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In vitro Gas Production and Dry Matter Digestibility of Tannin-Containing Forges of Semi-Arid Region of North-Eastern Nigeria

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Abstract: A study was conducted to determine the chemical composition, *in vitro* dry matter digestibility and *in vtro* fermentation of four dorminant browse species in the region of experimentation (*Leuceana lecocephala, Moringa Oleafera, Acacia tortilis* and *Ziziphus mucronata*). Crude Protein (CP) (from 13.96% DM for *A. tortilis* to 19.42% DM for *L. leucocephala*) except *M. oleifera* which had the highest (21.42% DM). The range of lignocelluloses (acid detergent fibre, ADF) was from 21.16 g/100 g DM for *A. tortilis* to 31.39 g/100 g DM for *M. oleifera*. The netral detergent fibre ranged from 33.31 g/100 g DM for *M. oleifera* to 58.81 g/100 g DM. CF, EE and ash had a range of 20.00% DM for *Z. mucronata* to 32.53% DM for *M. oleifera*, 3.03% DM for *A. tortilis* to 5.33% DM for *Z. mucronata* and 10.76% DM for *A. tortilis* to 17.76% DM for *L. leucocephala*. The Total Condensed Tannin (TCT) for the browses ranged from 0.25 mg/g DM for *M. oleifera* to 2.96 mg/g DM for *L. leucocephala*. *Z. mucronata* had the highest value for Calcium (Ca), Magnesium (Mg), Sodium (Na) and Potassium (K) except for phosphorus. *In vitro* gas and methane production was highest in *Z. mucronata*. The IVDMD ranged from 70.66-72.00%. CP and TCT showed a positive relationship with IVDMD.

Key words: *In vitro*, Browse, semi-arid, tannin, digestibility, forages

INTRODUCTION

The utilization of browse is limited by the high lignin content and the presence of anti-nutritional factors, which may be toxic to ruminants. Many browse species have chemicals that appear to be produced for the purpose of deterring invasion or consumption of their leaves by microbes, insects and herbivorous animals. The most important cited is tannin, which is shown to decrease the digestibility in browse fodders. Tannins are a group of polyphenol substances with the ability to bind protein in aqueous solution. They are classified into two groups: Hydrolyzable or condensed tannins and are considered to have both adverse and beneficial effects depending on their concentration and nature and also animal species, physiological state of the animal and the composition of the diet (Makkar, 2003). Silanikove *et al.* (1996) concluded that goats have the ability to consume large amounts of tannin rich plants without exhibiting toxic syndromes (due to a detoxifying enzyme in the saliva), which is not the case for other ruminant species. The negative effect of tannin is seen in lowered feed intake, directly due to the astringent properties of tannin rich feed and indirectly by reducing the digestibility of the feed. However, the level of digestibility reduction varied depending on the level and the activities of tannin (Ebong, 1995). A level of tannin below 5% seems to be tolerable for ruminant animals. While tannins are the best known of the anti-nutritional factors of browse, there is a long list of secondary compounds: cyanide, nitrate, fluoroacetate, cyanogenic glucosides, saponins, oxalates, mimosine and various sterols (Leng, 1997). However, the toxic compounds seem to become of significance nutritionally only when the plant constitutes a high proportion of the diet. Hence, the effects of high protein forage could override the effect of the toxic compounds when used as supplement in the diets.

The *in vitro* gas production technique as modified by Menke and Steingass (1988) is widely used to evaluate the nutritive value of feeds resources consumed by ruminants especially tree and shrub legume forages, particularly to estimate energy value of straws (Makkar *et al.* (1999), agro industrial by-products (Krishna and Gunther, 1987), compound feeds (Aiple *et al.*, 1996) and various types of tropical feeds (Krishnamoorthy *et al.*, 1995). The use of *in vitro* gas method to estimate the digestion of feed is based on measured relationships between the *in vitro* digestibility of feeds and *in vitro* gas production, in combination with the feeds chemical composition (Menke and Steingass, 1988). The main objective of the present study was to investigate changes in chemical composition and *in vitro* digestibility of leaves of semi-arid browses. The present study therefore examines the nutrient composition, *in vitro* gas production and *in vitro* dry matter digestibility of some browsable forages in semi-arid region of Nigeria.

MATERIALS AND METHODS

Forage samples: Four indigenous browse samples (leaves) were used in this study. The species were: *Leuceana lecocephala, Moringa Oleafera, Acacia tortilis* and *Ziziphus mucronata*. All forages were harvested from Gwoza local government area of Borno State, Nigeria. The area is located at 11.05° North and 30.05° East and at an elevation of about 364 above sea level in the North Eastern part of Nigeria. The ambient temperature ranges between 30°C and 42°C being the hottest period (March to June) while its cold between November to February with temperatures ranging between 19-25°C. The browse forages were harvested from at least 10 trees per each specie selected at random in four locations with the study area at the end of the season. The harvested sample were then pooled for each individual tree species and then oven dried at 105°C for 24 h to constant weight and ground to pass through a 1.0 mm, sieve. The samples were then sub-sample to obtain three samples for each tree species and used for the laboratory analysis.

Chemical analysis: Browse species were analyzed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crue Fibre (CF) and ash according to AOAC (2005). The leaves samples were analyzed for Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), 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 West African Dwarf (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 brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was as reported (Fievez *et al.*, 2005) using 120 ml calibrated syringes in three batch incubation at 39°C. Into 200 mg sample (n = 4) in the syringe was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$ + 3 Na_2HPO_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, 24, 27, 30, 33, 36, 39, 42, 45 and 48 h. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Methane production: In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 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.

***In vitro* Dry Matter Digestibility (IVDMD):** After 24 h digestion, the samples were transferred into test tubes and centrifuge 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 65°C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

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

Statistical analysis: Metabolizable 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 assess 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 proximate composition of the leaves of the browse forages is presented in Table 1. 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, those of Rittner and Reed (1992), Makkar and Becker (1998) and Njidda *et al.* (2009) 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. Calicium (Ca), Phosphosrus (P) and Magnesium (Mg) are within the range reported by Njidda *et al.* (2009) for semi-arid browses while Sodium (Na) and Potassium (K) falls within the range reported by Bamikole (2003). Generally, the minerals were within the range values and are adequate to meet the requirement for growth, reproduction and milk in sheep and goat as reported by Babayemi (2006).

The highest cumulative gas production was observed in *Ziziphus muconata* and lowest in *Acacia tortilis*. The variation in gas production between the browse species forages can be attributed to compositional differences of the browse forages, especially CP and fibre and may be other anti-nutritional components. These factors influence the amount of substrate OM that is fermented and the Short Chain Fatty Acids (SCFAs) produced upon fermentation. Other reason may be due to low NFE content for browses which has a positive correlation with gas production. On the other hand, cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. This may tend to reduce the microbial

Table 1: Proximate composition of some selected browse species forages of semi-arid (% DM); NDF, ADF (g/100 g DM); TCT (mg/g)

Browse forages	DM	CP	CF	EE	Ash	NFE	NDF	ADF	TCT
Acacia tortilis	72.33[c]	13.96[c]	30.50[b]	3.03[c]	10.76[c]	14.06[c]	48.62[b]	21.16[c]	0.32[b]
Leucaena leucocephala	89.63[b]	19.42[b]	31.50[a]	3.46[c]	17.96[a]	17.29[b]	58.81[a]	25.52[b]	2.96[a]
Moringa oleifera	88.22[b]	21.42[a]	32.53[a]	4.16[b]	17.60[a]	12.51[d]	33.31[c]	31.39[a]	0.25[b]
Ziziphus mucronata	92.26[a]	19.23[b]	20.00[c]	5.33[a]	15.43[b]	29.27[a]	58.67[a]	22.89[c]	0.72[b]
Means	85.61	18.51	28.63	3.99	15.44	18.28	49.85	25.24	1.06
SEM	2.19	0.73	0.89	0.26	0.95	1.66	3.42	0.97	0.55

[a,b,c]Means in the same column with different superscript differ significantly ($p<0.05$). Dry matter; CP = Crude Protein; EE = Ether Extract NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; TCT = Total Condensed Tannin

activity through increasing the adverse environmental conditions as incubation time progress. This is consistent with De Boever *et al.* (2005), who reported that gas production was negatively related with NDF content and positively with starch. The tannin content of the browse are low 0.25-2.96 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 tannins when forages containing low levels of tannins (Barry *et al.*, 1986) are fed could be due to the protection of protein from microbial degradation by tannins, thus increasing the amount of undegraded protein entering the small intestine. In addition, a higher flow of microbial protein to the intestine as a result of higher efficiency of microbial protein synthesis (Getachew *et al.*, 2000) has been observed. However, higher concentration of tannins in the diet is associated with reduction in organic matter digestibility (Silanikove *et al.*, 1997; Waghorn and Shelton, 1997). 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 got the nutrient requirement met. 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 Fig. 1 indicated that more degradation of dry matter were still possible beyond 48 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 *Ziziphus mucronata* for reason that was not clear since the secondary metabolites are all within the normal ranges 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. Methane (ml/200 mg DM) production (Fig. 2) ranged from 5-17 among the forages, the least and the highest being from

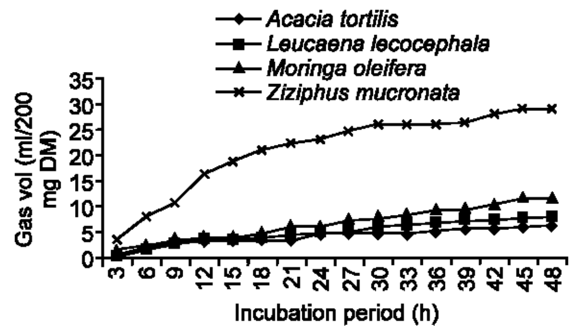

Fig. 1: *In vitro* gas production of semi-arid browse forages

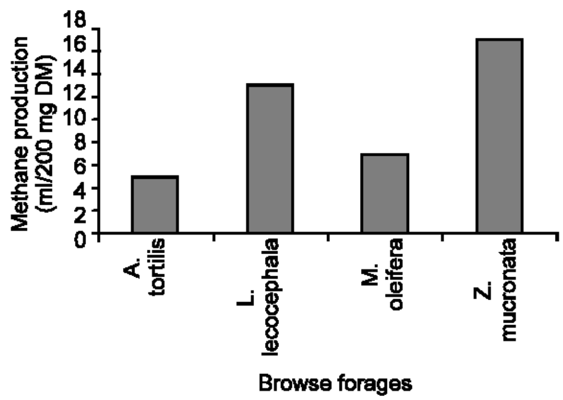

Fig. 2: Methane production of semi-arid browse forages

Z. mucronata and *A. tortilis* , 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 ruminant and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi and Bamikole, 2006) as an integrated part of carbohydrate metabolism (Demeyer and Van Nevel, 1975).

***In vitro* dry matter digestibility:** The result of the IVDMD is shown in Fig. 3. IVDMD was higher for all browses with *M. oleifera* having the highest (72%). 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 tannin in the browse plants which suggest that it could be a valuable protein supplement

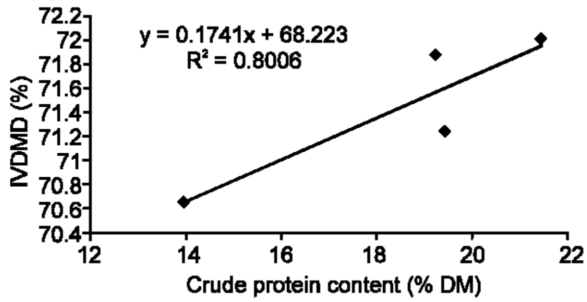

Fig. 3: Relationship between IVDMD and CP of semi arid browses

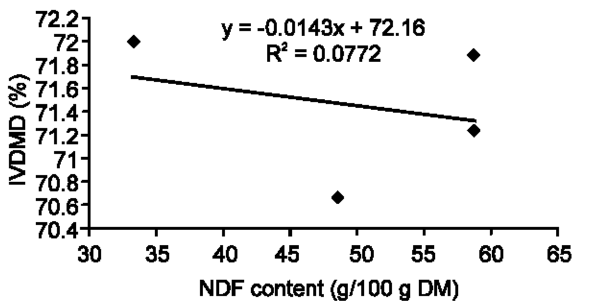

Fig. 4: Relationship between semi arid IVDMD and NDF of semi-arid browses

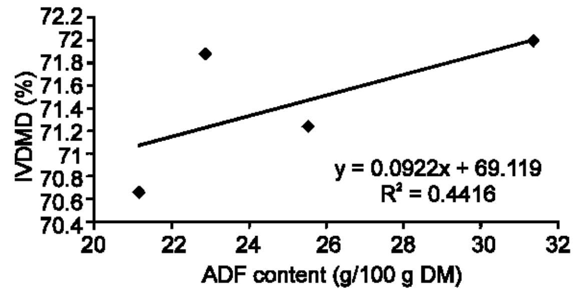

Fig. 5: Relationship between IVDMD and ADF of semi-arid browses

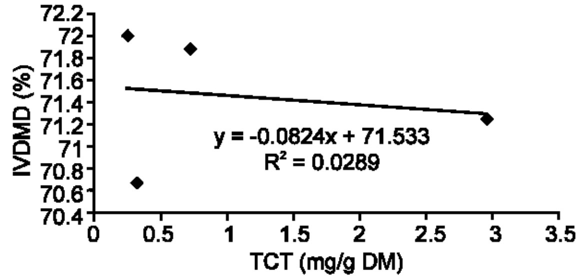

Fig. 6: Relationship between IVDMD and TCT of semi-arid browses

in ruminant diets (Aganga and Mosase, 2001). This was further manifested in methane production (Fig. 2) where methane production showed inhibitory features. This suggest that feeds containing high level of CP and low levels of tannin could generate more methane in the rumen. The *in vitro* DMD of leaves of all the browses under study were generally higher.

IVDMD and its relationship to chemical composition: Crude protein was positively correlated to IVDMD (R^2 = 0.0289, n = 4). CP in the present study is in the level permissible for optimal feed intake and rumen function considering the ranges of IVDMD (70.66-72.00%). A positive correlation between IVDMD and CP indicate that as the crude protein increase, there was an improvement in IVDMD. There were significant ($p<0.05$) negative correlations between *in vitro* DMD and cell wall contents (NDF and ADF) (Fig. 4 and 5). This result is consistent with the findings of Seresinhe and Iben (2003) and Ammar *et al.* (2004), indicate that the cell wall indices in the present group of samples were relatively poor predictors of IVDMD. Madibela and Modiakgotla (2004) reported that ADF has a negative effect on energy content of forages and this was consistent with a highly negative correlation observed between ADF and IVDMD. Irrespective of the maturity stage, leaves were always more digestible than stems, in agreement with Lambert *et al.* (1989). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification, as lignin is indigestible fraction and acts as a barrier limiting the access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and lignin were significant and negatively correlated with *in vitro* digestibility. It is well established that a low content of poorly digestible cell wall components (ADF and ADL) and a high CP content are indicators of a good forage quality (Van Soest, 1994). Therefore, at the light of our results, leaves have a higher nutritive value than stems. There was a positive correlation between *in vitro* DMD and TCT (r = 0.98, n = 4) (Fig. 6). The result is consistent with findings of Frutos *et al.* (2002) and Seresinhe and Iben (2003).

Gas production parameters: Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA) of the browse forages are presented in Table 3. The value for the ME, OMD and SCFA ranged from 3.31 in *L. leucocephala* to 6.23 (MJ/Kg DM) in *Z. mucornata*, 30.64 in *L.leucocephala* to 55.44 (%) in *Z. mucornata*, -0.03 in *L.leucocephala* to 0.55 (mmol) *Z.mucornata,* 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 with certain anti-nutritive factor had been reported to be low in metabolizable energy and organic matter digestibility (Aregheore and Abdulrazak, 2005).

Table 2: Composition of macro minerals (% DM)

Browse forages	Ca	P	Mg	Na	K
Acacia tortilis	0.78[bc]	26.57[b]	0.25[c]	0.20	2.00
Leucaena leucocephala	0.90[b]	10.25[c]	0.17[d]	0.30	2.20
Moringa oleifera	1.06[a]	30.52[a]	0.40[b]	0.30	2.10
Ziziphus mucronata	1.24[a]	11.25[c]	0.72[a]	0.50	2.22
Means	0.99	19.64	0.39	0.32	2.13
SEM	0.04	3.52	0.05	0.06NS	0.54NS

[a,b,c]Means in the same column with different superscript differ significantly ($p<0.05$).

Ca = Calcium; P = Phosphorus; Mg = Magnesium; Na = Sodium and K = Potassium

Table 3: Net Gas volume, metabolizable energy, organic matter digestibility, short chain fatty acid of semi-arid browse forages

	Gas production parameters				
Browse forages	NGV	ME	OMD	SCFA	IVDMD
Acacia tortilis	2.83[c]	3.47[c]	30.68[b]	0.01[c]	70.66
Leucaena leucocephala	1.16[c]	3.31[c]	30.64[b]	-0.03[d]	71.24
Moringa oleifera	8.16[b]	4.33[b]	40.94[a]	0.13[b]	72.00
Ziziphus mucronata	25.50[a]	6.23[a]	55.44[a]	0.55[a]	71.88
Means	9.41	4.33	39.42	0.17	71.44
SEM	2.43	0.28	3.35	0.06	0.19NS

Net Gas Volume (NGV = ml/200 mg DM), Metabolizable Energy (ME = MJ/Kg DM), Organic matter digestibility (OMD = %), Short Chain Fatty Acids (mmol), IVDMD = *In vitro* dry matter digestibility (%), CH_4 = ml/200 mg DM)

The predicted ME profile were varied in four forages particularly high in *Z.mucronata*. *A. tortilis* and *L. leucocephala* had significantly lower values of ME. The data showed that there was no significant difference among *A. tortilis and L. leucocephala*. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1988). The Organic Matter Digestibility (OMD) was highest for *Z. mucronata* and lowest for *A. tortilis* and *L. leucocephala* though there was no significant difference ($p<0.05$) between *M. Oleifera and Z. mucronata*. The OMD was no significant difference among *M. Oleifera and Z. mucronata* and *A. tortilis* and *L. leucocephala*. Menke *et al.* (1979), Steingass and Menke (1986), Menke and Steingass (1988) and Chenost *et al.* (1997) concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. Also, there are significant correlation between *in vitro* gas measurement and *in vivo* digestibility. 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* (Menke and Steingass, 1988). Ranges of gas production characteristics reported in this study may partly due to difference in CP, NDF and ADF contents. 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. Cone and Van Gelder (1999) used different proportions of casein and carbohydrate sources (glucose and starch) and reported a poor correlation between gas measured and calculated from SCFA. These poor correlations could be due to the highly fermentable carbohydrate sources that drastically changed the molar proportions of SCFA, indicating the pattern of fermentation of pure substrate does not reflect the normal fermentation pattern that occurs in the rumen.

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

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