



Evaluation of chemical composition and *in vitro* gas production of browses collected from Tanzania

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Abstract

Browse plays an important role in providing feed for livestock in tropics. Chemical composition and *in vitro* gas production of nine browse species commonly utilized by smallholder farmers in Mvomero district Morogoro, Tanzania, are presented. The forages had a wide range of crude protein content ranging from 89.73 in *C. africana* to 276.0 g kg⁻¹ DM in *A. tortilis*. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) varied significantly (P<0.05) among the browse species. High contents (g kg⁻¹ DM) were recorded in *L. camara* for NDF (666.6), ADF (555.6) and ADL (229.71). *Balanites aegyptiaca* had the highest (g kg⁻¹ DM) hemicellulose (206.02) and the lowest cellulose (75.45) contents. The browse species varied widely in the phenolics compositions. The highest total extractable phenolics and total extractable tannin (TEPH and TET) values were recorded in *A. nilotica* (168.36 and 176.15 mg g⁻¹ DM, respectively), while the lowest values were recorded in *B. aegyptiaca* (11.34 and 11.89 mg g⁻¹ DM, respectively). *In vitro* gas production (ml gas/200 mg DM) at 96 h ranged from 41.51 in *S. siamea* to 23.3 in *A. tortilis*. *Commiphora africana* had the highest potential gas production and *B. aegyptiaca* had high rate of gas production. Addition of polyethylene glycol (PEG = 6000) increased 24 h gas production, organic matter degradability and metabolizable energy in *C. africana*, *A. tortilis* and *A. nilotica*. Based on chemical composition, gas production and *in vitro* degradability, the studied browse forages have high potential nutritive value, especially as protein supplements to low quality forages in the tropics. However, presence of tannins in some species adversely affects their potential.

Key words: Browse species, chemical composition, gas production, metabolizable energy, organic matter degradability.

Introduction

Browse species play an important role in maintaining year-long productivity of livestock in the tropic dry areas, especially in the dry season. During these dry periods, forage trees remain green and maintain a relatively high crude protein (CP) content¹, and their foliage is commonly used as a protein and energy supplement to ruminant fed low quality forages². Most of these leguminous forages are both readily available and affordable by poor peasants. The utilization of browse species is limited by the lignin content and/or presence of antinutritional factors which may be toxic for ruminants. The most important antinutritional factor cited is tannin, which binds to and form complex with proteins, carbohydrates, minerals and other dietary nutrients during feed digestion, resulting in deficiencies of both protein and energy to animal^{3,4}. Following establishment of level of antinutritional factors and tannins in browse foliages, the fodder could be used as protein supplements to livestock fed on low quality roughages in the tropics, especially during dry seasons.

Information on the nutritive potential of indigenous browse species in Tanzania are limited⁵⁻⁸ despite their key role as livestock feeds in many pastoral communities in the tropics. Therefore, the objective of this study was to evaluate chemical composition and *in vitro* fermentation characteristics of browse species native to Morogoro, Tanzania. Also the estimation of extent and rate of

organic matter (OM) degradation and assessment of effect of polyethylene glycol on feed digestibility was done.

Materials and Methods

Collection of browses: Browse leaves of *Accacia nilotica*, *Accacia tortilis*, *Balanites aegyptiaca*, *Commiphora africana*, *Ficus sycomorus*, *Lantana camara*, *Lawsonia inermis*, *Senna siamea* and *Trichilia emetia* were harvested from 4 wards in Mvomero district lying approximately 380-500 m above sea level. The areas receive bimodal rainfall ranging between 600 and 2000 mm per annum, with most of rain falling between February and May. Average temperatures range from 15 to 29°C per annum.

Young and mature leaves from 8 to 10 trees for each species in each ward were selected randomly from five linear transects of 35 m and manually harvested at the end of the rain season of year 2005. The samples were pooled for each individual tree species, dried at 50°C in a forced air oven for 48 h to a constant weight and ground to pass 2 mm sieve. The samples were then sub-sampled into 27 bulk samples (three samples for each species) for further laboratory analysis. Samples for phenolics and *in vitro* gas production analyses were ground to pass 1 mm sieve.

Chemical analyses: Organic matter (OM), crude protein (CP),

neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined^{9,10}. Cellulose (CEL) and hemicellulose (HCEL) contents were calculated from the difference between ADF and ADL and between NDF and ADF respectively.

The extraction of phenolics was done using 70% aqueous acetone. Total extractable phenolics (TEPH) were assayed using Folin-Ciocalteu method¹¹. Total extractable tannin (TET) was estimated indirectly after being absorbed to insoluble polyvinyl pyrrolidone (PVPP). The concentration of TET was calculated by subtracting the TEPH remaining after PVP treatment from TEPH. For both the TEPH and TET, the absorbance was measured using a spectrophotometer (Quatification-2 program pack, Shimadzu Corporation, Kyoto, Japan).

In vitro gas production study

Animals and rumen fluid extraction: Rumen liquor for the *in vitro* gas production and tannin bioassay was obtained from three mature healthy Japanese Corriedale sheep fitted with permanent rumen fistula. The animals were fed on standard diet of 800 g DM Timothy hay and 200 g DM concentrate twice daily at 09.00 and 16.00 h in equal sized meals. The animals had free access to water and mineral lick throughout the experimental period. The rumen liquor was withdrawn prior morning feeding, mixed, strained through four layers of cheesecloth and kept at approximately 39°C in water bath flushed with CO₂ before use and diluted with culture media¹⁶.

***In vitro* gas production:** Samples were incubated *in vitro* with rumen fluid in calibrated glass syringes¹². About 200 mg of sample (milled through 1.0 mm sieve) were weighed in duplicate into 100 ml calibrated glass syringes. Vaseline oil was applied to the piston to ease movement and to prevent escape of gas. The syringes were pre-warmed at 39°C before addition of 30±1 ml of rumen liquor buffer mixture (ratio 1:2) into each syringe. Blanks with buffer rumen fluid without feed sample were also included. All the syringes were incubated in a water bath maintained at 39±0.1°C. The syringes were gently shaken every hour during the first 8 hours of incubation and readings were recorded after 3, 6, 12, 24, 48, 72 and 96 h of incubation.

The gas production characteristics were estimated by fitting the mean gas volumes to the exponential equation¹³ $p = a + b(1 - e^{-ct})$, where p is the gas production (ml g⁻¹ OM) at time t, a is the gas production (ml g⁻¹ OM) from immediate soluble OM fraction, b is the gas production (ml g⁻¹ OM) from the insoluble degradable fraction, a + b is the potential gas production and c is the gas production (ml g⁻¹ OM) rate constant from b.

Effect of polyethylene glycol (PEG) addition on digestibility:

About 500 mg DM of the feed samples were incubated with or without 0.1 g polyethylene glycol (PEG, MW = 6000)¹⁴. The syringes were pre-warmed at 39°C for 1 h before addition of 40±0.5 ml rumen-liquor buffer mixture (ratio 1:3) into the syringes and incubated in triplicate in a water bath maintained at 39±0.1°C. Blanks were also included in the incubation. The syringes were gently shaken every hour during the first 4 h and thereafter, every 2 h up to 8 h of incubation. The gas production readings (ml) were recorded after 2, 4, 6, 8, 12, 16 and 24 h of incubation.

Feed organic matter digestibility (OMD) (%) and metabolizable energy (ME) (MJ kg⁻¹ DM) were estimated from the following equation^{12,15} based on 24 h gas production (Gv, ml) and crude protein content (CP, DM %):

$$\text{OMD}(\%) = 14.88 + 0.889 \text{ Gv} + 0.45 \text{ CP}$$

$$\text{ME}(\text{MJ kg}^{-1} \text{ DM}) = 2.20 + 0.136 \text{ Gv} + 0.057 \text{ CP}$$

Statistical analysis: Gas production data were fitted to the asymptote exponential model using Neway Excel computer program (Chen X.B., Rowett Research Institute, Aberdeen). Analysis of variance (ANOVA) was carried out on chemical composition, phenolics, *in vitro* gas production characteristics, degradability and digestibility estimates data using the General Linear Model (GLM) procedure¹⁶. Significance difference between means was tested using the least significance differences (LSD).

Results and Discussion

The browse species used in the present study varied (P<0.05) significantly (Table 1). Feed protein contents ranged from 276 in *A. tortilis* to 89.73 g kg⁻¹ DM in *C. africana*. The fibre fractions differed significantly among browse species. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) varied (P<0.05) between browse species. *Lantana camara* had the highest contents (666.7, 555.6 and 229.71 g kg⁻¹ DM; NDF, ADF and ADL, respectively). *Balanites aegyptiaca* had the highest hemicellulose (206.02 g kg⁻¹ DM) and the lowest cellulose (75.45 g kg⁻¹ DM) contents. The highest TEPH and TET contents were recorded in *A. nilotica* (168.36 and 176.15 mg g⁻¹ DM, respectively), and the lowest values were recorded in *B. aegyptiaca* (11.34 and 11.89 mg g⁻¹ DM, respectively).

The chemical composition of the browse species varied within a wide range. Many authors have reported a large variation in chemical composition of African browse species^{7,8,17-19}. The variation in chemical composition evaluated in browse species

Table 1. Chemical composition of browse forages.

Species	g kg ⁻¹ DM							mg g ⁻¹ DM	
	OM	CP	NDF	ADF	ADL	HCEL	CEL	TEPH	TET
<i>A. nilotica</i>	950.4 ^a	118.1 ^{bc}	339.1 ^d	303.2 ^{cd}	176.1 ^b	35.9 ^c	127.1 ^{bc}	168.4 ^a	176.2 ^a
<i>A. tortilis</i>	927.2 ^{bc}	276.8 ^a	587.6 ^{ab}	364.1 ^{bc}	187.2 ^b	223.5 ^a	177.0 ^b	62.5 ^c	66.4 ^c
<i>B. aegyptiaca</i>	818.0 ^d	131.5 ^b	450.6 ^c	244.8 ^d	169.1 ^b	206.0 ^a	75.5 ^c	11.3 ^e	11.9 ^e
<i>C. africana</i>	932.3 ^b	89.7 ^c	621.9 ^a	469.5 ^b	202.8 ^{ab}	152.4 ^{ab}	266.7 ^a	82.4 ^{bc}	88.1 ^{bc}
<i>F. sycomorus</i>	892.1 ^{cd}	130.7 ^b	576.6 ^{ab}	519.9 ^{ab}	218.2 ^a	106.7 ^b	251.1 ^a	27.9 ^e	29.8 ^{de}
<i>L. camara</i>	918.2 ^c	142.4 ^b	666.7 ^a	555.6 ^a	229.7 ^a	111.1 ^b	225.9 ^a	21.0 ^{de}	22.4 ^{de}
<i>L. inermis</i>	928.6 ^{bc}	148.7 ^b	521.9 ^{bc}	349.8 ^c	108.2 ^{cd}	172.1 ^a	241.5 ^a	35.1 ^d	37.2 ^d
<i>S. siamea</i>	950.9 ^a	175.0 ^b	491.3 ^c	323.7 ^c	108.5 ^{cd}	167.6 ^a	215.2 ^{ab}	38.6 ^d	40.45 ^d
<i>T. emetia</i>	916.2 ^c	135.6 ^b	414.0 ^{cd}	363.1 ^{bc}	104.7 ^d	56.9 ^c	258.4 ^a	102.6 ^b	108.3 ^b
Mean	910.4	158.3	500.4	377.2	159.1	167.2	125.8	54.9	58.0
SEM	0.83	12.3	20.6	21.7	11.9	13.4	12.7	9.9	10.4

SEM = Standard error of the means; Means in the same column with different superscripts differ significantly (p<0.05)

may be due to species, soils, stage of maturity and type (i.e. twigs, leaves or soft tem) of foliage sampled. Also climatic influence on foliage growth and plant nutrient accumulations may have contributed to the inconsistency in nutrient compositions among browses²⁰.

These browse species could form potential feed resources mainly as protein supplements to ruminants and could correct the deficient nitrogen in basal roughages that have CP values (30-70 g kg⁻¹ DM) especially during dry seasons. The CP levels in the current study ranged from 89.7 to 276 g kg⁻¹ DM which is adequate for maintenance and growth of ruminant livestock and could correct the deficient nitrogen in basal roughages. However, optimal utilization of CP in browse species is dependent on soluble phenolic compounds such as tannins and insoluble proanthocyanidins²¹.

Overall, the browse forages had moderate to high fibre (NDF, ADF and ADL) content. This can be attributed to presence of antinutritional factors which limit microbial degradation and inhibit the activity of rumen microbes. With exception of *B. aegyptiaca*, all studied browse species had high cellulose (CEL) contents which suggest their high digestibility potential. However, high CEL contents of tanniferous browse should be interpreted with precaution due to presence of soluble polyphenolic compounds which could lead to over estimation of digestibility. For example in the present study, *C. africana*, *T. emertia* and *A. nilotica* had highest CEL content (266.67, 258.44 and 127.08 g kg⁻¹ DM; respectively) but also high total phenolics contents (82.41, 102.55 and 168.36 g kg⁻¹ DM; respectively).

There were differences between levels of TEPH and TET in the studied browse species (Table 1) compared to what has been reported by others. For example Osuga¹⁸ reported TEPH and TET levels between 10.0-165.8 and 1.5-125.7 mg g⁻¹ DM, respectively. This may be due to variability in chemical composition of polyphenolics among species²², stage of plant growth and/or season of collection²³, site of sampling and/or proportion of foliage materials sampled.

The *in vitro* cumulative gas production and the fermentation characteristics of the browse forages are presented in Table 2. The high cumulative gas production observed in *S. siamea* could be explained by its relatively high CP and low fibre content. Crude protein represents a large proportion of an immediately fermentable feed fraction at initial incubations and decreases at subsequent incubations. Cell wall content (NDF and ADF) are negatively correlated with gas production at all incubation times and estimated parameters. This may tend to reduce microbial

activity through increasing the adverse environmental conditions as incubation time progress. The relatively high level of ADL in *L. camara* and *F. sycomorus* as shown in Table 1 explains in part the limited *in vitro* degradation and therefore the lower amount of gas produced. Low gas production from *Acacia* species in the present study can be explained by tannin content binding to the carbohydrates causing inhibition of enzymes or microorganisms, complexing with ligninocellulose thus preventing microbial digestion. Tropical browses have been shown to contain considerable amounts of phenolic compounds that reduce *in vitro* gas production^{7, 23-25}. The highest potential gas production was recorded in *C. africana* while *B. aegyptiaca* had high rate of gas production.

The studied browse forages did not give similar responses on PEG supplementation (Table 3). *Commiphora africana*, *A. tortilis* and *A. nilotica* showed the highest response on PEG treatment on 24 h gas production while *S. siamea* and *B. aegyptiaca* had the lowest response. The nature and magnitude of the effect of PEG is thought to depend on factors such as tannin structure and level of tannin in the foliage¹⁹. In the present study increased *in vitro* gas production on addition of PEG was observed in browses with high tannin content. This is probably due to high tannin activity in the browse species. The low increase in gas production in *S. siamea* (1.29%) can be explained by its relatively low fibre (NDF and ADF) and tannin content suggesting their suitability of being good supplements to low quality forages. However, the antinutritional effect of tannins in the browses can be partially or completely neutralized by additional of PEG²⁶.

The increased OMD and ME upon additional of PEG was observed in *C. africana* (23.48% and 25.34%, respectively) (Table 3). Current findings showed that the effect of PEG on OMD and ME in *A. nilotica* and *A. tortilis* are lower than those of *Acacia* species reported⁸. Leaves of shrub and tree did not give the same response for PEG addition, possibly due to differences in chemical composition of tannins, variation in tannin antinutritive activity between foliage species, the nature of tannin and chemical structure and degree of polymerization²⁷. Marginal effect of PEG on other browse species could be due to low level of tannin and/or absence of adverse effect of tannin on feed degradability. Addition of PEG could overcome adverse effects of tannins on nutrient availability as indicated by gas production OMD and ME parameters in studied browse species. However, effects were not uniform among forages, suggesting the presence of intrinsic factors other than phenolics.

Table 2. *In vitro* gas production and fermentation characteristics of the browse forages (ml gas/200 mg DM).

Species	Incubation intervals, h						Gas production parameters				
	3	6	12	24	48	72	96	A	B	A+B	C
<i>A. nilotica</i>	7.1 ^b	10.6 ^d	14.8 ^d	16.1 ^c	21.3 ^c	22.1 ^d	24.1 ^d	5.8 ^a	76.8 ^b	82.6 ^b	0.01 ^{cd}
<i>A. tortilis</i>	5.8 ^{bc}	9.8 ^d	14.3 ^d	15.8 ^c	20.6 ^{cd}	22.3 ^d	23.3 ^d	4.9 ^{ab}	59.0 ^{bc}	63.8 ^b	0.01 ^{cd}
<i>B. aegyptiaca</i>	9.0 ^a	17.0 ^b	25.0 ^{ab}	27.5 ^{ab}	29.8 ^b	31.5 ^{bc}	32.5 ^{bc}	-6.7 ^c	38.4 ^d	31.7 ^{cd}	0.11 ^a
<i>C. africana</i>	6.0 ^{bc}	11.0 ^{cd}	14.5 ^d	16.0 ^c	22.5 ^c	25.8 ^c	27.3 ^c	5.3 ^a	151.6 ^a	157.0 ^a	0.004 ^c
<i>F. sycomorus</i>	5.1 ^c	12.6 ^c	20.6 ^b	29.1 ^a	33.1 ^a	35.3 ^{ab}	37.1 ^b	-0.2 ^d	45.9 ^{cd}	45.7 ^c	0.04 ^c
<i>L. camara</i>	7.5 ^b	15.3 ^{bc}	21.3 ^b	25.3 ^b	30.0 ^b	30.0 ^{bc}	30.8 ^{bc}	1.7 ^c	30.4 ^{de}	32.1 ^{cd}	0.06 ^{ab}
<i>L. inermis</i>	9.3 ^a	15.3 ^{bc}	19.8 ^{bc}	20.8 ^{bc}	25.0 ^{bc}	25.8 ^c	26.3 ^c	3.7 ^{ab}	23.3 ^e	27.0 ^a	0.07 ^{ab}
<i>S. siamea</i>	12.0 ^a	21.5 ^a	28.5 ^a	32.5 ^a	36.8 ^a	38.5 ^a	41.5 ^a	6.9 ^a	35.4 ^d	42.2 ^c	0.05 ^{ab}
<i>T. emertia</i>	7.3 ^b	12.8 ^c	18.3 ^{bc}	26.0 ^b	30.3 ^{bb}	31.0 ^{bc}	32.0 ^{bc}	3.4 ^b	49.2 ^{cd}	52.6 ^{bc}	0.03 ^c
Mean	7.4	13.7	20.1	23.7	28.3	29.8	31.2	2.2	55.3	57.5	0.04
SEM	0.5	0.8	1.1	1.4	1.3	1.4	1.5	1.2	9.7	10.03	0.01

SEM = Standard error of the means; Means in the same column with different superscripts differ significantly (p<0.05), A: gas production from quickly soluble fraction, B: gas production from insoluble but degradable fraction, c: gas production rate (%).

Table 3. The effect of PEG treatment on 24 h cumulative gas production, estimations for *in vitro* organic matter digestibility (OMD) and metabolizable energy (ME) content.

Species	24 h gas production (ml/500mg DM)			24 h response in OMD, %			24 h response in ME, MJ kg ⁻¹ DM		
	-PEG	+PEG	*Incr.	-PEG	+PEG	*Incr.	-PEG	+PEG	*Incr.
<i>A. nilotica</i>	50.83 ^c	67.12 ^d	32.05	85.45 ^{cf}	99.94 ^e	16.96	12.33 ^c	14.54 ^e	17.92
<i>A. tortilis</i>	47.06 ^{cd}	66.35 ^d	41.0	181.28 ^a	198.42 ^a	9.45	24.38 ^a	27.00 ^b	10.75
<i>B. aegyptiaca</i>	88.87 ^a	91.12 ^a	2.56	153.08 ^{bc}	155.08 ^{bc}	1.31	21.78 ^{ab}	22.09 ^b	1.42
<i>C. africana</i>	44.14 ^d	67.65 ^d	55.39	89.07 ^c	109.98 ^{de}	23.48	12.63 ^{de}	15.83 ^{de}	25.34
<i>F. sycomorus</i>	62.03 ^b	75.03 ^{bc}	22.32	124.61 ^d	136.16 ^d	9.27	17.55 ^c	19.32 ^d	10.09
<i>L. camara</i>	70.90 ^b	73.16 ^{cd}	3.19	141.98 ^c	143.98 ^{bcd}	1.41	19.96 ^{bc}	20.26 ^{cd}	1.50
<i>L. inertia</i>	69.65 ^b	76.91 ^b	10.74	143.71 ^c	150.16 ^{bc}	4.49	10.15 ^{def}	21.14 ^{bc}	10.28
<i>S. siamea</i>	76.82 ^{ab}	77.81 ^b	1.29	161.94 ^{ab}	162.82 ^{ab}	0.54	22.62 ^{ab}	22.76 ^b	0.62
<i>T. emetia</i>	61.63 ^{bc}	74.15 ^{bc}	21.32	132.07 ^{cd}	143.19 ^{bcd}	8.42	18.49 ^{bc}	20.19 ^{cd}	9.19
Mean	65.17	74.6		140.74	149.14		19.67	20.95	
SEM	2.95	1.47		7.26	6.55		0.95	0.84	

SEM = Standard error of the means; Means in the same column with different superscripts differ significantly (P<0.05). * Increase (ml) = {(+PEG)- (-PEG)}x 100/-PEG.

Conclusions

Appreciable quantities of crude protein in all studied species make them potential as supplement to poor quality feeds typical of the long dry periods experienced in the study area. However, the effect of chemical components, such as fibre and phenolic compounds, in some of the browse forages like *C. africana*, *A. tortilis*, *A. nilotica* and *T. emetia* will determine availability of CP for efficient fermentation of fibrous low quality feedstuffs. Improved digestibility due to PEG treatment represents nutritive potential previously depressed by tannins' antinutritive activity. Leaves of *S. siamea* and *L. inermis* could be valuable ruminant feeds due to their *in vitro* fermentation characteristics, relatively low fibre and high CP content. Further research on assessment of browse forages nutritive potential *in vivo* is recommended.

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