

Rumen degradation and *in vitro* gas production parameters in some browse forages, grasses and maize stover from Kenya

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Abstract

Forages from five browse species *Bauhinia alba*, *Carisa edulis*, *Lantana camara*, *Sesbania sesban* and *Tithonia diversifolia*; two grass species *Chloris gayana* and *Pennisetum purpureum* and crop residue maize (*Zea mays*) stover were analysed for chemical composition including phenolics and rumen degradation characteristics. The rumen fermentation characteristics with and without polyethylene glycol (PEG) were studied *in vitro* by gas production. The crude protein content was more than 200 g/kg dry matter (DM) in *S. sesban* and *T. diversifolia* while was lowest in *C. gayana* hay and maize stover. The organic matter (OM) (g/kg DM) was high in *C. edulis* (945.3) and low in *P. purpureum* (837.3) while *C. gayana* hay and maize stover contained the highest neutral detergent fibre (NDF). Total extractable phenolics (TEPH) and total extractable tannins (TET) tended to be high in *B. alba* and *C. edulis*. The DM disappearance after 24 h of rumen incubation ranged from 44.1 in *C. gayana* hay to 82.4% in *T. diversifolia*. The effective degradability (ED) was high in the browse forages than the grasses and maize stover. The gas produced after 96 h of incubation ranged from 23.9 in *B. alba* to 52.8 ml/200 mg DM in maize stover. The grasses and maize stover produced more gas than the browse forages at all incubation h after 24 h. Use of PEG indicated that tannins had inhibitory effect on rumen microbial fermentation and that this depends on the amount and activity of the tannins present. The estimated *in vitro* OM digestibility and metabolizable energy also increased numerically with PEG addition. The results of this study indicates that such browse forages have the potential to be used as feed supplements for ruminants especially during the dry season when feeds such as hay and crop residues are the only feed resources available to the farmers.

Key words: Chemical composition, *in situ* degradation, *in vitro* gas production, PEG.

Introduction

Evaluating the nutritional value of locally available feed resources including shrubs and trees is important as these feeds make an important contribution to the nutrition of livestock. This is especially important in the tropics including Kenya, where forage quality and availability may be severely limited during lean periods^{1,2}. Indigenous vegetative species such as *Bauhinia alba*, *Carisa edulis*, *Lantana camara*, *Sesbania sesban* and *Tithonia diversifolia* are widely distributed in Kenya and form a significant portion of the diet consumed by ruminants. These feed resources are used by the farmers in conjunction with grass species such as *Pennisetum purpureum* and *Chloris gayana* and crop residues such as maize (*Zea mays*) stover. However, information on the nutritional value of such feed resources that are accessible to the farmers in terms of their digestible and fermentative characteristics is still scarce.

The *in situ* degradation and *in vitro* gas production techniques are useful tools for determining the potential nutritional value of feed resources consumed by ruminants. The *in situ* technique uses time series measurements of degradability of the feed in the rumen³. The *in vitro* gas production technique measures the volume of gas produced, which reflects the end result of the fermentation of the feed substrate to volatile fatty acids (VFAs), microbial biomass and the neutralization of the VFAs produced. In both techniques, application of models allows the fermentation

characteristics of the soluble and readily degradable fraction of the feeds and the insoluble but slowly degradable fraction to be described⁴. In addition, the gas production technique can be used to demonstrate and quantify the adverse effect of some chemical constituents such as tannins in browse forages. Therefore, the objective of this study was to estimate the *in situ* degradation and *in vitro* gas production characteristics of the feed resources and to assess the adverse effects of tannins present in the browse forages.

Materials and Methods

Forage samples: Leaves from five browse species, two grass species and a crop residue were used in this study. The browse species used include: *Bauhinia alba*, *Carisa edulis*, *Lantana camara*, *Sesbania sesban* and *Tithonia diversifolia*. The grasses were: *Chloris gayana* (hay) and *Pennisetum purpureum* and maize (*Zea mays*) stover. All the samples were harvested from International Centre for Research in Agroforestry, Maseno station (0°0'S; 34°35'E) in western Kenya. The area is located at an altitude of 1600 m above sea level with an average annual rainfall and temperature of 1500-2000 mm and 20-23°C respectively.

The feed samples were selected randomly from four sub-regions within the study area during the dry season (December/January). The browse forages from each sub-region were harvested from

at least 10 different trees⁵. The harvested samples were then pooled for each individual species and then oven dried at 50°C for 48 h to constant weight and ground to pass through a 2.0 mm sieve. The samples were then sub-sampled to obtain three samples for each species and used for laboratory analysis. However, for analysis of phenolics and *in vitro* gas production experiments, the samples were further ground to pass through a 1.0 mm sieve.

Chemical analysis: Organic matter (OM) and crude protein (CP) contents were measured according to the official methods of Association of Official Analytical Chemists⁶. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al.*⁷. Total extractable phenolics (TEPH) and total extractable tannins (TET) were determined by the method described by Makkar⁸. Tannic acid (Sigma-Aldrich Chemie, Steinheim, Germany) was used as standard.

In situ DM degradation: The nylon bag technique as described by Ørskov⁹ was used to measure the kinetics of DM degradation of the feeds. Three mature sheep fitted with permanent rumen fistula were used in this study. The sheep were fed on a standard diet of 800 g timothy hay and 200 g concentrate (2 parts wheat bran and 1 part rolled barley). The ration was divided into two equal meals fed at 8:30 and 16:00 h daily. The animals had free access to water and mineral lick throughout the experiment period. Three grams of each feed sample, dried and milled through 2.0 mm sieve, were weighed into nylon bags and incubated for 4, 8, 16, 24, 48 and 72 h in the rumen of the sheep. Zero time washing loss was measured by soaking the nylon bags with sample at 39°C for 1 h. After removal from the rumen and water, the nylon bags were thoroughly washed with cold water for 15 minutes in a domestic washing machine without spinning. The washed bags with residue were then dried for 48 h at 60°C in a forced air oven and weighed to determine the DM loss.

The DM disappearance data was fitted to the exponential equation³: $P = a + b(1 - e^{-ct})$, where p is the disappearance of DM (%) during time t , a is the rapidly soluble DM, b is the insoluble DM which is degradable and c is the degradation rate of b (/h). The effective DM degradability (ED) was calculated by applying the equation¹⁰: $ED = a + b \times [c/(c+0.05)]$, where a constant 0.05/h is the rumen outflow rate of small ruminant with low level of production as recommended by AFRC¹¹. The index value (IV) was calculated from the equation¹²: $IV = a + 0.4b + 200c$.

In vitro gas production: Rumen liquor for *in vitro* degradability (*in vitro* gas production and tannin bioassays) was also obtained from the three mature fistulated sheep used in *in sacco* study. The rumen liquor was withdrawn at 14 h post feeding, mixed, strained through four layers of cheesecloth and kept at 39°C under a CO₂ atmosphere.

Samples were incubated *in vitro* with rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass¹³. About 200 mg of sample (milled through a 1.0 mm sieve) were weighed into 100 ml calibrated glass syringes in duplicate. Vaseline oil was applied to the pistons 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 buffered rumen fluid

without feed sample were also included in triplicate. All the syringes were incubated in a water bath maintained at 39°C. The gas production 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 equation³: $G = a + b(1 - e^{-ct})$, where G is the gas production (ml/200 mg DM) at time t , $a + b$ is the potential gas production (ml/200 mg DM), c is the rate constant of gas production.

Tannin bioassay: Incubation was carried out using the method of Menke and Steingass¹³ as modified by Makkar *et al.*¹⁴. Makkar *et al.*¹⁴ increased the sample size from 200 mg to 500 mg and consequently increased the amount of buffer two-fold. The incubation volume was also increased from 30 ml to 40 ml of the rumen-buffer mixture. About 500 mg DM of the feed samples were incubated with or without 1.0 g polyethylene glycol (PEG, MW=6000).

The syringes were pre-warmed at 39°C for 1 h before addition of 40±0.5 ml rumen liquor-buffer mixture into the syringes and incubated in triplicate in a water bath maintained at 39°C. Blanks were also included in the incubation. The gas production readings were recorded after 2, 4, 6, 8, 12, 16 and 24 h of incubation.

Feed *in vitro* OM digestibility (OMD, %) and metabolizable energy (ME, MJ/kg DM) content were estimated based on 24 h gas production (Gv, ml), CP content (% DM) and crude ash content (CA, % DM) from the equations^{13,15}:

$$\text{OMD (\%)} = 14.88 + 0.889 \text{ Gv} + 0.45 \text{ CP} + 0.0651 \text{ CA.}$$

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \text{ Gv} + 0.057 \text{ CP.}$$

Statistical analysis: *In sacco* and *in vitro* gas production data were fitted to the asymptote exponential model using Neway-Excel computer program (Macaulay Institute, Aberdeen, UK). Analysis of variance (ANOVA) was carried out on chemical composition including phenolics, *in vitro* gas production, *in sacco* DM degradation and degradability characteristics and digestibility estimates and ME contents using the General Linear Model procedure¹⁶. Significance between means was tested using the least significance difference (LSD).

Results and Discussion

Chemical composition: The chemical compositions of the feedstuffs are shown in Table 1. There were significant differences between the chemical compositions of the feeds analyzed in this study. The OM content ranged from 837.3 to 945.3 g/kg DM. The CP content ranged from 53.3 to 287.3 g/kg DM while the NDF content ranged from 302.9 to 741.2 g/kg DM. The TET content ranged from 1.1 to 57.9 g/kg DM. Some of the forages used in this study have been investigated in previous studies¹⁷⁻²⁰. The chemical compositions of the forages were within the published values for such feeds. The CP content is a very important index of nutritional quality of a feed. The browse forages tended to have higher CP content while the grasses especially *C. gayana* and maize stover had low levels of CP content. This indicates that the browse forages would be good protein supplements for the low quality feeds such as the hay and stover.

The cell wall analysis, based on detergent extraction, is a good indicator for predicting the nutritional worthiness of fibrous feed resources. This is because the voluntary feed DM intake and DM digestibility are dependent on cell wall constituents

especially the NDF and lignin²¹. In the present study, the browse forages had low to moderate content of fibre (NDF and ADF) while the grasses had high fiber contents. Tegua *et al.*¹⁹ also found higher fiber content in grasses than the browse forages, which is in agreement with the trend in this study. Presence of simple phenolics and polyphenolic affects the nutritive value of feeds²² especially the browse forages. This tends to limit their use especially as protein supplements. This is because in ruminants, the polyphenolics tend to alter the composition of the rumen micro-organisms, complex and inhibit microbial enzymes, complex with feed nutrients (protein, carbohydrates and minerals) and their metabolic products may be absorbed from the rumen and produce toxic effects at the tissue level²³.

In this study, *B. alba* and *C. edulis* tended to have moderate levels of both TEPH and TET while the other browse species had low contents of phenolics. However, the effect of polyphenolics in browse species is influenced by other factors such as the reactivity, structure, molecular weight and interactions with other secondary metabolites²⁰.

In situ DM degradation: Table 2 presents the *in situ* DM disappearance and estimated degradation kinetics. Significant differences in the DM disappearance were observed between the various types of feeds evaluated. At 24 h of rumen incubation, *T. diversifolia* had the highest DM degradability while both *T. diversifolia* and *S. sesban* had the highest DM degradability at 48 h of rumen incubation. The browse species tended to have higher soluble fraction (a) while *P. purpureum* had the highest potentially degradable fraction (b). Both *T. diversifolia* and *S. sesban* had the highest rate of degradation (c). The ED and IV were higher in both *S. sesban* and *T. diversifolia*. *P. purpureum* had higher degradability than the other grasses.

The differences in degradability of the various feeds evaluated can be attributed to the differences in the chemical composition of the various feeds. *S. sesban* and *T. diversifolia* had higher *in situ* DM degradation parameters than the other feeds. The two species contained higher content of CP and lower contents of NDF, ADF, ADL, TEPH and TET. The higher degradability of *S. sesban* has been reported in various studies^{19,20}. Many studies have indicated a negative correlation between the fiber fractions, polyphenolics and the degradability of fibrous feeds^{5,20,24}. *P. purpureum* also tended to show higher degradability than the other grasses due to its higher CP content and low NDF, ADF and ADL. This is in agreement with the results of Tegua *et al.*¹⁹ who also reported high degradability of *P. purpureum*. The low rates, extents as well as potential and effective degradability of DM of *C. gayana* and maize stover indicates that they are poor quality feeds that needs to be supplemented with feeds of better degradability in order to support meaningful animal production.

In vitro gas production: The *in vitro* cumulative gas production and the fermentation characteristics of the evaluated feeds are summarized in Table 3. There were significant differences in the cumulative gas production and fermentation kinetics among the various feeds. Maize stover and *P. purpureum* produced the highest gas at 24 h of incubation. Among the browse forages, *S. sesban* produced the highest gas at all h of incubation. The potential gas production was highest in maize stover and *C. gayana* while among the browse forages, the potential gas production was highest in *S. sesban*. *S. sesban* and *L. camara* had the highest rate of gas production. In general, the grasses and maize stover, tended to produce more gas and had more potential gas production than the browse forages.

The significant differences among the browse samples for their

Table 1. The chemical composition of the forages.

Species	Organic matter (OM)	Crude protein (CP)	Neutral detergent fibre (NDF)	Acid detergent fibre (ADF)	Acid detergent lignin (ADL)	Total extractable phenolics (TEPH)	Total extractable tannins (TET)
	g/kg of DM						
<i>B. alba</i>	939.6 ^b	153.6 ^d	522.0 ^d	380.2 ^d	134.2 ^d	87.5 ^a	57.9 ^a
<i>C. edulis</i>	945.3 ^a	127.3 ^e	533.6 ^d	476.3 ^a	291.4 ^a	61.4 ^b	47.4 ^b
<i>L. camara</i>	898.5 ^d	179.8 ^c	474.5 ^e	392.9 ^c	226.7 ^b	17.4 ^c	4.4 ^c
<i>S. sesban</i>	923.4 ^c	287.3 ^a	302.9 ^g	206.8 ^f	56.9 ^e	23.5 ^c	5.9 ^c
<i>T. diversifolia</i>	859.2 ^g	211.5 ^b	401.0 ^f	358.4 ^e	179.0 ^c	5.1 ^d	1.1 ^c
<i>C. gayana</i>	892.3 ^e	55.0 ^f	741.2 ^a	460.9 ^b	57.1 ^e	ND	ND
<i>P. purpureum</i>	837.3 ^h	173.2 ^c	590.3 ^c	360.5 ^e	28.7 ^f	ND	ND
Maize stover	867.6 ^f	53.3 ^f	666.0 ^b	403.4 ^e	37.1 ^f	ND	ND
SEM	9.4	18.9	33.9	19.9	23.7	10.3	8.1

SEM, Standard error of the means. Means with different superscripts in a column differ significantly (P<0.05). ND, not determined.

Table 2. *In situ* DM degradation characteristics of the forages.

Species	24 h	48 h	a	b	c	Undegradable fraction (UDF)	Effective degradability (ED)	Index value (IV [†])
	← % →		→ %/h →			← % →		
<i>L. camara</i>	65.4 ^c	74.6 ^b	30.1 ^c	56.3 ^b	2.8 ^{bc}	13.6 ^e	50.3 ^c	58.1 ^d
<i>S. sesban</i>	76.6 ^b	86.7 ^a	43.0 ^a	51.3 ^d	3.5 ^a	5.6 ^e	64.5 ^a	70.7 ^a
<i>T. diversifolia</i>	82.4 ^a	87.0 ^a	38.0 ^b	56.5 ^b	3.9 ^a	5.5 ^e	63.1 ^a	68.4 ^b
<i>C. gayana</i>	44.1 ^e	55.4 ^d	22.2 ^e	54.6 ^c	1.9 ^d	23.2 ^a	37.2 ^c	47.8 ^f
<i>P. purpureum</i>	66.0 ^c	77.9 ^b	30.8 ^c	59.9 ^a	2.9 ^b	9.3 ^d	52.8 ^b	60.5 ^c
Maize stover	50.4 ^d	66.6 ^c	25.9 ^d	56.3 ^b	2.4 ^c	17.8 ^b	44.0 ^d	53.1 ^e
SEM	3.3	2.7	1.7	0.6	0.0	1.6	2.4	1.9

SEM, Standard error of the means. Means with different superscripts in a column differ significantly (P<0.05). a, b and c are degradation constants; †Index value, for details see equation in *in situ* degradation section.

in vitro gas production and fermentation characteristics are in agreement with previous reports on tropical browses^{5, 18-20}. The high rate and extent of fermentation of *S. sesban* could be related with its high CP content and low content of NDF, ADF, ADL and polyphenolics. Kamalak *et al.*²⁴ and Abdulrazak *et al.*⁵ reported that gas production and estimated parameters are negatively correlated with NDF and ADF. A negative correlation has also been reported between gas production and polyphenolics present in the browse forages^{18, 24}. In this study, *C. edulis* and *B. alba* which had high fiber and polyphenolics contents, had low gas production and fermentation characteristics.

The higher extent of gas production in the grasses and maize stover than the browse species, though contrary to expectations, has previously been reported^{19, 20}. Melaku *et al.*²⁰ suggested that this phenomenon could be due to the rapid rate of gas production in the browse forages leading to substrate exhaustion and limitation on the extent of gas production in the browse forages compared to the grasses. The presence of polyphenolics in the browse forages may also have a strong negative influence on the extent of gas production. Blümmel *et al.*²⁵ suggested that protein in the feeds as a depressing effect on gas production and that this could be due to a change in the partitioning of the fermented substrate between microbial cells (microbial biomass) and short chain fatty acids (SCFAs) and gas. Blümmel and Becker²⁶ pointed to a possible inverse relationship between the gas production (and SCFA production) and microbial cell yield when both are related to a unit of substrate truly fermented. Hence, less microbial yield and higher SCFA production from the grasses and maize stover would, therefore, result in higher gas volumes for the grasses and maize stover.

Tannin bioassay: The effect of PEG-6000 addition on the gas production, OMD and ME of the browse forages is presented in Table 4. The highest and significant increase in gas production, OMD and ME was obtained with *C. edulis* and *B. alba*. The increase in gas production, OMD and ME due to PEG-6000 addition was small and not significant for *L. camara*, *S. sesban* and *T. diversifolia*.

The *in vitro* gas method, together with use of tannin-inactivating agents such as PEG-6000 has been widely used to evaluate tannin activity in browse forages²⁷. Makkar *et al.*¹⁴ demonstrated that PEG binds to the tannins forming inert PEG-tannin complexes, which results in increase in gas production. Getachew *et al.*²⁸ noted that the increase in gas production upon addition of PEG correlated with protein precipitation capacity of tannins, total phenols and tannins. Hence, the increase in gas production on addition of PEG is considered as a measure of the biological anti-nutritive activity of the tannins^{14, 28}. In the present study, *C. edulis* and *B. alba* tended to show high tannin anti-nutritive activity than the rest of the species evaluated. However, the tannins in *C. edulis* seem of higher anti-nutritive activity than those in *B. alba* as the chemical assay detected low content of tannins in *C. edulis* than in *B. alba* but the response to PEG addition was higher in *C. edulis* than in *B. alba*. Our earlier study with the species¹⁸ also found it to contain moderate content of tannins but high anti-nutritive activity of the tannins. The other browse species had low tannin contents and tended to show minimal response in gas production, OMD and ME to PEG addition.

Table 3. *In vitro* gas production (gas ml/200 mg DM) and fermentation characteristics of the forages.

Species	24 h	48 h	72 h	96 h	a+b	c
<i>B. alba</i>	17.9 ^c	19.5 ^f	22.8 ^f	23.9 ^g	22.9 ^g	5.7 ^b
<i>C. edulis</i>	20.8 ^d	25.1 ^e	25.6 ^e	26.7 ^f	26.4 ^f	6.1 ^b
<i>L. camara</i>	29.5 ^b	31.7 ^d	31.7 ^d	32.2 ^d	31.9 ^e	11.4 ^a
<i>S. sesban</i>	34.2 ^a	36.9 ^c	39.1 ^c	40.7 ^c	38.6 ^d	11.6 ^a
<i>T. diversifolia</i>	15.3 ^f	25.1 ^e	27.3 ^e	29.5 ^e	31.8 ^e	2.9 ^d
<i>C. gayana</i>	25.8 ^c	38.3 ^c	42.8 ^b	46.4 ^b	48.7 ^b	3.1 ^d
<i>P. purpureum</i>	34.1 ^a	41.7 ^b	44.0 ^b	47.6 ^b	45.7 ^c	6.3 ^b
Maize stover	32.6 ^a	45.0 ^a	49.6 ^a	52.8 ^a	53.2 ^a	4.1 ^c
SEM	1.8	2.2	2.4	2.6	2.7	0.8

SEM, Standard error of the means. Means with different superscripts in a column differ significantly ($P < 0.05$). a, b and c are constants in the equation $G = a + b(1 - e^{-c})$.

Table 4. The effect of PEG addition on gas production (gas ml/500 mg DM), estimated organic matter digestibility (OMD, %) and metabolizable energy (ME, MJ/kg DM) of the browse forages.

Species	Gas production, OMD and ME response at 24 hours of incubation.								
	24 h gas production			OMD			ME		
	-PEG	+PEG	% Increase	-PEG	+PEG	% Increase	-PEG	+PEG	% Increase
<i>B. alba</i>	51.6 ^c	62.4 ^d	20.9 [*]	40.1 ^c	44.0 ^c	9.7 [*]	5.9 ^c	6.4 ^c	8.5 [*]
<i>C. edulis</i>	50.9 ^c	80.3 ^b	57.8 ^{**}	38.7 ^c	49.2 ^b	27.1 ^{**}	5.7 ^c	7.3 ^b	28.1 ^{**}
<i>L. camara</i>	72.3 ^b	73.0 ^c	1.0	48.7 ^b	48.9 ^b	0.4	7.2 ^b	7.2 ^b	0.0
<i>S. sesban</i>	94.7 ^a	98.3 ^a	3.8	61.5 ^a	62.7 ^a	2.0	9.0 ^a	9.2 ^a	2.2
<i>T. diversifolia</i>	37.7 ^d	38.3 ^e	1.6	37.8 ^c	38.0 ^d	0.5	5.5 ^c	5.5 ^d	0.0
SEM	6.7	6.7		3.0	2.7		0.4	0.4	

SEM, Standard error of the means. Means with different superscripts in a column differ significantly ($P < 0.05$); * significant ($P < 0.05$), ** significant ($P < 0.01$). PEG, polyethylene glycol.

Conclusions

Based on chemical composition, rumen degradation, *in vitro* gas production, most of the browse forages were found to have potential as supplements to low quality feeds such as *C. gayana* hay and maize stover. However, the higher content of anti-nutritional factors such as tannins, poor *in situ* DM degradability parameters, low rates and extents of *in vitro* gas production in some of the browse forages like *C. edulis* and *B. alba*, make them less favorable supplements compared with *S. sesban*, *T. diversifolia* and *L. camara*.

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