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Content of phenolics and tannins in leaves and pods of some *Acacia* and *Dichrostachys* species and effects on *in vitro* digestibility*

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ABSTRACT

Four browse legume foliages (leaves and pod fruits) from three species of *Acacia* (*A. polyacantha*, *A. tortilis*, *A. nilotica*) and *Dichrostachys* sp. native to Tanzania were evaluated for nutritive potential to establish levels and effect of normal phenolics and tannins antinutritive factors (ANFs) on *in vitro* feed digestibility (IVD). Total extractable phenolics (TP), tannins (TT) were estimated by Folin-Ciocalteu assay, and condensed tannins (CT) by butanol/HCl assay. In leaves, total proanthocyanidins were assayed into constituent anthocyanidins' flavonoids by high performance liquid chromatography (HPLC). Organic matter degradability (OMD) was estimated *in vitro* by gas production technique. Adverse effects of tannin's ANF on IVD were assessed by polyethylene glycol (PEG) tannin bioassay. Crude protein (CP) varied ($P<0.05$) between fodder species and foliages. Leaves had ($P<0.05$) higher CP (141-194 g/kg DM) in *Dichrostachys* sp. and *A polyacantha*, respectively, compared to pods (133-142 g/kg DM) in *A. tortilis* and *Dichrostachys* sp., respectively. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) va-

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ried (P<0.05) between fodder species and foliages. *A. nilotica* had lowest (P<0.05) NDF, ADF and ADL (182, 68 and 44 g/kg DM, respectively). *A. polyacantha* and *Dichrostachys* sp. had highest (P<0.05) NDF and ADF (416 and 146 g/kg DM, respectively). Pods had higher (P<0.05) fibre values than leaves. Leaves had higher (P<0.05) TP, TT, and CT (162, 138, and 55 mg/g DM), than pods (112, 105 and 35 mg/g DM), respectively. Proanthocyanidin flavonoids (mg/g DM) varied (P<0.05) from 0.062 (*A. nilotica*) to 5.288 (*A. tortilis*), 0.188 (*A. tortilis*) to 4.179 (*A. polyacantha*), and 0.009 (*A. nilotica*) to 4.392 (*Dichrostachys* sp.) for delphinidins, cyanidins and pelargonidin, respectively. The browse foliages had relatively high tannin content greater than 5% DM, a beneficial level in animal feeding and nutrition, and thus could impair feed digestibility. OMD varied significantly (P<0.05) both between fodder species and foliage components. Pods had higher (P<0.05) gas production (GP) and OMD than leaves. Addition of PEG improved (P<0.05) gas production, IVD and metabolizable energy (ME) in leaves and pods by binding tannins. Leaves had higher response on gas production and IVD due to PEG treatment compared to pods except *A. nilotica*. Improved gas production and digestibility due to PEG treatment indicate nutritive potential in browse fodder previously depressed by tannin ANFs.

Therefore, phenolics and tannins ANFs could limit utilization of browse fodder nutritive potential as supplements to ruminants consuming low quality roughages. Utilization of browse legume fodder could be optimized through reduction of tannin antinutritive activity. Under farmers' conditions, optimal utilization of browse could be achieved through feeding a mixture of tanniferous browse with other feeds especially high in nitrogen to dilute tannin antinutritive activity.

INTRODUCTION

Browse tree legume fodder represents cheap protein supplement for livestock in tropical countries where both feed quality and quantity pose a great constraint on animal production. In most tropical region ruminant production depends on low quality forages that are deficient in essential nutrients especially protein and minerals that fluctuate with seasons (Crowder and Chheda, 1982). In the dry season forage quality declines tremendously, CP drops to as low as 3.0 to 5.0% of DM (Kakengi et al., 2001), a level that is too low to meet minimum normal nitrogen requirements of 8% CP for optimal rumen microorganisms function (Hungate, 1966; Annison and Bryden, 1998). In turn, basal roughages alone could not meet animal requirements for maintenance and production unless supplemented with nitrogen (protein) sources (Leng, 1990). Conventional protein and energy supplements (concentrates, fish meal) are expensive and unaffordable by most low capital traditional farmers. Browse fodder could improve animal nutrition through increased CP content (110 to 250 g/kg DM) (Le Houérou, 1980). However, utilization of nutritive potential from browse legume fodder is limited by presence of anti-nutritive factors (ANFs) such as phenolics and tannins (Mangan, 1988; Kumar and D'Mello, 1995). Tannins refer to naturally occurring high molecular weight soluble polyphenolic secondary plant compounds that bind to, and form complex with proteins, carbohydrates, minerals, and other dietary nutrients during feed digestion (Mangan, 1988), rendering them unavailable to the animal. Phenolics and tannins cause reduced digestibility through

inhibition of digestive enzymes, complex formation with rumen microorganisms, and toxic effects to animal body (Mangan, 1988; Makkar et al., 1995). Subject to establishment of level of phenolics and tannins ANFs in browse foliages, the fodder could be used as protein supplements to ruminants fed on low quality basal roughages. *Acacia* spp. and other browsable tree leaves, fruits (fresh and dry pods) and seeds represent an important protein supplements for livestock fed on low quality roughages (standing hay, straw and stover) basal feeds in most arid and semiarid regions, or even in the tropics especially during dry seasons. However, utilization of browse as protein supplement has not been optimized following presence of polyphenolics antinutritive factors.

The current work aimed to develop a low cost and sustainable dry season protein feed supplements from promising tree legume fodder species native to semi-arid western Tanzania. A study was therefore conducted to determine essential chemical composition (feed protein and dietary fibres), phenolics and tannin levels in three species of *Acacia* and one species of *Dichrostachys* leaves and pods. Also, to estimate extent and rate of organic matter (OM) degradation, and to assess adverse effects of tannin on feed digestibility *in vitro*.

MATERIAL AND METHODS

Study area

Browse leaves and pod fruits from three species of *Acacia* (*A. nilotica*, *A. tortilis* and A. *polyacantha*), and *Dichrostachys* sp. were harvested from five administrative districts (Bariadi, Kahama, Meatu, Shinyanga Urban and Shinyanga Rural) in Shinyanga region, north-western Tanzania (2 - 3^{o}S; 31-31.5^{o}E). Shinyanga region forms a semiarid agro-ecological zone (SAEZ) located at 1100-1300 m above sea level. Minimum and maximum temperatures vary from 15 to 18.3^{o}C and 27.6 to 30.2^{o}C, respectively. This SAEZ receives low annual rainfall of 600 to 800 mm raining from November to mid May. Dry season is from June to October. Common vegetations include short grasses and scattered shrubs and trees mostly dominated by *Acacia* spp.

Forage sample collection and processing

For each four species, leaves and soft twigs samples were hand plucked from three positions of a tree canopy: on the lower, middle and upper parts of the tree trunk. The foliage samples were harvested from 8 to 10 trees selected at random in each of four sub-plots (70 x 70 m) in four rangelands in the five districts in the study area. Collected browsable fodder samples constituted a mixture of leaves

and thin twigs (midrib and twigs less than 5 mm), there after referred to as "leaves and soft twigs", and pod fruits. Pod fruits were collected while still intact to the plants just before drying when had turned to yellowish and were separated from pods with inflorescence as well as from dry pods either still attached on the tree or already dropped on the ground. The leaves and pod foliages were harvested at advanced maturity stage in late rainy season between mid April and mid May in 2001. Harvested browse samples were pooled for each individual tree species and foliage components (leaves and soft twigs separated from pod fruits). The samples were dried at 50°C in a forced air oven for 48 h to constant weight, then ground to pass through a 2.00 mm sieve and sub-sampled into 24 bulk samples (three samples for each species and foliage components) for further laboratory analyses. Samples for phenolics, tannins and for *in vitro* degradability assays were ground to pass through 1 mm-sieve.

Chemical analyses

Chemical composition: dry matter (DM), organic matter (OM), ash and crude protein (CP) (N x 6.25) (Kjeldahl technique) were estimated based on AOAC (1990) standard procedures. Fibre components (NDF, ADF, ADL or lignin) were estimated from detergent solvents (Van Soest et al., 1991).

Phenolics and tannin assays

Approximately 200 mg DM fine ground (1.00 mm) sample was extracted in 10 ml of aqueous acetone (7:3 v/v) in a water bath maintained at 40°C rotating at 130 cycles rpm for 90 min. The supernatants were carefully transferred into 10 ml centrifuge tubes, and centrifuged at 3 000 rpm (4°C) for 20 min. The aqueous aliquots were assayed for total phenolics and tannins, and the residue discarded. Total extractable phenolics (TP) were assayed by Folin-Ciocalteu's reagent (Sigma-Aldrich Chemie, Steinhein, Germany) based on regression equation of known concentrations of tannic acid standard (Sigma-Aldrich Chemie, Steinhein, Germany) as described by Jolkumen-Tiito (1985) and Makkar (2000). Total extractable tannins (TT) were estimated gravimetrically as a difference of phenolics remaining from total phenolics after binding tannins with polyvinyl polypyrrolidone (PVPP; Sigma-Aldrich Chemie, Steinhein, Germany) as described by Makkar et al. (1993). Total extractable condensed tannins (CT) or proanthocyanidins were assayed by butanol/HCl/Fe^{3+} assay (Porter et al., 1986). Concentrations of TP and TT were expressed as tannic acid equivalent, while CTs were expressed as leucocyanidins equivalent. In leaves CTs were assayed into different leucoanthocyanidin flavonoid constituents by high performance liquid chromatography (HPLC) based on Hedqvist et al. (2000) and Stewart et al. (2000) techniques. A 1.00 g (1.00 mm) sample

was extracted for 30 min in an ultrasonic water bath with 4.0 ml aqueous acetone (7:3 v/v) containing 1 g/l ascorbic acid, centrifuged at 3,000 rpm for 15 min. 0.5 ml supernatant was vortexed with 0.25 ml of dichloromethane and centrifuged again. A 50 µl aliquot of the aqueous upper layer was combined with 3.0 ml of butanol/HCl (95:5, v/v) and heated at 95°C for 1 h. Butanol/ HCl was evaporated to dryness under nitrogen stream with the tubes kept at 50°C. The residue was redissolved in 0.5 ml of methanol/HCl (99:1, v/v), and filtered through a 0.02 polytetrafluoroethylene membrane, followed by injection of 10.0 µl aliquot into Inertsil ODS-80A (C 18) column, 150 x 4.6 mm (Shimadzu Co., Kyoto, Japan). Water/acetic acid (96:4, v/v; solvent A) and methanol (solvent B) were used for gradient elution at 2 ml/ min. The gradient profile was 5-40% B (0-5 min); 40-50% B (5-10 min), 50-100% B (10-15 min) and 100-5% B (15-20 min). The absorbance at 525 nm was recorded using a LC-10AT HPLC system (Shimadzu Co., Kyoto, Japan) fitted with a CR-6A data processor (Shimadzu Co., Kyoto, Japan) and a SPD-10A variable wavelength detector (Shimadzu Co., Kyoto, Japan). The peaks were identified using cyanidin chloride, delphinidin chloride and pelargonidin chloride (Extrasynthese Co., Genay, France), which had retention times of 7.48, 10.24 and 12.64 min, respectively.

In vitro organic matter degradability study

Animals and management. Rumen fluid for *in vitro* gas production, digestibility and tannin bioassay studies was obtained from three healthy mature Japanese Corriedale female sheep fitted with permanent rumen cannulae (70 mm). The fistulated animals were kept on standard daily ration of 800 g timothy hay and 200 g concentrates (2-parts wheat bran and 1-part rolled barley) divided into two equal meals fed at 8.00 and 16.00 h daily. The animals were supplemented with minerals to meet body requirements according to ARC (1990), and had free access to water throughout the experimental duration.

In vitro gas production. Rumen fluid was obtained from the three sheep early in the morning before feeding. Preparation of an *in vitro* mineral buffer media for gas test was conducted as described by Menke and Steingass (1988). Rumen fluid was filtered through a cheese cloth and mixed (1:2 v/v) with an anaerobic buffer-mineral solution containing, per litre, g: $NaHCO_3$, 8.75; NH_4HCO_3, 1.00; Na_2HPO_4, 1.43; KH_2PO_4, 1.55; $MgSO_4 \cdot 7H_2O$, 0.15; Na_2S, 0.52; $CaCl_2 \cdot H_2O$, 0.017; $MnCl_2 \cdot 4H_2O$, 0.015; $CoCl_2 \cdot 6H_2O$, 0.002 and sodium resazurin, 0.125. The mixture was kept stirred under CO_2 flushing at 39°C using a magnetic stirrer fitted with a hot plate.

Browse legume fodder test feed samples were incubated *in vitro* with buffered rumen fluid in 100 ml graduated glass syringes following the procedure described by Menke and Steingass (1988). Approximately 200 mg DM (1 mm milled feed samples) were weighed into 100 ml syringes in triplicate, and in each series a blank,

buffered rumen fluid without test feed sample, was run in triplicate. Vaseline oil was applied to the piston to ease movement and prevent escape of gas. The syringes were pre-warmed (39°C) for 1 h, before addition of 30 ± 0.5 ml of rumen buffer mixture into each syringe. 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 h of incubation. Gas production readings (ml) were recorded after 3, 6, 12, 24, 48, 72 and 96 h. The OM degradability characteristics, extent and rate of feed degradation were estimated by fitting gas production readings (ml) into degradability curve (Ørskov and McDonald, 1979) based on mathematical model:

$$y = a + b (1 - e^{-ct})$$ (McDonald, 1981),

where y is the potential gas production (ml/g OM), and a is gas production (ml/g OM) from immediately soluble OM fraction. Constant b is gas production (ml/g OM) from the slowly but potentially degradable feed fraction; and c is gas production rate constant (ml/h) from slowly fermentable feed fraction, b.

Effect of polyethylene glycol (PEG) treatment on digestibility. Adverse effects of tannins ANFs on *in vitro* OM digestibility (IVD) were assessed by incubation of approximately 500 mg DM test feed samples with or without 1.0 g polyethylene glycol (PEG) molecular weight (MW, 4 000) (Wako Pure Chemicals Industries Ltd., Japan). The feed samples were incubated in 100 ml glass syringes essentially by the procedure of Menke and Steingass (1988). The PEG tannin bioassay was conducted according to Makkar et al. (1995) as detailed by Makkar (2000). Rumen fluid for tannin bioassay experiment was obtained from similar sheep handled and treated in a similar procedure as for *in vitro* gas production test above except composition of the buffer medium. As the amount of feed incubated was 500 mg, composition of the rumen buffer medium was according to Tilley and Terry (1963). Reduced buffer medium composition, per litre, were, g: $NaHCO_3$, 35.0; NH_4HCO_3, 4.00; Na_2HPO_4, 5.7; KH_2PO_4, 6.2; NaCl, 2.22; $MgSO_4 \cdot 7H_2O$, 0.6; Na_2S, 0.52; $CaCl_2 \cdot H_2O$, 13.2; $MnCl_2 \cdot 4H_2O$, 10.0; $CoCl_2 \cdot 6H_2O$, 1.0 and sodium resazurin, 0.01 and, 60 ml freshly prepared reduction solution containing 580 mg $Na_2S.9H_2O$ and 3.7 ml 1 M-NaoH. A portion (40.0 ± 0.5 ml) of the rumen fluid medium was transferred into each syringe and incubated into water bath at 39 ± 0.1°C as described by Blümmel and Ørskov (1993). The syringes were pre-warmed (39°C) for 1 h, before addition of 40 ± 0.5 ml of rumen buffer mixture into each syringe, and incubated in a water bath maintained at 39 ± 0.1°C. The syringes were gently shaken every hour during the first 8 h of incubation. Gas production readings (ml) were recorded after 2, 4, 6, 8, 12, 16 and 24 h for both PEG treated samples and blank samples. Feed organic matter digestibility (OMD) (%) and metabolizable energy (ME) (MJ/kg DM) were estimated from equation of Menke and Steingass (1988) and Makkar and Becker (1996) based on 24 h gas production, (GAS, ml) and crude protein content (CP, % DM):

OMD (%) = 14.88 + 0.889GAS + 0.45CP, and
ME (MJ/kg DM) = 2.20 + 0.136Gv + 0.057CP.

Statistical analyses

Data on chemical compositions, *in vitro* gas production, degradability characteristics and digestibility estimates were subjected to analysis of variance (ANOVA) using the General Linear Model Procedure (GLMP) (SAS/ Statview, 1999). Data were analyzed based on statistical model:

$$Y_{ij} = \mu_{ij} + S_i + F_j + (S+F)_{ij} + e,$$

where, Y_{ij} is the general observation on chemical composition, gas production (GP), degradability characteristics, and *in vitro* digestibility parameters. μ_{ij} is the general mean common for each parameter under investigation; S_i is the i^{th} effect of browse fodder species on the observable parameters; F_j is the j^{th} effect of foliage components (leaves or pods), $(S+F)_{ij}$ represent i^{th} and j^{th} interaction effects of fodder species and foliage component on the observed parameters, and *e* is the standard error term.

RESULTS

Chemical compositions

Results on chemical compositions are presented in Table 1. Feed protein (CP), ash content, fibre components (NDF, ADF, and ADL) were significantly ($P<0.05$) different between both fodder species and between leaves and pods. Leaves harvested from *A. polyacantha* and *A. tortilis* had higher ($P<0.05$) CP (194 and 188 g/kg DM, respectively), compared to *A. nilotica* and *Dichrostachys* sp. that had lower ($P<0.05$) CP (144 and 141 g/kg DM, respectively). There was no significant ($P>0.05$) difference in CP between leaves and pods except in *A. polyacantha* (194 vs 133 g/kg DM) and *A. tortilis* (188 vs 133 g/kg DM) (Table 1). Ash varied ($P<0.05$) between both leaves and pod foliages (Table 1), and was influenced ($P<0.001$) by fodder species and foliages interaction. There was no difference ($P>0.05$) in ash content (g/kg DM) between leaves and pod foliages except A. *polyacantha* (121 vs 85) and *Dichrostachys* sp. (96 vs 67), respectively. Leaves harvested from *A. polyacantha* and *Dichrostachys* sp. had higher ($P<0.05$) ash content than pod fruits. In leaves, ash varied ($P<0.05$) from 70 (*A. nilotica*) to 121 g/kg DM (*A. polyacantha)*. *A. nilotica* pods had the lowest ash content (63 g/kg DM), and was not different ($P>0.05$) from pods harvested from other fodder species except *A. polyacantha* (85 g/kg DM). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) varied ($P<0.05$) both between fodder species and between

leaves and pod foliages (Table 1). In both leaves and pod foliages, *A. nilotica* had lowest (P<0.05) NDF (182 vs 187 g/kg DM, respectively) compared to *A. polyacantha* (416 vs 488 g/kg DM, respectively). Leaves and soft twigs had lower NDF fraction though not statistically different from pod fodder except *Dichrostachys* sp. (Table 1). ADF ranged from 68 (*A. nilotica*) to 150 g/kg DM (*A. tortilis)* in leaves, and from 86 to 214 g/kg DM in *A. nilotica* and *A. polyacantha* pods, respectively. ADL ranged from 44 to 123 g/kg DM in *A. nilotica* and *Dichrostachys* sp. leaves, respectively, and from 37 to 162 g/kg DM in *A. nilotica* and *A. polyacantha* pods, respectively. There was no difference (P>0.05) in ADL contents between leaves and pods except *A. polyacantha* (162 vs 107 g/kg DM) and *Dichrostachys* sp. (74 vs 123 g/kg DM) (Table 1).

TABLE 1

Chemical compositions in selected browse tree leaves and pod fruits, g/kg DM

Fodder species	Foliages	CP	Ash	NDF	ADF	ADL
A. polyacantha	leaves	194[a]	121[a]	416[a]	136[a]	107[a]
	pods	133[b]	85[bd]	488[b]	214[b]	162[b]
A. tortilis	leaves	188[a]	82[b]	361[c]	150[a]	101[a]
	pods	133[b]	65[b]	383[c]	200[b]	111[ad]
A. nilotica	leaves	144[b]	70[c]	182[d]	68[c]	44[c]
	pods	137[b]	63[c]	187[d]	86[c]	38[c]
Dichrostachys sp.	leaves	141[b]	96[d]	380[c]	146[a]	123[d]
	pods	142[b]	67[c]	348[e]	149[a]	74[e]
Mean	leaves	167	92	335	125	94
Mean	pods	136	70	352	162	87
Significance of effects						
browse species		***	***	***	***	***
foliage component		***	***	***	***	ns
species and foliages		***	**	***	***	***

[a,b,c,d,e] different letters in the same column indicate significant differences (P<0.05)

*** P<0.001

ns = not significant (P>0.05)

Phenolics and tannins compositions

Table 2 presents total phenolics and tannin levels in browse legume leaves and pod fruits. Foliages harvested from three species of *Acacia* and *Dichrostachys* sp. had detectable total extractable phenolics (TP), total extractable tannins (TT) and total extractable condensed tannins (CT), and proanthocyanidins' flavonoid constituents. The polyphenolics varied significantly (P<0.05) between both browse

fodder species and foliage components (leaves and pods). Fodder species and foliage components interaction had influence (P<0.001) on TP, TT, CT, and proanthocyanidins (delphinidin, cyanidin and pelargonidins). In leaves, *A. nilotica* had highest (P<0.05) TP and TT (237 and 236 mg/g DM, respectively). *Dichrostachys* sp. leaves had highest (P<0.05) CT (74 mg/g DM) compared to *A. tortilis* leaves (51 mg/g DM). In leaves, TP, TT and CT varied (P<0.05) from 98 (*A. polyacantha*) to 237 (*A. nilotica*); 95 (*A. polyacantha*) to 236 (*A. nilotica*), and from 51 (*A. tortilis*) to 74 mg/g DM (*Dichrostachys* sp.), respectively. In pod foliages, TP, TT and CT varied (P<0.05) from 45 (*A. polyacantha*) to 180 (*A. nilotica*), 41 (*A. polyacantha*) to 163 (*A. nilotica*), and from 10 to 45 mg/g DM, respectively. Compared to leaves, pods harvested from *A. polyacantha*, *A. nilotica* and *Dichrostachys* sp. had lower (P<0.05) TP and CT content. Fractionation of CTs in leaves revealed detectable leucoanthocyanidins (delphinidins, cyanidins and pelargonidins). *A. tortilis*, *A. polyacantha* and *Dichrostachys* had the highest (P<0.05) delphinidin, cyanidin and pelargonidin concentrations of 5.288, 4.179 and 4.392 mg/g DM, respectively. *A. nilotica* had lowest (P<0.05) delphinidin (0.062 mg/g DM) and low pelargonidins (0.009 mg/g DM), though not (P>0.05) from *A. polyacantha* and *A. tortilis*. *A. tortilis* had the lowest cyanidins (0.188 mg/g DM).

TABLE 2

Phenolics and tannins compositions in selected browse leaves and pod fruits, mg/g DM

Fodder species	Foliages	Total phenolics	Total tannins	Condensed tannins	Delphinidins	Cyanidins	Pelargonidins
A. polyacantha	leaves	98^{a}	95^{a}	46^{ab}	0.454^{a}	4.179^{a}	0.098^{a}
	pods	45^{b}	41^{b}	41^{a}	n.a	n.a	n.a
A. tortilis	leaves	127^{c}	121^{ad}	51^{ab}	5.288^{b}	0.188^{b}	0.018^{a}
	pods	95^{a}	91^{a}	45^{ab}	n.a	n.a	n.a
A. nilotica	leaves	237^{d}	236^{c}	55^{b}	0.062^{a}	0.243^{b}	0.009^{a}
	pods	180^{e}	163^{cd}	10^{c}	n.a	n.a	n.a
Dichrostachys sp.	leaves	184^{e}	100^{a}	74^{d}	1.630^{c}	1.424^{c}	4.392^{b}
	pods	129^{c}	123^{ad}	43^{a}	n.a	n.a	n.a
Means	leaves	162	138	55	1.859	1.509	1.129
Means	pods	112	105	35			
Significance of effects							
species		***	***	***	***	***	***
foliages		***	***	***	***	***	***
species and foliages		***	***	***	***	***	***

^{a,b,c,d,e} different letters in the same column indicate significant differences (P<0.05)

*** P<0.001

ns = not significant (P>0.05)

n.a = condensed tannins in pod foliages were not fractionated for flavonoid compositions

Gas production potential and forage degradability characteristics in vitro

In vitro gas production varied significantly (P<0.05) both between legume fodder species and between foliage components (Table 3). Fodder species and foliages components had a significant (P<0.01) influence on gas production and OM fermentation characteristics. Gas production increased with feed incubation intervals. Pod fruits had higher (P<0.05) gas production potential compared to leaves at all incubations. In leaves, *A. nilotica* had highest (P<0.05) gas production at all incubations (3-96 h) compared to *A. tortilis* and *A. polyacantha* that had low (P<0.05) gas productions at 3-6 h, and 12-96 h incubations, respectively. Gas production (ml/g OM) at 3 and 6 h incubations ranged from 22.3 to 39.7 (*A. tortilis)*, and from 49.0 to 74.4 (*A. nilotica*). In leaves, *A. polyacantha* had lowest (P<0.05) gas production potential at 12, 24, 48, 72 and 96 h (75.5, 101.2, 111.7, 126.8 and 135.9 ml/g OM, respectively), compared to *A. nilotica* (109.2, 148.7, 183.6, 195.8 and 200.7 ml/g OM, respectively). Other species' foliages had intermediate gas production potential. Pods harvested from *Dichrostachys* sp. had lowest gas production at 3 and 6 (37.5 and 66.2 ml/g OM), respectively, compared to *A. polyacantha* (51.4 ml/g OM), and *A. tortilis* (78.1 ml/g OM). *A. polyacantha* pods had low (P<0.05) gas production at 12, 24 and 48 h, receptively, compared to *A. tortilis* pods, and *A. nilotica* pods. Gas production from pods at 72 h ranged from 180.0 (*A. polyacantha*) to 193.7 ml/g OM (*Dichrostachys* sp.), and was not different (P>0.05) between pod species. Similarly, *A. polyacantha* pods had low gas production at 96 h (186.0 ml/ g OM) compared to *Dichrostachys* sp. pods (199.5 ml/g OM), respectively.

The OM fermentation characteristics varied (P<0.05) between fodder species and between leaves and pods. Laves had lower (P<0.05) OM fermentation characteristics compared to pods. *A. tortilis* had lowest (P<0.05) a value (-6.5 ml/g OM) compared to *A. polyacantha*, that had high a (33.0 ml/g OM). Other browse fodder species and foliages (leaves and pods) had intermediate a values. Gas production from slowly degradable OM fraction, b varied (P>0.05) from 110.0 (*A. polyacantha*) to 181.0 ml/g OM (*A. tortilis*) in leaves, and from 152.0 (*A. polyacantha*) to 191.0 ml/g OM (*A. tortilis*) in pods. Pod foliages had higher (P<0.05) potential gas production (a+b) than leaves. *A. polyacantha* had lowest (P<0.05) a+b (131.0 ml/g OM) compared to *A. nilotica* leaves (197.5 ml/g OM). In pods, a+b varied (P<0.05) between fodder species. In pod fruits, the a+b ranged from 185.0 (*A. polyacantha*) to 194.5 ml/g OM (*Dichrostachys* sp.), respectively. The gas production rate constant, c, varied (P<0.05) from 5.8 (*Dichrostachys* sp.) to 5.9 ml/h (*A. tortilis*) leaves. In pods, *A. polyacantha* had the lowest c constant (5.8 ml/h) compared to *A. tortilis* (9.0 ml/h).

Effect polyethylene glycol (PEG) treatment on in vitro digestibility

Addition of PEG resulted in significant (P<0.05) improvement in gas production (Table 4), OM digestibility and metabolizable energy (ME) estimates (Table 5).

TABLE 3

Cumulative *in vitro* gas production potential (ml gas/g OM) in browse legume foliage leaves and pod fruits at different incubations, h, OM fermentation characteristics, a: b and (a+b) (ml gas/g OM); OM degradation rate constant, c (ml gas/h)

Forages and components		Incubation interval, h								Degradability characteristics			
Forage species	Foliages	n	3	6	12	24	48	72	96	a	b	a+b	c
A. polyacantha	leaves	3	37.4^{ab}	49.8^{ab}	75.5^{a}	101.2^{a}	111.7^{a}	126.8^{a}	135.9^{a}	21.0^{a}	110.0^{a}	131.0^{a}	5.9^{a}
	pods	3	51.4^{a}	76.2^{a}	106.5^{bc}	139.7^{b}	167.9^{bc}	180.0^{bc}	186.0^{b}	33.0^{b}	152.0^{b}	185.0^{b}	5.8^{a}
A. tortilis	leaves	3	22.3^{a}	39.7^{bc}	80.4^{a}	124.9^{c}	155.8^{b}	170.0^{b}	171.1^{c}	- 6.5^{c}	181.0^{c}	174.5^{c}	6.0^{b}
	pods	3	42.6^{b}	78.1^{a}	122.2^{b}	166.2^{df}	183.4^{c}	189.5^{cd}	196.5^{bd}	1.5^{d}	191.0^{d}	192.5^{d}	9.0^{a}
A. nilotica	leaves	3	49.0^{a}	74.4^{a}	109.2^{b}	148.7^{e}	183.6^{c}	195.8^{d}	200.7^{d}	18.5^{a}	179.0^{c}	197.5^{d}	5.8^{a}
	pods	3	41.5^{ab}	74.5^{a}	118.0^{b}	156.6^{f}	175.0^{c}	187.4^{cd}	196.3^{d}	10.5^{e}	182.0^{c}	192.5^{d}	7.8^{c}
Dichrostachys sp.	leaves	4	39.5^{a}	60.0^{ac}	93.7^{ac}	130.3^{c}	157.4^{b}	169.1^{b}	175.7^{c}	17.0^{a}	156.0^{b}	173.0^{c}	5.8^{a}
	pods	4	37.5^{a}	66.2^{a}	109.4^{b}	146.8^{be}	173.6^{c}	193.7^{cd}	199.5^{d}	12.5^{e}	182.0^{c}	194.5^{d}	6.1^{a}
Significance of effects													
species			ns	ns	**	***	***	***	***				
foliages			ns	**	***	***	***	***	***				
species x foliages			ns	ns	*	***	***	***	**				

^{a,b,c,d,e,f} different superscripts letters in the same column indicate significant differences (P<0.05)

* P<0.05; ** P<0.01; *** P<0.001

ns = not significant (P>0.05)

Addition of PEG had no effect on gas production, OMD and ME values in blank samples. In both leaves and pod foliages, *A. nilotica* showed the highest relative response to PEG treatment on gas production, OMD and ME estimates. Increase in gas production at 24 h due to PEG treatment ranged from 28.7 to 46.6% in *A. polyacantha* and *A. nilotica* in leaves, respectively; and from 14.4 (*A. polyacantha*) to 74.9% (*A. nilotica*) in pods. In leaves, increase in OMD due to PEG treatment ranged from 14.7 (*A. polyacantha*) to 23.4% (*A. nilotica*). Pod foliages had lower response (8.2 - 22.2%) on OMD due to addition of PEG except in *A. nilotica* pods (40.6%). Response on ME due to addition of PEG varied ($P<0.05$) between both leaves and pod foliages in three species of *Acacia* and *Dichrostachys* sp.. Increase in ME due to PEG treatment varied from 15.3 to 24.3% in A. *polyacantha* and *A. nilotica* leaves, respectively. *A. polyacantha* pods had the lowest response on ME (8.5 %) due to addition of PEG compared to *A. nilotica* (42.0%).

TABLE 4

Effect of polyethylene glycol (PEG) treatment on *in vitro* gas production (ml/g OM) at different incubation intervals, h

Forages		Gas production response at different incubation intervals, h							
Fodder species	Foliages	16 h				24 h			
		- PEG	+ PEG	increase	%	- PEG	+ PEG	increase	%
A. polyacantha	leaves	59.1[a]	73.9[b]	14.8	24.9	63.6[a]	81.8[b]	18.2	28.7
	pods	65.2[a]	73.9[b]	8.7	13.3	31.0[a]	35.5[b]	4.5	14.4
A. tortilis	leaves	47.8[a]	64.1[b]	16.3	34.1	57.6[a]	82.6[b]	25.0	43.0
	pods	71.9[a]	97.9[b]	26.0	36.1	83.1[a]	112.7[b]	29.6	35.6
A. nilotica	leaves	46.2[a]	62.4[b]	16.1	34.9	58.1[a]	82.6[b]	24.7	46.6
	pods	43.9[a]	85.0[b]	41.3	93.7	59.7[a]	104.4[b]	44.7	74.9
Dichrostachys sp.	leaves	60.0[a]	78.9[b]	18.9	31.5	68.9[a]	94.4[b]	25.6	37.1
	Pods	56.9[a]	80.9[b]	24.0	42.2	65.8[a]	88.8[b]	23.0	35.0
Significance of effects									
species		***	***	***	***	***	***	***	***
foliages		**	**	***	***	**	**	***	***
species x foliages		***	**	***	***	***	**	***	***

[a, b] different superscripts letters in the same row indicate significant differences ($P<0.05$) in gas production at 16 h and 24 h

** $P<0.01$; *** $P<0.001$

TABLE 5

Effect of polyethylene glycol (PEG) treatment on *in vitro* organic matter digestibility (IVD) (%) and metabolizable energy (ME) content, MJ/kg DM

Forages		Response in ME and OMD at different incubation intervals							
Fodder species	Foliages	24 h response in OMD, %				24 h response in ME, MJ/kg DM			
		- PEG	+ PEG	increase	%	- PEG	+ PEG	increase	%
A. polyacantha	leaves	48.5[a]	55.6[b]	7.1	14.7	7.11[a]	8.20[b]	1.09	15.3
	pods	48.4[a]	52.4[b]	4.0	8.2	7.17[a]	7.79[b]	0.61	8.5
A. tortilis	leaves	46.9[a]	57.1[b]	10.2	21.7	6.88[a]	8.44[b]	1.56	22.6
	pods	55.6[a]	67.9[b]	12.4	22.2	8.27[a]	10.16[b]	1.89	22.9
A. nilotica	leaves	45.4[a]	55.6[b]	10.2	23.4	6.69[a]	8.26[b]	1.56	24.3
	pods	46.0[a]	64.7[b]	18.7	40.6	6.80[a]	9.65[b]	2.86	42.0
Dichrostachys sp.	leaves	48.8[a]	59.0[b]	10.2	21.0	7.22[a]	8.78[b]	1.56	21.7
	pods	48.5[a]	58.0[b]	9.5	19.6	7.17[a]	8.63[b]	1.46	20.3

[a, b] different superscripts letters in the same row indicate significant differences ($P<0.05$) in OMD and ME response due to addition of PEG

DISCUSSION

Browse leaves and pod foliages from *Acacia* spp. and *Dichrostachys* sp. could form potential feed resources mainly as protein supplements to ruminants fed on low quality basal forages such as standing hay and crop by-products (straw, stover) that have low CP values (30-70 g/kg DM) especially during dry seasons. High CP values in browse (133-194 g/kg DM) could correct deficient nitrogen in basal roughages. Presented results on browse chemical compositions (Table 1) were comparable to other workers (Le Houérou, 1980; Topps, 1997; Abdulrazak et al., 2000). The latter authors reported high CP contents (103 to 336 g/kg DM) in browse. However, some variations in CP could be due to proportion of foliage sampled for analyses as well as stage of maturity. For example, Topps (1997) reported low CP contents of 153, 153 and 219 g/kg DM in *A. tortilis*, *A. hockii* and *A. senegal* old leaves compared to high CP values (210, 194 and 319 g/kg DM) in newly emerged leaves, respectively. Low CP contents in pod fruits for example those harvested from *A. polyacantha* and *A. tortilis* compared to the respective species' leaves, is partly explained by genotype factors that control differential accumulation of nutrients in leaves and fruits (pods) as related to stage of maturity. Pods were harvested before drying (when had started turning to yellow) at their advanced maturity stage. Therefore, browse legume fodder represent a potential feed resource that could be utilized as a protein supplement to correct deficient feed nitrogen to animals fed on poor quality basal roughages in tropical regions especially during dry seasons.

High ash contents in browse to a large extent could be related to browse fodder nutritive potential.

Lower fibre compositions (NDF, ADF and ADL or lignin) indicate promising nutritive potential in browse legume fodder. Low NDF content suggests feed of high cell contents (CC) that could be related to high feed digestibility. Other workers (Reed, 1986) had similarly reported low fibre compositions in East African *Acacia* spp. However, low fibre fraction in tanniferous browse should be interpreted with precaution. Most browse had low NDF, for example *A. nilotica* leaves and pods (182 vs 187 g/kg DM) that would suggest high cell contents (CC). High CC in some *Acacia* spp. foliages for example *A. nilotica* could be due to soluble polyphenolic compounds, that would lead to underestimation of NDF, ADF, and therefore over estimation of digestibility. *A. nilotica* had high total phenolics and tannins (Table 1) that possibly could had interfered with fibre determination.

High phenolics and tannins compositions in browse would depress feed nutritive values. The browsable fodder (leaves and pods) had tannins greater than lower beneficial level (5 % DM) in animal feeding and nutrition (Mangan, 1988). High levels of phenolics and tannins ANFs could have adverse effects (depressed feed intake, impaired feed digestibility and toxic effect on rumen microbes) especially when fed in high proportions in ruminants' diets depending on nature and tannin activity. Relatively low polyphenolics composition in pod foliages suggests its superiority as a protein supplement to animals fed on low quality basal forages compared to browse leaves and soft tender twigs. Accumulation of polyphenolics secondary plant metabolites represents a self-defence and adaptation mechanism in plants against foraging herbivores and insects, and even moisture stress. High polyphenolics in *Acacia* spp. has been reported elsewhere (Reed, 1986; Abdulrazak et al., 2000), or even *Dichrostachys* sp. (Shayo and Udén, 1999).

Delphinidin, cyanidin and pelargonidin are products of leucodelphinidins, leucocyanidins and leucopelargonidins flavonoids, respectively, following cleavage of polymerized (condensed) tannin flavan in hot mineral acid-alcohol mixture. Delphinidins, cyanidins and pelargonidins represent flavan-3-diol and flavan-3,4-diols flavonoids or a mixture of the two, are the depolymerized constituents of leucoanthocyanidins (condensed tannins). Detected delphinidin, cyanidin and pelargonidin flavonoids in *A. polyacantha, A. tortilis, A. nilotica*; and *Dichrostachys* sp. leaves elucidate the structure, type and nature of tannin as related to tannin biological activity. The flavan-3-diol and flavan-3,4-diols molecules elucidate the tannin molecule steriochemistry, and suggest presence of several isomers in the polymerized tannin molecule. For example, a flavan-3,4-diols flavonoids possess asymmetric carbon at C-2, C-3 and C-4, a structure that indicate presence of eight isomers. Tannin molecules with different isomeric forms in their flavan have different tannin structure and possibly different biological activity, and would have variable tannin antinutritive activity whether *in vitro* or *in vivo*.

Gas production is a result of feed fermentation *in vitro* that simulates the rumen degradability phenomenon. Gas production originates from feed fermentation and indirectly from CO_2 released from buffer mixture by volatile fatty acids (acetates, butyrates and propionates). The extent of gas production partly reflects efficiency of fermentation or extent of degradability of feed OM. Feed OM is fermented to microbial mass (microbial protein) and gases (CO_2, methane, CH_4) and short chain fatty acids (acetates, butyrates and propionates). Proportion of fermentation products depends on nature of feed, especially CP and fibre contents. Most of CP is degraded to microbial mass and gives less gas.

High gas production potential in *A. nilotica*, *A. tortilis* leaves and in pods suggest high extent and rate of feed fermentation. On the other hand, variable gas production within fodder species' foliages could be due to nature and proportion of fibre in the feed sample. Decreased degradability could mostly explained by phenolics and tannins binding feed nutrients in the process of fermentation. Makkar et al. (1995), Makkar and Becker (1996) and Getachew et al. (2000) reported similar observations. High feed *in vitro* degradability estimates indicated by gas production in *A. nilotica* different from expectations due to high phenolics and tannins could be attributed to specific nature of tannin activity as related to biological anti-nutritive activity. Some of variations could be due to plant genotypic characteristics in relation to type of phenolics and tannins' activity on digestibility. Makkar and Becker (1993) reported a variable nature of tannins between and within plant species as related to their nature and biological activity.

Similarly, observed differences on degradability both between fodder species and foliage components could be due to fibre type and extent of lignification (Van Soest, 1994; Fonseca et al., 1998). High degradability values in pod foliages could be due to lower phenolics and tannin contents and that could be related to decreased anti-nutritive activity. High gas production from *A. polyacantha* at initial incubations (3 and 6 h) could be explained by high CP. CP represents a large proportion of an immediately fermentable OM feed fraction at initial incubations, and decreases at subsequent incubations.

The reflected high gas production from *A. nilotica* could be explained by low fibre composition (Table 1), though had high total phenolics and tannins (Table 2). Results suggest that polyphenolics in *A. nilotica* had less depressive effect on gas production. Also low fibre could probably had less effect on depressed OM degradability *in vitro*. Based on gas production potential in leaves, *A. polyacantha* ranked lowest followed by *Dichrostachys* sp., *A. tortilis* and *A. nilotica* (the highest). This trend could be due to amount of lignin and extent of lignification and possibly due to level of tannin and related biological activity. The constant a, represent the immediately fermentable OM fraction. The b constant denotes a slowly fermentable feed fraction with time. The constants a and b are mainly related to type of fibre and extent of fibre lignification, and plant genotype as related to factors that control the a and b constants.

PEG binds tannins and deactivates tannin anti-nutritive activity on lowered nutritive values. Therefore, observed responses on improved gas production, OMD and ME values due to PEG treatment mainly reflects deactivation of tannin activity in tanniferous browse. Makkar et al. (1995) and Getachew et al. (2000) reported improved gas production and digestibility estimates due to PEG binding tannins ANFs in *Dichrostachys* sp. For example, incubation of browse fodder with PEG MWT 4 000 and 6 000 improved OMD from 25.3 to 39.5%, and from 25.3 to 42.5% in *Dichrostachys cinerea*, respectively (Makkar et al., 1995). Improved OMD and ME due to addition of PEG represent nutritive potential in browse foliages previously depressed by tannin activity. This follows the fact that PEG has high affinity for tannins (Makkar et al., 1995). Relatively lower response in pod foliages due to PEG treatment indicate relatively higher nutritive potential, that could be due to lower phenolics amount compared to those in leaves.

CONCLUSIONS

From this study it was concluded that both leaves and pod foliages from *A. polyacantha*, *A. tortilis*, *A. nilotica* and *Dichrostachys* sp. could be used as protein supplements to ruminants fed on poor quality roughages due to high protein and low fibre contents compared to basal roughages. However, high tannin (>10% DM) in these browse legumes could limit optimal utilization of browse fodder due to depressed feed digestion as was demonstrated by PEG tannin bioassay. Browse pods could be regarded of superior nutritive potential due to low phenolics and tannins contents. Pod fruits harvested from *A. polyacantha*, *A. tortilis*, *A. nilotica* and *Dichrostachys* sp. produced significantly more ($P<0.05$) gas and had higher degradability values *in vitro* compared to respective species' leaves. Improved digestibility due to PEG treatment represents nutritive potential previously depressed by tannins' antinutritive activity. PEG binds tannins, deactivates tannin antinutritive activity and recovers nutrients previously bound by tannins. The PEG bioassay demonstrated what could be appropriate utilization of tanniferous browse by reducing tannin levels. Therefore, utilization of browse fodder could be optimized by either reduction of phenolics and tannins levels in browse, or feeding of a mixture of feeds with readily available nitrogen together with tanniniferous browse to dilute tannin antinutritive activity. Further studies are recommended on assessment of browse fodder nutritive potential *in viv*o through palatability, intake and animal growth performance trials.

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STRESZCZENIE

Zawartość fenoli i tanin w liściach i strąkach niektórych gatunków *Acacia* i *Dichrostachys* oraz ich wpływ na strawność *in vitro*

Badano wpływ fenoli i tanin jako czynników antyodżywczych (ANFs) zawartych w liściach i strąkach trzech gatunków *Acacia* (*A. polyacantha*, *A. tortilis*, *A. nilotica*) oraz jednego gatunku *Dichrostachys* na strawność *in vitro* (IVD). Sumę dających się wyekstrahować fenoli (TP) i tanin (TT) oznaczano metodą Folina-Ciocalteu, skondensowane taniny (CT) stosując butanol/HCl. Ogólną zawartość proanthocjanidyn w liściach oznaczano metodą HPLC jako flawonoidy antycjanidyn. Strawność masy organicznej (OMD) oznaczano mierząc produkcję gazu metodą *in vitro*. Ujemne oddziaływanie tanin ANF na IVD określano biotestem polietylenoglikolowym (PEG). Zawartość białka ogólnego (CP) oraz NDF, ADF i ADL różniła się ($P<0,05$) w zależności od gatunku roślin, a także między liśćmi i strąkami. Strąki zawierały więcej ($P<0,05$) włókna niż liście, natomiast w liściach było więcej TP, TT i CT. Zawartość flawonoidów proanthocjanidynowyh: cyjanidyn, delfinidyny i pelar-

gonidyny, była podobnie zróżnicowana. Udział tanin w liściach był większy niż 5% w s.m., który to poziom uważany jest za korzystny z punktu widzenia żywienia zwierząt, i dlatego mógł wpływać ujemnie na strawność paszy. OMD różniła się istotnie ($P<0,05$) zarówno między gatunkami roślin, jak między liśćmi i strąkami. Produkcja gazu i OMD strąków były większe ($P<0,05$) niż liści. Dodatek PEG zwiększał ($P<0,05$) produkcję gazu, a tym samym IVD i zawartość energii metabolicznej w liściach i strąkach, dzięki wiązaniu tanin.

W podsumowaniu autorzy stwierdzają, że fenole i związki antyodżywcze tanin mogą ograniczać wykorzystanie krzewiastych roślin motylkowych jako potencjalnego źródła składników pokarmowych dla przeżuwaczy otrzymujących niskiej jakości pasze objętościowe. Wartość pokarmową tych pasz można poprawić poprzez obniżenie w nich zawartości ANFs. Użytkowanie tych roślin w warunkach farm może być poprawione przez skarmianie ich razem z innymi rodzajami pasz bogatymi w azot, celem zmniejszenia ujemnego działania ANFs.