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RESEARCH ARTICLE

IN VITRO GAS PRODUCTION AND ACCEPTABILITY EVALUATION OF SOME PLANT SPECIES IN SOUTHERN GUINEA SAVANNAH ZONE OF NIGERIA BY RED SOKOTO GOATS

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ABSTRACT

This study was conducted to estimate the fermentation characteristics and acceptability of some selected plant species using West African dwarf (WAD) goats in a completely randomized design. The experimental samples were incubated *in vitro* with rumen liquor taken from three WAD goats in 24 h fermentation. The volume of gas produced at the end of incubation was highest in ECA (27 ml/200mg DM.) and lowest in PRA (24 ml/200mg DM.). The methane ranged from 16 to 5.33 in ECA and PRA respectively. The cumulative gas produced and the nutrients composition of the forages were used to predict the Organic Matter digestibility (OMD), Metabolisable Energy (ME) and Short Chain Fatty Acids (SCFA). The values obtained for the OMD, ME and SCFA ranged from 37.44 to 51.95 %, 2.61to 6.80 MJ/Kg and 0.23 to 0.60µmol respectively. The forage acceptability revealed that the daily DM intake increased as the day progressed. *Gliricidia sepium* had the highest DM intake from the inception of experiment while *Prosopis africana* was the least. Coefficient of Preference (COP) varied from 2.20 in GLS to 0.93 in PRA. It was indicated that *Gliricidia sepium* was best accepted amongst the forages. The rank is as follows: GLS > DLO > PAB > ECA > FIC > FIP > PRA. In conclusion, the result in this study showed that *in vitro* gas production and acceptability trial may be useful methods of evaluating feed stuffs in ruminant production to avert feed scarcity experienced during the lean period of the year.

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INTRODUCTION

The challenge of inadequate and nutritive feedstuffs in the southern guinea savannah area of the country has been a perennial issue. This is a region of poor pasture. The livestock, most especially cattle, goat and sheep suffer a set back as the weight gained during surplus period is often lost during the time of feed scarcity (Ogunbosoye *et al*., 2015). Meanwhile profitable and sustainable livestock production depends on many factors among which are regular supply of good quality feeds, housing and health program. Proper nutrition is the pivot on which a productive livestock development in any country stands. The reason is that at any given management disease control, livestock production is a function of quality feeding. For ruminant animals, forage is the cheapest source of feedstuff in the world. The challenge of insufficient feedstuff for ruminant animals through the year due to seasonal variations calls for special attention by the researchers and ruminant farmers in such a way that profit is maximized, taking into consideration the animal welfare. In Nigeria, dry season poses a serious forage management and feeding the livestock becomes very difficult, when available native pastures could no longer supply the minimum nutrients requirement to the grazing animals. At this period, the standing pastures are dried and lignified. Adebambo (2000) reported that animal productivity per head in Nigeria is below global standards with milk and milk products from cattle averaging only 13 and 39 kg/head respectively and 3.5 and 7.9 kg/head from sheep and goats. The consequent effect of this low production on the populace is the poor nutritional status. Browse trees (leguminous and non- leguminous) are important because they provide better quality feed to ruminants throughout the year (Ogunbosoye and Babayemi, 2008). Browse plants are humanly inedible and no competition with other livestock. Ruminants have a unique digestive system that enables them to use a number of non-conventional feedstuffs, which cannot be utilized as nutrient sources by any animal. There are number of forages in Nigeria that could be good feed resources for ruminants but are yet to be earnest for better productivity.

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Therefore, it is pertinent to screen these less utilized feed materials to ameliorate the difficulty often encountered in ruminant animal production. The *in vitro* gas method is one of the techniques use to screen feeds. It is more efficient than the *in sacco* method in evaluating the effects of tannins or other anti-nutritive factors (Makkar *et al*., 1995). It is less expensive, less time consuming and allows incubation conditions to be maintained more precisely than others.

It utilizes small amount of test feeds making them applicable to screening of feeds that are not available in sufficient quantity for *in vivo* method (Getachew *et al*., 2002). Some researchers have also reported that *in vitro* gas method is a good tool for predicting organic matter digestibility, metabolisable energy and short chain fatty acid of any feedstuff (Makkar *et al*., 1999; Babayemi *et al*., 2004., Fievez *et al*., 2005). Preference assessment is also another method that could be used to further evaluate feed samples. The method is a fast and cheaper means of evaluating fodder plants that may be used as feed by ruminant animals in a short period of time using animals (Bamikole *et al*., 2004; Ogunbosoye, 2011), Therefore, this study was designed to evaluate the *in vitro* fermentation characteristics and voluntary intake of some fodder trees that may be relished by ruminants in the Southern guinea savannah region of Nigeria.

MATERIALS AND METHODS

Collection of forages

The legumes (*Parkia biglobosa, Gliricidia sepium, Prosopis africana,*) and non legume (*Daniella oleiveri, Eclipta alba, Ficus polita, Ficus cogensis, Polyalthia longifolia*) browse trees were collected at the peak of dry season February, 2014 from Malete and its environs.

Proximate composition

Crude protein, crude fibre, ether extract and ash concentrations of the plant species were estimated (AOAC, 2005)

In vitro gas production

Rumen fluid was obtained from three West African Dwarf goats through suction tube before the morning feed and sieved with four layered cheese cloth. The animals were previously fed 40% concentrate feed and 60% guinea grass. Incubation was carried out in 120 ml calibrated syringes in three batches at ±39º C. To 200 mg sample in the syringe was added 30 ml inoculum that contained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na2HPO_4 + 0.57g KCl + 0.47g NaCl + 0.12g $MgSO_4$. $7H_20$ + 0.16g $CaCI_2$. $2H_2O$ in a ratio (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 (Menke and Steingass 1988). The average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. The volume of gas production characteristics were estimated using the equation $y = a + b (1 – e^{-ct})$ (Ørskov and McDonald, 1979), where y = volume of gas produced at time't', a = intercept (gas produced from the soluble fraction), b = gas production from the insoluble fraction, (a+b) = final gas produced, c = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy (ME, MJ/Kg DM), organic matter digestibility (OMD %) and short chain fatty acids (SCFA) were estimated at 24h incubation ME = 2.20 + 0.136 GV + 0.057 CP + 0.0029CF (Menke and Steingas 1988). OMD = 14.88 + 0.88GV + 0.45CP +0.651XA (Menke and Steingas 1988). SCFA = 0.0239GV – 0.0601 (Getachew *et al*., 1999). Where GV, CP, CF and XA are net gas production (ml/200mg, DM) at 24 h incubation time, crude protein, crude fibre and ash contents of incubated samples respectively.

Methane production

At the end of 24 hours incubation, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe as reported by Fievez *et al*. (2005). The content was inserted into the silicon tube, which was fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration.

Acceptability study

The browse plants collected at the peak of dry season were introduced to the animals and monitored for 2 hours (08:00-10:00). The collection was done daily for the period of 10 days which the acceptability study lasted.

Experimental site

The experiment was conducted at the goat unit of Teaching and Research Farm of the Department of Animal Production, Kwara State University, Malete.

Experimental goats

Twelve (12) Female Red Sokoto goats with average weight of 25.6 kg were used for the study. The animals (already adapted to the environment) were taken out of their pens and the individual pen was cleaned and disinfected with Asuntol and allowed to rest for two days. The animals were introduced to the forages in four replicates of 3 animals per replicate in a completely randomized design (CRD).

Feeding of Animal

Two kg of each sample of browse plant was air dried over night before feeding to the goat. Feed was served by hanging on the wall of the pen for convenient. The position of feed was changed to prevent adaptation of the animal to particular forage. The forages were fed to the animals at 8.00hrs and withdrawn at 10.00hrs. The left-over of both feeds were weighed and recorded separately. The difference between the quantity offered and the left over was recorded as intake. The feed resources preferred was assessed from coefficient of preference (COP) value, calculated from the ratio between the intake of individual feed resource and the average intake of the feed resources. Forage was inferred to be relatively acceptable provided COP was greater than unity (Bamikole *et al.,* 2004).

Statistical Analysis

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., 1999) options.

RESULTS AND DISCUSSION

Proximate composition

The result revealed that there were variations in the proximate composition of the plant species. The CP values of these forages were very much above the critical range of 8 to 10% reported (Isah *et al*., 2012). However, the 15.34% and 20.74% obtained for *Ficus polita* and *Daniella oleiveri* respectively were within the range of 17.4 to 20.68% reported for browse plants (Isah *et al*., 2012). Meanwhile, all the browse species being examined have their CP levels within the acceptable range (7-14%) for ruminant (Meissner, 1991). It proves that the forages under review can be used as a protein supplement to low quality feed stuffs to enhance rumen microbial activities (Table 1)

Table 1. % Proximate composition of the foliages of plant species

Plant species	CP	CF	EE	Ash
FIC	18.22	25.56	3.68	6.77
ECA	15.86	22.05	6.23	7.36
GLS	20.16	16.20	3.23	9.14
FIP	20.74	24.23	3.86	7.84
PAB	19.90	29.36	3.89	8.23
DAO	15.34	21.64	6.30	7.23
PRA	16.20	17.54	4.02	7.15
POL	17.33	26.74	4.05	6.68

CP = Crude protein, CF = Crude fibre, and EE = Ether extract
Parkia biglobosa (PAB), *Daniella oleiveri* (DAO), *Eclipta alba* (ECA), *Gliricidia sepium* (GLS), *Prosopis africana* (PRA), *Ficus cogensis* (FIC), *Ficus polita* (FIP) and Polyalthia longifolia (PLO)

IN VITRO gas production

Table 2 presents the *in vitro* gas production pattern of the forages. There was a significant variation in the gas volume produced and ranged from 12 (PRA) to 27.67 (ECA) ml/200mg DM. This is an indication that more degradation is still possible after 24 hours of incubation due to rises in the gas at every three hours. The observation in the gas being produced suggests that some of typical forages take longer time to degrade in the rumen, most especially during dry season when most of the forages contain high lignin contents. 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 feedstuff if the donor animal from which rumen liquor for incubation was collected receives high quality feed that supply all the essential nutrients needed. 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 method has also been widely used to evaluate the energy value of several classes of feed (Getachew *et al*., 2002; and Akinfemi 2014). Presented in Fig. 1 is the methane production of forages. Significant differences ($P < 0.05$) were obtained among the plant species. The amount of methane produced depends on the level of fermentation. It varies from 5.33 ml/200mg DM. (PRA) to 16 ml/200mg DM. (ECA). It is observed from this study that forage with high gas production and produces more methane. Methane production is a colossal waste but it is a parameter to measure feedstuff digestibility in the rumen of an animal (Babayemi and Bamikole, 2006; Njidda and Nasiru, 2010). There were a difference ($P < 0.05$) in gas production characteristics among forages (Table 3). Potential gas production (*b*) differed ($P < 0.05$) among plant species. ECA, FIP GLS and FIC recorded higher values of insoluble carbohydrate (b) than the others. The high value of b during fermentation is an indication that there is possibility of further degradation of these forages if incubation time is elongated (Njidda and Nasiru, 2010). This may possibly be influenced by the carbohydrate fractions readily available to the rumen microbial population (Chumpawadee *et al*., 2007). While there was a wide range in rates of gas production (*c*) among feedstuffs but no significant difference was observed among the plant species. Significant difference was also observed in incubation time (t) which ranged from 6 (ECA and GLS) to 20 (PRA). The less gas produced forages had the longest period of fermentation. Many factors may be responsible for this such as high fiber concentration, presence of anti-nutritional factors which hinder digestibility of feedstuffs in the rumen ((Mebrahtu and Tenaye 1997; Iantcheva *et al*., 1999). Organic matter digestibility (OMD), Metabolizable energy (ME), and short chain fatty acids (SCFA) of the forages are presented in Table 4. The values for the OMD, ME and SCFA ranged from 37.44 in PRA and to 51.95 in GLS, 2.61 (PRA) to 6.80 (GLS) and 0.23 in PRA to 0.60 in ECA respectively. The concentrations of OMD and ME obtained in the investigated forages were similar to the values reported elsewhere (Njidda and Nasiru, 2010). Meanwhile, SCFA values observed by these researchers were at variance with what was obtained in this present study. The variation could be as a result of the different nutrient composition of these forages and gas volume produced. Menke and Steingass, (1988) reported that there was a positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with ME value of conventional feeds measured *in vivo*. Akinfemi and Ladipo, (2014) also indicated that the SCFA is an indicator of energy availability to the animal and since higher values were inherent in the forages, it suggests more energy potential for these forages.

Acceptability study

Table 5 shows dry matter intake of forages which significantly different ($p<0.05$) from day 1 to day 10, the highest dry matter intakes shows the high level of consumption rate of the species. GLS has the highest dry matter intake (0.69kg) and PRA has the least value (0.27kg). Forage moisture level has been studied as a possible determinant of voluntary dry matter intake. Minson (1990) observed that feeding either fresh or dried or frozen to sheep shows no significant difference in voluntary intake resulting from method of preparation.

Table 2. Gas volume produced at different hours of incubation of foliages of leaves of forages for ruminants

Forage	3hrs	6hrs	9hrs	12hrs	15hrs	18hrs	21hrs	24hrs
FIC	3.67	7.33ab	10.33a	12.00ab	13.67ab	22.33a	23.67a	26.00a
ECA	3.33	9.33ab	11.67a	12.33ab	13.67ab	19.00a	25.67a	27.67a
GLS	3.00	12.00a	13.33a	14.00a	15.33ab	18.67a	22.00a	25.00a
FIP	1.67	8.00ab	10.33a	13.00ab	17.00a	22.33a	22.33a	24.53a
PAB	2.00	5.67bc	9.67a	8'33abc	10.67b	13.67b	16.33b	17.67b
DAO	1.33	6.33bc	7.00ab	8.00bc	9.33bc	12.00b	15.00d	17.00b
PRA	1.00	2.00c	3.00b	3.00c	4.00c	6.00c	9.00c	12.00c
SEM	0.96	1.43	1.97	1.69	1.86	1.41	1.35	1.20

abc = Means with the same letters within the column are not significantly different (P> 0.05)

Table 3. *IN VITRO* characteristics of foliages plant species for ruminants

Samples	a	a+b	b	c	t	y
PAB	2.00abcd	17.67b	15.67b	0.069	8.00bc	9.00b
ECA	3.33ab	27.67a	24.33a	0.083	15.00abc	19.00a
POL	1.33cd	16.33b	15.00b	0.056	14.00abc	9.00b
DAO	3.00abc	17.00b	14.00bc	0.079	15.00abc	12.00b
FIP	1.67bcd	24.33a	22.67a	0.055	6.00c	8.00b
GLS	3.00abc	25.00a	22.00a	0.088	6.00c	12.00b
FIC	3.67a	26.00a	22.33a	0.082	17.00ab	20.33a
PRA	1.00d	12.00c	11.00c	0.059	20.00a	8.33b

abcd = Means with the same letters within the column are not significantly different (P> 0.05)

Table 4. Calculated OMD (%), ME (MJ/Kg) and SCFA (µmol) of the foliages of forage plants

Forage species	OMD	ME	SCFA
PAB	44.80bc	5.82a	0.36
ECA	51.12a	6.93a	0.60
DAO	41.49cd	5.45a	0.35
FIP	50.90a	6.76a	0.53
GLS	51.95a	6.80a	0.54
FIC	50.41ab	5.95a	0.56
PRA	37.44d	2.61b	0.23
SEM	1.87	0.48	0.57

OMD= organic matter digestibility, ME=Metabolizable energy, SCFA= short chain fatty acids (SCFA)

Table 5. Daily dry matter intake (kg) of the browse species by Red Sokoto Goats

	PAB	GLS	DAO	ECA	FIP	FIC	PRA
Day 1	0.09a	0.06bc	0.08ab	0.06bc	0.04dc	0.01de	0.00e
Day 2	0.11a	0.11a	0.11a	0.09ab	0.07b	0.02c	0.00c
Day 3	0.16a	0.17a	0.1a	0.14ab	0.09bc	0.06c	0.01d
Day 4	0.32a	0.31a	0.26ab	0.18b	0.23ab	0.08c	0.02c
Day 5	0.37ab	0.39a	0.34ab	0.29b	0.30ab	0.09c	0.04c
Day 6	0.39a	0.43a	0.42a	0.43a	0.34a	0.19b	0.05c
Day 7	0.45ab	0.52a	0.43bc	0.46ab	0.37c	0.25d	0.06e
Day 8	0.50a	0.56a	0.48ab	0.52a	0.49bc	0.37c	0.67d
Day 9	0.54abc	0.58ab	0.50bc	0.61a	0.45dc	0.39d	0.10e
Day 10	0.62a	0.69a	0.60ab	0.53ab	0.54ab	0.44b	0.27c

Table 6. Coefficient of preference of foliage of browse

Day	PAB	GLS	DLO	ECA	FIP	FIC	PRA
1	0.96	0.79ab	0.74ab	0.52b	0.00c	0.40c	0.00d
2	1.04	1.20a	1.16a	0.88b	0.34c	0.23c	0.00d
3	1.21	1.29a	1.22a	0.99cb	0.47c	0.67bc	0.02d
4	1.28	1.36a	1.25a	1.04b	0.55c	0.56c	0.07d
5	1.39	1.39a	1.39a	1,14b	0.73c	0.68c	0.08d
6	1.46	1.42a	1.39a	1.17b	0.79c	0.77c	0.12d
7	1.58	1.54a	1.57a	1.22b	0.87c	0.86c	0.18b
8	1.67	1.58a	1.60a	1.25b	0.89c	0.98c	0.31d
9	1.75	1.64a	1.77a	1.34b	1.02c	1.06c	0.51d
10	1.90	2.20a	1.98a	1.43b	1.19bc	1.26bc	0.93c

abcde = Means with the same letters within the column are not significantly different (P> 0.05). *Parkia biglobosa* (PAB), *Daniella oleiveri* (DAO), *Eclipta alba* (ECA), *Gliricidia sepium* (GLS), *Prosopis africana* (PRA), *Ficus cogensis* (FIC), *Ficus polita* (FIP)

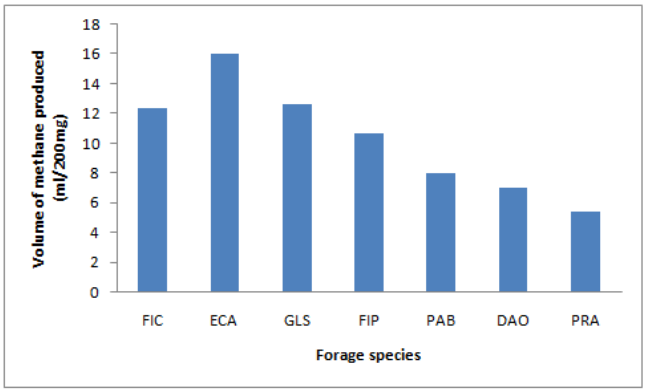


Figure 1. Methane production of the foliages of forage plants

However, moisture levels may be having a secondary role to organic acids or other substances which are found in higher moisture silage and which may influence dry matter and intake in some manner. Presented in Table 6 is the coefficient of preference of the browse plants among the plants. *Gliricidia sepium* (GLS) was highly preferred, this may be because the animals are used to this forage and it is the most existing browse species. PAB, GLS and DAO seemed to have been preferred equally but were not initially consumed in the first day of offering to the animals. During the study period the preference was not affected by the CP, probably because N was not limiting in these animals (Van Soest 1994) as all the tree fodders had CP above requirement level. *Prosopis africana* was least consumed and least preferred despite the fact that it has 16.62% CP which is within the tolerable level and the fibre composition is within the acceptable level (Meissner *et al*., 1991).

Refusal of it might be due to some factors like taste, odour, presence of anti nutrition factors, plant morphology and structure (Ortega and Provenza, 1993; Omokanye *et al* 2001; Babayemi and Bamikole, 2006). The order of preference is GLS>DAO>PAB>ECL>FIC>FIP respectively. The reason for the preferred six tree fodders and refused one might be due to one of the previously mentioned factors but toward the end of the experiment, the intake of *Prosopis africana* was increasing as the days were progressing, indicating that the animals need a few days to get accustomed to the forages. This is in agreement with Grovum (1988) that animals need some time to get used to a new feed. In conclusion, *in vitro* digestibility and acceptability can be considered useful tools for the evaluation of the nutritive value of any feedstuff. These browses are forages with high protein concentrations, moderate *in vitro* DM digestibility and of high acceptance by the goats, suggesting they have potential as forages for farmers during the long period of dry season.

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