

Assessment of Nutritive Value of some Indigenous Plants Consumed by Ruminants in the Humid and Sub-Humid Region of Nigeria using *In Vitro* Technique.

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ABSTRACT

The study was conducted to assess the nutritive quality of twelve indigenous browse plants consumed by small ruminants in the humid and sub humid region of Nigeria. A thorough examination of the chemical composition (DM, CP, CF, Ash EE), fiber fraction (ADF, NDF and ADL), *in vitro* gas production, *in vitro* gas characteristics (organic matter digestibility (%), Metabolisable energy (ME, MJ/Kg DM), short chain fatty acid ($\mu\text{mol}/200\text{mgDM}$), as well as methane production) was investigated. Dried, milled samples of browse plants were incubated using 200mg/30ml inoculum from West African dwarf goats for 3, 6, 9, 12, 15, 18, 21, 24, 36, and 48 hours.

The CP contents of the browse plants ranged from (9.27 –19.63%), the highest value ($P < 0.05$) of CF was observed in *Ficus thonningii* (34.65%) while the least value was recorded in *Tithonia diversifolia* (14.72%). *Newbouldia laevis* was observed to be significantly higher in NDF(66.97%) and ADF (47.72%) among the browse plants. *Tithonia diversifolia* recorded the highest methane production (22.67 ml/200mgDM) while the least methane production was recorded in *Mangifera indica* (18.67 ml/200mgDM). *In vitro* gas production of the various plants varied significantly ($P < 0.05$) from 3 hours to 48 hours of incubation except 9 hours and 15 hours of incubation where no significant variation occurred ($P > 0.05$). Gas production by *Azadiracta indica* was consistently high ($p < 0.05$) throughout the incubation period from 3 – 48 hours. 36 hour gas volume produced was similar ($P > 0.05$) to 48 hour gas volume production in the various plants examined except for *Tithonia diversifolia* which recorded different ($P < 0.05$) value.

(Keywords: ruminants, browse plants, nutrient values)

INTRODUCTION

Browse plants, besides grasses, constitute one of the cheapest sources of feed for ruminants (Ahamefule *et al.*, 2006). These plants are important for animal production owing to their potentially good nutritive value. Ramirez (1998) was in opinion that there is a need for more research into ways of managing browse plants to balance forage quality and quantity. *In vitro* fermentation has been used to evaluate digestibility and nutritional value of feed as it is cheaper, less laborious and most importantly, allows experimental conditions more accurately than the *in vivo* techniques (Getachew *et al.*, 2002; Ajayi and Babayemi, 2008). It also allows a large number of feed samples to be handled simultaneously. It is based on the quantification of substrate degraded and of gas produced in rumen fermentation system based on syringes. The focus of this research was therefore designed to assess the nutritive value of some indigenous plants consumed by ruminants in humid and sub humid region of Nigeria using *in vitro* technique.

MATERIALS AND METHODS

Study location

The leaf samples were collected at the arboretum of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. The study location lies within the savanna agro-ecological zone of southwest Nigeria (Latitude: 7° N, Longitude 3.5°). Abeokuta has an average annual rainfall of 1037 mm. The town also has a bimodal rainfall pattern that characteristically peaks in July and September with a break of 2–3 weeks in August. Temperatures are fairly uniform with daytime values of 28–30°C during the rainy

season and 30 to 34°C during the dry season with the lowest night temperature of approximately 24°C during the Harmattan period (December and February). Relative humidity of the study site is high during the rainy season with values ranging between 63 and 96% as compared to dry season values of 55 to 84% (Anele *et al.*, 2011).

Sample Collection and Preparation

Twelve indigenous forage plants (Table 1) were used for this study. Leaf samples were harvested from mature plants within the arboretum of the Federal University of Agriculture, Abeokuta, Nigeria. Approximately 0.2 kg of the leaves was collected from each plant species.

Analysis of the Browse Samples

The foliage samples were sub-sampled, weighed fresh in the field and then oven-dried to a constant weight at 65°C. The dried foliage samples were hammer-milled through a 1 mm sieve. Crude protein (% Nitrogen X 6.25), ash and ether extract were analyzed according to the standard methods of AOAC (1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.*, (1991). The difference between NDF and ADF was designated as hemicellulose, and the differential between ADF and ADL was labeled as cellulose.

Determination of the *in vitro* Gas Production

The *in vitro* gas production was determined according to Menke and Steingass (1988). West African dwarf (WAD) rams fed a mixed diet of fresh *Panicum maximum* (60% DM) and concentrates (40% DM) daily were used. The concentrate feed consisted of (as fed basis) 4% 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. Feeds were offered in two equal meals at 07:00 and 19:00 h respectively. Rumen fluid was collected prior to feeding with the use of suction tube from three rumen-fistulated West African dwarf (WAD) rams. The fluid was strained through two layers of cheese cloth into a pre-warmed, insulated bottle. All laboratory handling of rumen fluid was carried out under a continuous flow of carbon IV oxide.

Samples (200 mg) of the oven dried and milled leaves were accurately weighed into 100ml glass syringes fitted with plungers. *In vitro* incubation of the samples was conducted in triplicates. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution. Three blanks containing 30 ml of medium only were included. The syringes were placed in a rotor inside the incubator (39°C) with a speed of about one rotation per minute. The gas production was recorded after 3, 6, 9, 12, 15, 18, 21, 24, 36, and 48 hours of incubation.

Methane Determination

The volume of methane gas produced by each browse sample was determined by dispensing 4 ml of 10N sodium hydroxide into each incubated sample at the end of 48 h. of incubation periods. Sodium hydroxide was added to absorb carbon-dioxide produced during the process of fermentation and the remaining volume of gas was recorded as methane according to the method of Fievez *et al.*, (2005).

Determination of Percentage Dry Matter Degradability (DMD)

500 mg samples were weighed into 125-ml Erlenmeyer flasks. The samples were then incubated in a buffered medium containing rumen liquor (40 ml). Dry matter degradability was estimated after 24 and 48 h. incubation; contents of the flasks were then treated with neutral detergent solution according to the procedure of van Soest and Robertson (1985) to obtain NDF. The determination of dry matter degradability was calculated thus: %DMD = 100 – neutral detergent residue.

Calculations and Statistical Analysis

The data obtained from *in vitro* gas production was fitted to the nonlinear equation (Larbi *et al.*, 1996): $V \text{ (ml/0.2 g DM)} = GV (1 - e^{-ct})$ where V is the potential gas production, GV is the volume of gas and c is the fractional rate of gas production. Other post incubation parameters determined were:

i) Organic matter digestibility (OMD) was estimated as:

OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 ash (Menke and Steingass, 1988).

ii) Total gas volume (GV) is expressed as ml/0.2 g DM, CP and ash as g/kg DM.

Data collected from the chemical composition, gas production kinetics, percentage dry matter degradability, and organic dry matter degradability were subjected to one-way analysis of variance (ANOVA) procedure using SAS (1990). Significant differences between individual means were determined using the Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical Composition

Result of proximate composition (Table 1) revealed that *P. maximum* which is a tropical natural grass in humid and sub humid region of Nigeria recorded ($P < 0.05$) least CP value (9.27%) and the highest CP content was recorded in *M. aegyptia* (19.63%). CF was significantly ($P < 0.05$) low and fall within the range of 14.72% and 34.64% in *T. diversifolia* and *F. thonningii* respectively. Ash content in *M. indica* was significantly ($P < 0.05$) higher (13.66%) than other plants.

There were significant ($P < 0.05$) variations in the NDF value ranging from 36.08 – 66.97% while least ADF value of 34.47% was recorded for *M. indica* and highest value for *N. laevis* (47.72%). Highest ADL (34.53%) was recorded in *T. diversifolia* lowest ADL (6.46%) was observed in *G. arborea*, *C. odorata* recorded intermediate value (15.55). The high value of CP recorded in all the browse plants is an indication that these various browse plants could serve as potential protein supplements to enhance the intake and utilization of low quality grass and fibrous crop residues by ruminants. This revealed that more forages could be added to farmers feed resource base from local resources available in the study area.

The result is in consonance with the report of Getachew *et al.*, (2000) which stated that browse forages are higher in CP than tropical grasses and roughages such as hay, straw and stover. However, the CP content of the various plants studied were all above 8% CP required to satisfy maintenance requirement of ruminant animals

(Norton, 2003) and above the minimum level necessary to provide sufficient nitrogen required by rumen microorganisms to support optimum activity (Mc Donald *et al.*, 2002) and for adequate intake of forages. (Ranjhnan (2001) have shown that intake of forages is limited when their CP content is less than 10%.

Voluntary feed intake also rapidly falls if CP content of forages is below 6.2% (Nasrullah *et al.*, 2003). The optimum concentration level of rumen bacteria is reached at a CP level of 13.0% CP in the diet. The crude fiber content of the various browse plants is within the range of 15-20% CF recommended for improved intake and production in finishing ruminants (Buxton, 1996), except for *F. thonningii* and *Panicum maximum*. Also, the NDF contents of the different browses except *Newbouldia laevis*, *Ficus thonningii* and *Panicum maximum* are within the range of 55- 60% threshold level in tropical grasses beyond which DM intake is affected (Meissner *et al.*, 1991). Tree forages with low NDF content of 20 – 35% are usually of high digestibility (Norton, 1994). Feeds that contained high proportion of ADF have low availability. Ether extracts content of browses fall within the range of 4 – 10% EE recommendation (Preston, 1995 and Campbel *et al.*, 2006).

In vitro Gas Production

In vitro gas production of the various plants (Table 2) varied significantly from 3 hour to 48 hours of incubation except 9 hours and 15 hours of incubation where no significant variation occurs. At 3 hours incubation *Spondia mombin* had highest ($P < 0.05$) volume of gas production (2.00 ml/200mg) while *Ficus thonningii* did not record any gas production. However, *Mangifera indica* produced high volume of gas from 3 hours incubation until 36 hours of incubation after which no more gas was added.

Ficus thonningii commenced gas production at 6 hours of incubation and ended up producing the highest ($P < 0.05$) volume of gas (17.00 ml/200mg) at 48 hour of incubation. Gas production by *Azadiracta indica* was consistently high ($P < 0.05$) throughout the incubation period from 3 – 48 hours. 36 hour gas volume produced was similar ($P > 0.05$) to 48 hour gas volume production in the various plants examined except for *Tithonia diversifolia* which recorded different ($P < 0.05$) values.

Gas volume produced by *Panicum maximum* was consistently low ($P < 0.05$) throughout the incubation period.

The variation in cumulative gas production at 48 hour observed could be attributed to differences in their CP and fiber component. This is a reflection of the amount of substrate organic matter fermentation and production of Volatile Fatty Acids.

Significant increase in the volume of gas produced in almost all the browse plants could be attributed to the high level of crude protein content. Gas production is positively related to microbial protein synthesis (Hillman et al., 1993) while Marricco et al., (1990) and Murrillo et al., (2011) were of the opinion that gas production is a wasteful product but still it could be used to predict ME, OMD and SCFA of feedstuffs.

The amount of gas produced is also affected by the nature of feed and presence of secondary metabolites. However, the presence of secondary metabolites was not determined in this study (Babayemi et al., 2004a). Generally gas production is a function and a mirror of degradable carbohydrate and the amount depends on the nature of the carbohydrate (Demeyer and Van Nevel, 1975, Blumanel, 1997).

Methane Production

Methane production from the various browse plants are shown in Figure 1. *Mangifera indica* produced least ($P < 0.05$) volume of methane gas (18.67 ml/200mgDM) while *Tithonia diversifolia* (22.67 ml/200mgDM) produced the highest ($P < 0.05$) volume similar ($P > 0.05$) to that produced by *Azadiracta indica* (22.33 ml/200mgDM) and *Panicum maximum* (22.00 ml/200mgDM). Isah et al., (2012) reported lower *In vitro* methane production from *Azadiracta indica* and *Ficus exasperata* this might be as a result of the fiber content of the browse due to the seasonal effect. In this study, it was observed that feedstuffs with high NDF content and high IVDMD showed high methane production.

Methane production is an energy loss to ruminant and also has environmental implication on the greenhouse gas contributing to global warming (Johnson and Johnson, 1995).

***In vitro* Gas Production Characteristics:** As presented in Table 3, the result of the *In vitro* gas production characteristics (OMD SCFA, IVDM and ME) of the various plants estimated from gas production. Highest ($P < 0.05$) IVDMD was observed in *Chromolena odorata* (68.67%) while *Mangifera indica* recorded least value of IVDMD (33.33%) Albizia saman produced lowest ($P < 0.05$) SCFA, *Ficus thonningii* (0.35) and *Azadiracta indica* (0.32) had highest but similar values of SCFA. Also *in vitro* organic matter digestibility (OMD) of *Azadiracta indica* (44.11%) and *Ficus thonningii* (44.17%) was highest while *Newbouldia laevis* (35.68%) recorded least value of OMD.

Estimated ME values of the various browse plants ranged from (4.44) in *Newbouldia laevis* to (5.41) in *Ficus thonningii*. The ME, OMD and SCFA observed among browse plants were lower than the report of Babayemi et al., (2009) on some forage seeds. The ME, OM and SCFA could be translated to DM intake in ruminants. When IVDMD falls below 55.0%, physical limitation on the rate of eating, rate of digestion and passage through the gastro intestinal tract is restricted (SCA, 1990) it has been reported that cell wall components, NDF, ADF, and lignin were negatively correlated with IVDMD in tree leaves (Kundu and sharma,1988, Perveen, 1998).

Tree forages with a low NDF content are usually of high digestibility (Norton, 1994); High ADL can limit voluntary feed intake, digestibility and nutrient utilization of ruminant animals (Khanal and Subba, 2001).

Table 1: Nutrient composition of the various Browse plants.

Sample	DM	CP	CF	ASH	EE	NDF	ADF	ADL
<i>Newbouldia laevis</i>	90.05 ^e	12.67 ^g	15.37 ^e	8.41 ^c	12.59 ^a	66.97 ^a	47.72 ^a	12.88 ^c
<i>Albizia saman</i>	90.52 ^{de}	17.26 ^c	16.29 ^e	11.38 ^{ab}	4.87 ^d	48.93 ^g	37.68 ^e	6.52 ^h
<i>Gmelina arborea</i>	91.57 ^{bc}	18.25 ^b	15.56 ^e	11.95 ^{ab}	5.60 ^d	46.51 ^h	35.34 ^f	6.46 ^h
<i>Spondia mombin</i>	92.39 ^a	15.44 ^e	17.58 ^e	8.36 ^c	10.36 ^b	54.32 ^d	39.63 ^d	7.59 ^g
<i>Mangifera indica</i>	91.66 ^{abc}	12.77 ^g	27.45 ^c	13.66 ^a	5.59 ^d	47.06 ^h	34.47 ^g	13.24 ^c
<i>Azadiracta indica</i>	91.88 ^{ab}	16.18 ^d	16.52 ^e	11.68 ^{ab}	5.47 ^d	50.87 ^f	39.00 ^d	7.49 ^g
<i>Ficus exasperate</i>	91.67 ^{abc}	19.38 ^a	16.48 ^e	11.84 ^{ab}	6.74 ^c	36.08 ^j	33.07 ^h	7.79 ^g
<i>Tithonia diversifolia</i>	91.95 ^{ab}	19.39 ^a	14.72 ^e	12.58 ^a	4.75 ^d	45.43 ⁱ	40.42 ^c	34.53 ^a
<i>Chromolena odorata</i>	91.97 ^{ab}	18.10 ^b	15.30 ^e	13.33 ^a	2.65 ^e	51.99 ^e	42.49 ^b	15.55 ^b
<i>Merremia aegyptia</i>	90.89 ^{cd}	19.63 ^a	20.65 ^d	11.40 ^{ab}	7.15 ^c	55.51 ^c	38.27 ^e	11.29 ^e
<i>Ficus thonningii</i>	90.91 ^{cd}	14.34 ^f	34.65 ^a	12.02 ^{ab}	10.47 ^b	64.19 ^b	47.29 ^a	12.15 ^d
<i>Panicum maximum</i>	91.89 ^{bc}	9.27 ^h	31.25 ^b	9.78 ^{bc}	2.80 ^e	64.46 ^b	39.50 ^d	9.51 ^f
SEM	0.12	0.53	1.15	0.34	0.51	1.47	0.74	1.24

^{Abcdeghij} Means on the same column with different superscripts are significantly different (P<0.05)

Table 2: *In vitro* Gas Production (ml 200/200mgDM) of the Various Browse Plants Incubated at Forty-Eight Hours.

Forages	3h	6h	9h	12h	15h	18h	21h	24h	36h	48h
<i>Newbouldia laevis</i>	1.33 ^{abc}	2.00 ^{abc}	3.33	4.00 ^{ab}	5.67	7.33 ^{bc}	9.00 ^c	9.67 ^b	11.00 ^{de}	11.00 ^d
<i>Albizia saman</i>	1.00 ^{abc}	2.00 ^{abc}	3.33	4.00 ^{ab}	5.67	7.00 ^{bc}	8.00 ^c	10.00 ^b	10.67 ^{de}	10.67 ^d
<i>Gmelina arborea</i>	1.33 ^{abc}	2.67 ^{abc}	4.00	5.67 ^{ab}	7.00	8.67 ^{abc}	10.33 ^{abc}	11.67 ^{ab}	13.00 ^{abc}	13.00 ^c
<i>Spondia mombin</i>	2.00 ^a	3.33 ^{ab}	4.33	6.00 ^{ab}	7.33	8.67 ^{abc}	9.67 ^{bc}	11.00 ^b	10.67 ^{de}	11.67 ^d
<i>Mangifera indica</i>	1.67 ^{ab}	3.67 ^a	4.00	6.33 ^a	8.67	10.33 ^a	12.67 ^a	13.33 ^a	14.00 ^{ab}	14.00 ^{bc}
<i>Azadiracta indica</i>	1.67 ^{ab}	3.00 ^{abc}	4.33	5.67 ^{ab}	8.67	9.33 ^{ab}	12.00 ^{ab}	13.33 ^a	14.33 ^a	16.00 ^{ab}
<i>Ficus exasperate</i>	1.33 ^{abc}	2.33 ^{abc}	3.33	4.67 ^{ab}	6.33	6.67 ^c	9.33 ^{bc}	10.33 ^a	12.33 ^{bcd}	12.33 ^{cd}
<i>Tithonia diversifolia</i>	1.00 ^{abc}	2.00 ^{abc}	3.67	4.67 ^{ab}	6.67	7.33 ^{bc}	8.67 ^c	9.67 ^b	10.33 ^e	10.67 ^d
<i>Chromolena odorata</i>	1.33 ^{abc}	1.33 ^c	4.00	5.33 ^{ab}	6.33	8.00 ^{abc}	9.67 ^{bc}	10.67 ^b	12.00 ^{cde}	12.00 ^{cd}
<i>Merremia aegyptia</i>	1.00 ^{abc}	1.33 ^c	3.33	5.00 ^{ab}	6.67	6.67 ^c	9.00 ^c	9.67 ^b	10.67 ^{de}	11.00 ^d
<i>Ficus thonningii</i>	0.00 ^c	2.00 ^{abc}	3.33	6.00 ^{ab}	7.00	8.00 ^{abc}	9.33 ^{bc}	10.67 ^b	13.00 ^{abc}	17.00 ^a
<i>Panicum maximum</i>	0.33 ^{bc}	1.67 ^{bc}	2.67	3.67 ^b	7.67	6.33 ^c	8.33 ^c	10.33 ^b	11.00 ^{de}	12.00 ^{cd}
SEM	0.135	0.181	0.223	0.227	0.277	0.271	0.301	0.274	0.266	0.374

^{Abcde} Means on the same column with different superscripts are significantly different (P<0.05)

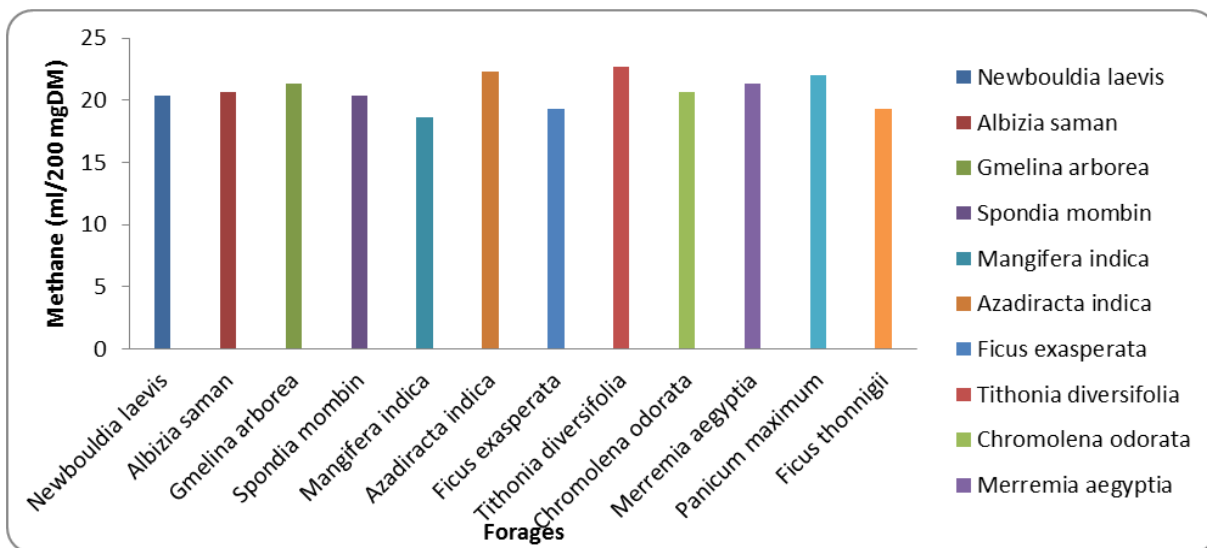


Figure 1: Methane Production from the Various Browse Plants.

Table 3: *In vitro* Gas Production Characteristics of the Various Browse Plants.

Samples	ME (MJ/KgDM)	OMD (%)	SCFA (mmol)	IVDMD (%)
<i>Newbouldia laevis</i>	4.44 ^t	35.68 ^e	0.20 ^{de}	43.33 ^{ab}
<i>Albizia saman</i>	4.68 ^{def}	40.30 ^c	0.19 ^e	40.00 ^{ab}
<i>Gmelina arborea</i>	5.05 ^{bc}	42.89 ^{ab}	0.25 ^{cd}	53.33 ^{ab}
<i>Spondia mombin</i>	4.74 ^{de}	38.03 ^d	0.22 ^{de}	56.67 ^{ab}
<i>Mangifera indica</i>	4.90 ^{cd}	42.99 ^{ab}	0.03 ^{bc}	33.33 ^b
<i>Azadiracta indica</i>	5.34 ^{ab}	44.11 ^a	0.32 ^{ab}	39.67 ^{ab}
<i>Ficus exasperate</i>	5.06 ^{bc}	43.04 ^{ab}	0.24 ^{cde}	53.33 ^{ab}
<i>Tithonia diversifolia</i>	4.81 ^{cd}	41.77 ^{bc}	0.19 ^e	58.33 ^{ab}
<i>Chromolena odorata</i>	4.93 ^{cd}	43.30 ^{ab}	0.23 ^{cde}	68.67 ^a
<i>Merremia aegyptia</i>	4.87 ^{cd}	40.98 ^c	0.20 ^{de}	56.67 ^{ab}
<i>Ficus thonningii</i>	5.41 ^a	44.17 ^a	0.35 ^a	68.33 ^{ab}
<i>Panicum maximum</i>	4.47 ^{ef}	36.36 ^{de}	0.23 ^{cde}	51.00 ^{ab}
SEM	0.05	0.50	0.01	2.93

^{abcdet} Means on the same column with different superscripts are significantly different (P<0.05)

CONCLUSION

In the present study the high CP content observed in all the browse plants indicates that the plants have the potential of being used as protein supplement by ruminants fed low quality roughages especially during the dry season, because of their high protein content. Browse intake improves digestibility of low quality feeds and leads to an overall increase in intake of digestible dry matter. Chemical composition and *in vitro* digestibility can be considered as useful indicators for the preliminary evaluation of the likely nutritive value of the browse plants These browse plants have potential as a forage for farmers during the long period of dry season when grasses are unavailable.

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