDM in *G. sepium*. The value 'a' used ideally reflect the fermentation of soluble fraction. The soluble fraction makes the attachment by rumen micro-organism to be done easily and lead to much gas production. The values obtained were significant ( $P < 0.05$ ) among the treatments. The extent of gas production 'a+b' ranged from 5.99 to 7.34 ml/200mgDM which was high in *G. sepium* and low in *A. indica*. Fievez *et al.* (2005) also reported *in-vitro* gas production to be synonymous with feed digestibility, so that the higher the gas production the higher the digestibility. The extent of gas production 'b' described the fermentation of insoluble but degradable fraction of the samples, the values ranged from 2.33 – 3.00ml/200mgDM. The highest value was observed in *L. leucocephala* while

*A. indica* recorded the lowest. Blummel and Orskov (1993) found that 'b' value could account for 88% for voluntary intake. The rate of gas production 'c' ranged from 0.05 – 0.14 ml/hr. *A. indica* recorded the lowest while the highest value was observed in *L. leucocephala*. The values observed were significantly ( $P < 0.05$ ) different among the browse plants. The values of 0.05 – 0.14ml/hr obtained for fractional rate of gas production of the browse plants were higher than the values: 0.028 – 0.046ml/hr reported by Anele *et al.* (2008) for multipurpose tree species and values of 0.0006 – 0.0655ml/hr obtained by Arigbede *et al.* (2008) for different parts of *Treculia africana*. The values are however similar to 0.056 - 0.169ml/hr reported by Getachew *et al.* (2004) for fractional rate of gas production in corn grain and canola meal. Higher values obtained in this study may be attributed to higher composition of nutrients in the leaves. It further shows that they are highly digestible as the rate at which feed or its constituents is digested in the rumen is as important as the extent of digestion. Babayemi *et al.* (2004) reported that factors that determine the rate of gas production during fermentation depend on the nature and level of the fibre, the presence of secondary metabolite and the potency of rumen liquor for incubation. Presented in Table 3 are the *in-vitro* gas production parameters. Highest value of metabolisable energy (ME MJkg/DM), organic matter digestibility (OMD%) and short chain fatty acid (SCFA) are consistently recorded for *G. sepium* in this study. This shows that the *Gliricidia sepium* could have the highest potential to make energy available to ruminant, this correlates with earlier report of Ajayi *et al.* (2008) that higher short chain fatty acid (SCFA) or volatile fatty acid (VFA) such as butyrate and acetate suggests a potential to make energy available to

ruminants. Methane production observed in this study was low (1.50ml) in *A. indica* but similar (2.50ml) in *L. Leucocephala*, *G. sepium* and *G. arborea*. Methane production is said to be energy loss to ruminants and also contribute to global warming resulting to destruction of ozone layer (Babayemi and Bamikole, 2006). In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production (Babayemi *et al.*, 2004; Babayemi and Bamikole, 2006). Dry matter intake of the browse ranged from 0.600kg/DM in *Azadiractha indica* to 5.99kg/DM in *Leuceana leucocephala*. Intake in *Azadiractha indica* (0.600g/DM) was very low but high in *L. leucocephala* (5.99kg) and *G. sepium* (5.54kg). An animal may find a specific feed or forage acceptable or unacceptable (Marten, 1978). Intake of green fodders is affected by plant species, stage of maturity and level of photochemical among others (Kalio, *et al.*, 2006). Coefficient of preference (COP) was used to rank the MPTS in order of preference: *L. leucocephala* > *G. sepium* > *G. arborea* > *A. indica*.

## CONCLUSION

The MPTS were found to have appreciable level of crude protein and may serve as valuable feed supplements to low quality diets. *Gliricidia sepium* and *Leuceana leucocephala* had higher rank value in terms of gas production characteristics and parameters. Thus, the browses have a higher potential nutritive value for ruminants in terms of rumen fermentation. They have potentials to contribute substantially to ruminant animal production in Nigeria.

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