Chemical Composition, Phenolic Concentration and *In Vitro* Gas Production Characteristics of Selected Acacia Fruits and Leaves

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ABSTRACT : The objective of this study was to evaluate the nutritive value of selected fruits (pods and seeds) and leaves of acacia tree species namely; *Acacia nubica* (nubica), *Acacia tortilis* (tortilis) and *Acacia brevispica* (brevispica), *Acacia reficiens* (reficiens) and *Acacia senegal* (senegal). A wide variability in chemical composition, polyphenolics and gas production was recorded. The crude protein (CP) ranged from 131 to 238 g/kg DM. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were higher in senegal and significantly different ($p<0.05$) from other species. The nitrogen bound to fiber tended to be higher in leaves than the fruits, ranging from 2.6 to 11.3 g/kg NDF and 1.6 to 3.2 g/kg ADF. The leaves of reficiens and senegal had higher concentrations of total extractable phenolics (TEPH), total extractable tannins (TET) and total condensed tannins (TCT), but lower in NDF, ADF and ADL than the fruits of nubica, tortilis and brevispica. Mineral concentrations varied among species; all were relatively poor in phosphorus, moderate in calcium and magnesium, and rich in microelements. A significant ($p<0.05$) variation in gas production after 12, 24, 48, 72 and 96 h was recorded between species. Nubica had the highest ($p<0.05$) rate of gas production (0.0925) while the highest potential gas production was recorded in tortilis. A strong negative correlation between TEPH and TET with gas production after 24, 48, 72 and 96 was established ($r=-0.72$ to -0.82). Crude protein and TCT correlated negatively but also weakly with gas production characteristics. Organic matter digestibility calculated from gas production after 48 h (OMD48) ranged between 465 g/kg DM in reficiens and 611 g/kg DM in tortilis. The results of this study indicate that acacia species have the potential to be used as feed supplements. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 7 : 935-940*)

Key Words : Acacia Forage, Gas Production, Chemical Composition, Polyphenolics

INTRODUCTION

Availability of feed throughout the year is major a constraint in animal production in the tropics. Natural pastures, especially during the dry season, and crop residues are low in crude protein and digestible nutrients and high in fiber (Leng, 1990). Multipurpose tree such as *Leucaena leucocephala* and *Gliricidia sepium* have been used as a supplementary feed to low quality diets with promising results (Abdulrazak et al., 1997; Goodchild and McMeniman, 1994; Devendra, 1993). Acacia tree species dominate in parts of Africa (Le Houerou, 1980) and Asia (Carter, 1994). Tree legumes provides not only fodder for livestock but also improve soil condition (Buresh and Tian, 1998), provide and fuel, timber and live hedges (Topps, 1992). Studies on evaluation of browse species have indicated their potential as feed supplements (Larbi et al., 1998), and a need to identify other species to be included in the list of potential fodder. *In vitro* gas production (Khaazal and Ørskov, 1994) has been used to assess the nutritive value of browse species. The presence of phenolic compounds in acacia species has negatively affected their nutritional value and their palatability to livestock (Degen et al., 1998). Tannins have been seen as a major reason why they are little used as livestock fodder (Makkar, 1993). Generally, tannins in fodder trees have been found to have a negative effect on intake and digestibility (Kumar and D'Mello, 1995). However, low tannins have been shown to have beneficial effects, by protecting proteins from rumen degradation (Barry and Duncan, 1984). Due to differences in nutritive value of browse species from various regions and also season variations, there is a need to evaluate the species in a particular region.

Therefore, the objective of this study was to assess the nutritive value and potential of some selected species of acacia from Kenya based on their chemical composition, polyphenolics, mineral concentrations and *in vitro* gas production.

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MATERIALS AND METHODS

Source of acacia samples

Three Acacia fruits, i.e. *Acacia nubica* (nubica), *Acacia tortilis* (tortilis) and *Acacia brevispica* (brevispica), and two acacia leaves; *Acacia reficiens* (reficiens) and *Acacia senegal* (senegal) were harvested from Marigat area (0° 31'S, 35° 78'E) Baringo district, Kenya. The area is located at an altitude of 1066 m above sea level. The annual mean rainfall and temperature are 700 mm and 24.0℃ respectively. Fruits (pods and seeds) and leaves samples were harvested and oven dried at 60℃ for 48 h before being shipped to Matsue, Japan.

Chemical analysis

Dry matter (DM), ash and nitrogen (N) content were measured according the official methods of the Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods of Van Soest et al. (1991). Mineral concentrations were determined by digesting samples in HNO_3 and $HClO_4$ (3:1) and inductively coupled plasma spectroscopy (ICP) measured Ca, Mg, P, S, Mn, Mo, Zn, Co, Cu and Fe. Fluorometric detection method using 2,3-diamono-naphthalene derivative was used for determination of Se, using the fluorescence spectro- photometer 204, (Hitachi Ltd, Tokyo, Japan) at 377 nm excitation and 520 nm emission (Watkinson, 1966).

Phenolic compounds

The extraction of phenolics was done using 70% aqueous acetone. Total extractable phenols (TEPH) were determined using Folin Ciocalteu according to Julkunen-Titto (1985). The concentration of TEPH was calculated using the regression equation of the tannic acid standard. Total extractable tannins (TET) were estimated indirectly after being absorbed to insoluble polyvinylypolyrrolidone (PVP). Concentration of TET was calculated by subtracting the TEPH remaining after PVP treatment from TEPH. The total condensed tannins were measured using the method of Porter et al. (1986).

***In vitro* gas production**

Samples were incubated *in vitro* with rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Rumen liquor was obtained from three sheep fed with 800 g DM timothy hay and 200 g DM concentrates, twice daily, and with free access to water and mineral mix. About 200 mg of 1 mm milled samples were weighed into 100 ml calibrated glass syringes in duplicate. Pure oil was applied to the piston to ease movement and to prevent escape of gas. The syringes were pre-warmed (39℃) for 1 h, before addition of 30±1.0 ml of rumen-buffer mixture into each syringe. All the syringes were incubated in a water bath maintained at 39±0.1℃. The syringes were gently shaken every hour during the first 8 h of incubation and readings were recorded after 3, 6, 12, 24, 48, 72 and 96 h. The mean gas volume readings were fitted to the exponential equation $p=a+b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979). where p=gas production at time t, a+b=the potential gas production, c=the rate of gas production, and a, b, and c are constants in the exponential equation using 'Neway' computer program (X. B. Chen, Rowett Research Institute, Aberdeen). Organic matter digestibility at 48 h was calculated from the equation OMD g/kg DM=185.3+9.239 GP+0.540 CP (Menke and Steingass, 1988). Where GP=gas production (ml/200 mg), CP=crude protein g/kg DM.

Statistical analysis

Analysis of variance (ANOVA) was carried out on chemical composition, phenolics, fiber content, *in vitro* gas production data using the general linear model (GLM) of Statistica for windows (Statistica, 1993). Significance between means were tested using the least significant difference (LSD). A simple correlation analysis was used to establish the relationship between chemical composition, polyphenolics concentration and *in vitro* gas production.

RESULTS AND DISCUSSION

Chemical composition and phenolic concentration of acacia species is presented in table 1. The ash content ranged from 41 to 91 g/kg DM. Senegal had a significantly ($p<0.05$) higher CP (238 g/kg DM) than the other species. There was a significant ($p<0.05$) difference in concentration of fiber among the species. The NDF, ADF and ADL were highest ($p<0.05$) in nubica and lowest in tortilis and reficiens. The nitrogen bound to fiber (NDF-N and ADF-N) varied between 2.9 to 11.3 g per kg NDF and 1.6 to 3.2 g per kg ADF respectively and was significantly ($p<0.05$) higher in senegal and reficiens. The TEPH and TCT ranged between 30 to 238 and 2.4 to 28.3 mg/g DM respectively. Reficiens had the highest TEPH concentration, which was significantly different ($p<0.05$) to all except senegal. Nubica and senegal had the lowest and highest ($p<0.05$) concentration of TCT respectively (table 1). Generally, the fruits tended to have higher ash, CP and phenolics compounds but lower fiber content.

The chemical composition was within the wide range of data reported for acacia and other browse species (Topps, 1992; Larbi et al., 1998; Balogun et al., 1998). The CP content of fruits agrees with the 127-143 g/kg DM range of whole acacia fruits (Tanner et al., 1990). Although the CP content was higher in the leaves, the proportion of N bound to fiber was also high. Apori et al. (1998) reported a range of 21 to 48% of NDF-N with Ghanaian browse species. The proportion of NDF-N and ADF-N of acacia leaves in this study was higher than those of *Sesbania sesban* and *Leucaena leucocephala* (Bonsi et al., 1995). A wide range of phenolic concentration in the acacia is consistent with the large range of phenolics reported in browse species. The range reported in this study agrees with those of Greek (Khazaal and Ørskov, 1994) and East African (Reed,

Table 1. The chemical composition and phenolic concentrations of acacia species

	DM	Ash	CP	NDF	ADF	ADL	NDF-N	ADF-N	TEPH	TET	TCT
	g/kg	g/kg DM					g/kg NDF	g/kg ADF	mg/g DM		
Fruits											
A. nubica	936	64	131	481	372	121	2.9	1.6	30.0	24.7	2.4
A. tortilis	850	41	141	195	169	42	4.7	2.4	88.4	74.4	10.3
A. brevispica	878	44	131	337	248	88	2.6	2.2	69.3	54.4	11.2
Leaves											
A. reficiens	879	91	156	186	111	51	6.6	3.2	237.9	212.9	16.1
A. senegal	878	77	238	245	141	52	11.3	2.6	188.8	145.4	28.3
SEM	9.4	6.5	14.4	36.7	31.2	9.9	1.06	1.77	25.9	22.7	2.9
LSD (p<0.05)	24.2	16.7	36.7	94.3	80.4	25.4	2.73	0.46	66.7	58.4	7.3

DM=Dry matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, ADL=Acid detergent lignin, NDF-N=Nitrogen bound to neutral detergent fiber, ADF-N=Acid detergent fiber, TEPH=Total extractable phenolics, TET=Total extractable tannins, TCT=Total condensed tannins.
SEM: Standard error of the means, LSD: Least significance differences.

Table 2. Mineral concentrations in acacia species

	Ca	Mg	P	S	Mn	Mo	Zn	Co	Cu	Fe	Se
	g/kg DM				mg/kg DM						
Fruits											
A. nubica	3.2	1.3	1.5	6.6	9.4	18.0	12.6	2.5	57.2	254	>100
A. tortilis	6.1	1.5	1.6	2.5	12.3	31.4	21.6	2.8	17.2	223	>100
A. brevispica	4.3	1.5	1.0	0.7	22.0	31.9	17.4	3.2	42.9	220	62.5
Leaves											
A. reficiens	12.1	2.3	0.7	3.9	67.8	23.4	22.9	3.9	54.5	745	>100
A. senegal	14.2	1.9	1.1	1.9	25.9	36.6	17.9	4.0	52.9	307	>100
SEM	1.5	0.11	0.14	0.68	7.0	2.2	1.2	0.22	7.3	74.1	5.0
LSD (p<0.05)	3.9	0.29	0.35	1.75	18.1	5.7	3.1	0.56	18.8	190.6	12.9

SEM: Standard error of the means, LSD: Least significant difference.

1986), but higher than those of Ghanaian (Apori et al., 1998) browse species. The probable reasons for this variation in polyphenolics concentration would be plant part, stage of maturity, seasonal variation and regions (Larbi et al., 1998; Makkar, 1993). Makkar and Becker (1998) reported relatively higher total phenols from African forages compared to Himalayan forages (15.7 vs 6.0%).

Table 2 presents the mineral concentrations in the acacia species. The leaves were higher ($p<0.05$) in calcium and magnesium than the fruits. Phosphorus levels were low in all species, resulting in Ca:P ratios of 2.1:1 to 17.3:1. Reficiens and brevispica had significantly ($p<0.05$) lower phosphorus and sulfur respectively. Except for copper, nubica tended to have a relatively lower concentration of microelements. The highest concentration of manganese was recorded in reficiens, which was significantly ($p<0.05$) different from other species. Cobalt ranged from 2.5 mg/kg DM in nubica to 4.0 mg/kg in senegal. Iron concentration was exceptionally higher in senegal, and significantly ($p<0.05$) different from other species. Values obtained in this study were consistent with the wide range of mineral data reported (Norton, 1994; Topps, 1992). There is very scanty information on mineral concentration of browse species. However, except for phosphorus and magnesium, the acacia species were rich in minerals, and the levels were above the critical requirement for ruminants (McDowell, 1985). The higher calcium content was comparable to those of *Leucaena leucocephala* from the Philippines (Orden et al., 1999). All but tortilis were high in copper. Zinc values were consistent with the values reported for other acacia species (Sawe et al., 1998). The copper and sulfur values in this study were higher and lower respectively than those reported for acacia species by Sawe et al. (1998). Generally browses are rich in microelements (Topps, 1992), but species differences, plant parts and seasonal changes will influence their mineral concentration (Little et al., 1989). Salawu et al. (1999) compared various parts of *Calliandra calothyrsus*, and reported differences between seeds,

Table 3. Gas production (ml/200 mg DM) after 12, 24, 48, 72, 96 h and gas production characteristics of acacia species

	12	24	48	72	96	*a*	*b*	*c*	*a+b*	OMD48 g/kg DM
Fruits										
A. nubica	24.7	28.1	32.4	33.4	34.4	5.2	27.9	0.0925	33.2	556
A. tortilis	26.9	30.5	37.9	40.7	41.6	4.0	36.7	0.0655	40.7	611
A. brevispica	15.3	28.2	32.8	37.0	39.3	-0.27	41.3	0.0500	38.5	559
Leaves										
A. reficiens	13.9	19.6	21.1	22.7	23.7	2.5	20.4	0.0699	22.9	465
A. senegal	17.7	22.3	29.5	30.1	32.9	-0.15	32.2	0.0564	32.1	587
SEM	1.8	1.5	1.9	2.1	2.2	1.2	2.5	0.00529	2.2	18.2
LSD ($p<0.05$)	4.7	3.9	5.0	5.6	5.6	3.0	6.4	0.01360	5.6	46.7

OMD48=Organic matter digestibility at 48 h.

a, b, c are constants in exponential equation $p=a+b\ (1-e^{-ct})$.

SEM: Standard error of the means, LSD: Least significant difference.

pods and leaves. Higher values of iron and selenium were comparable to those of *Leucaena leucocephala* (Orden et al., 1999) and iron concentration of 905 mg/g from *Calliandra calothyrsus* seeds (Salawu et al., 1999).

Table 3 presents the gas production parameters. Significant ($p<0.05$) variation among the species was recorded in gas production after 12, 24, 46, 72 and 96 h. Gas production at 48 h from tortilis and reficiens was significantly ($p<0.05$) higher and lower respectively than other species. The rate of gas production (c) and potential gas production (a+b) were significantly higher in nubica and tortilis respectively. The calculated organic matter digestibility at 48 h (OMD48) was subsequently higher in tortilis (611 g/kg DM) and lower in reficiens (465 g/kg DM). The fruits tended to have higher potential gas production than the leaves. The rate of gas production was within the range reported for other browse species (Apori et al., 1998; Larbi et al., 1998). Generally the higher gas production from fruits could be attributed to relatively lower phenols in these species. These results are consistent with of those of Tolera et al. (1997) reporting higher gas production in browses with relatively lower phenolic concentration.

Concentrations of TEPH, TET, TCT and CP fractions were negatively correlated with gas production (table 4). The phenolics concentration particularly TEPH and TET correlated strongly ($p<0.05$), with gas production characteristics, with an $r=-0.82$ at 24 h with TEPH and TET. The correlation between TET and gas production agrees with the results of Tolera et al. (1997), reporting a strong and negative correlation between gas production and phenolics compounds of browse species. Correlation coefficient tended to be significantly ($p<0.07$) different between TCT and gas production after 24 h. However, a poor relationship was recorded between poly-

Table 4. Correlation coefficients (*r*) of the relationships between concentration of phenolic compounds, crude protein and gas production volume (ml/200 mg DM) from the acacia species after 12, 24, 48, 72 or 96 h incubation

	Correlation coefficient with			
	TEPH	TET	TCT	CP
Gas production after				
H12	-0.59	-0.58	-0.48	-0.30
H24	-0.82**	-0.82**	-0.59(0.07)	-0.44
H48	-0.75*	-0.78**	-0.36	-0.19
H72	-0.73*	-0.75*	-0.34	-0.24
H96	-0.72*	-0.75*	-0.30	-0.23
Gas production characteristics				
a	-0.14	-0.10	-0.42	-0.20
b	-0.56	-0.61	-0.11	-0.08
c	-0.29	-0.23	-0.57	-0.37
a+b	-0.71	-0.74*	-0.29	-0.20

a, b, c are constants in exponential equation $p=a+b\ (1-e^{-ct})$.
* $p<0.05$, ** $p<0.01$.

phenolics compounds and the gas production characteristics (*b, c* and *a+b*) with an exception of TET and a+b, which was significant ($p<0.05$). Khazaal and Ørskov (1994) and Tolera et al. (1997) reported a significant correlation between the TCT and the gas production. However, the insignificant relationship with TCT in our study could probably be due to the wide variation of the TCT in these samples. Another possible reason could be differences in the nature of tannins between the browse species. Larbi et al. (1998) also reported a weak negative correlation ($r=-0.44$ and -0.42) between rate of gas production and insoluble phenolics with browse species in wet and dry season respectively. A wide variation of form and concentration of condensed in tropical browse species was reported (Balagun et al., 1998). Physiological state of plants at time of harvest, type of species and post harvest processes such as drying are known to affect the estimation of tannins in browse. Mwendia (1994) found that the condensed tannins of sun dried *Calliandra calothyrsus* were more toxic to rumen bacteria than the condensed tannins of other shrubs.

CONCLUSION

The chemical composition, mineral concentrations, phenolic compounds and gas production were within the ranges reported elsewhere with other browse species. The fruits tended to have relatively lower concentrations of phenolics, but higher fiber levels. Although the study focused on just a few selected sub-species, there is an indication of the potential of acacia as feed supplements. The results warrant a further investigation in evaluating other subspecies. Agronomic and animal studies are required to establish the nutritive value of these species.

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