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Effect of Tannins in *Acacia nilotica*, *Albizia procera* and *Sesbania acculeata* Foliage Determined *In vitro*, *In sacco*, and *In vivo*

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ABSTRACT : The nutritive value and the effect of tannins on the utilization of foliage from three commonly used legumes, *Acacia nilotica*, *Albizia procera*, and *Sesbania acculeata*, were determined. Three mature rumen-fistulated bullocks were used to study *in sacco* degradability and twelve adult sheep were randomly allocated on the basis of live weight to 4 groups of 3 in each to study the *in vivo* digestibility of the foliages. In all foliages, the contents of crude protein (17 to 24% of DM) were high. Fibre was especially high in Albizia (NDF 58.8% of DM vs. 21% in Sesbania and 15.4% in Acacia). Contents of both hydrolysable (4.4 to 0.05%) and condensed tannins (1.2 to 0.04%) varied from medium to low in the foliages. Acacia contained the highest level of total phenolics (20.1%), protein precipitable phenolics (13.2%) and had the highest capacity to precipitate protein (14.7%). Drying in shade reduced the tannin content in Acacia and Albizia by 48.6 and 69.3% respectively. The foliages ranked similarly for each of the different methods used to estimate tannin content and activity. Acacia and Sesbania foliage was highly degradable (85-87% potential degradability of DM *in sacco*), compared to Albizia (52%), indicating a minimal effect of tannins in Acacia and Sesbania. Yet, *in vitro*, the tannins in the Acacia inhibited microbial activity more than those in Albizia and Sesbania. Following the addition of polyethylene glycol to neutralise the tannins, gas production and microbial growth increased by 59% and 0.09 mg RNA equiv./dg microbial yield respectively in the Acacia, compared to 16-17% and 0.06 mg RNA equiv./dg microbial yield in the other foliages. There was a trend for low *in vivo* apparent digestibility of N in the Acacia (43.2%) and Albizia (44.2%) compared to the Sesbania (54.5%) supplemented groups. This was likely to be due to presence of tannins. Consistent with this was the low N retention (0.22 and 0.19 g N/g NI) in sheep supplemented with Acacia and Albizia compared to that for the Sesbania (0.32). Similarly, a trend for poor microbial N yield was observed in sheep fed these foliages. Across the foliages tested, an increase in tannin content was associated with a reduction in ruminal fermentation, N digestibility and N retention. For overall nutritive value, Sesbania proved to be the superior forage of the three tested. (**Key Words :** Acacia, Albizia, Sesbania, Polyphenolics, Digestibility, Purine Derivative)

INTRODUCTION

In Bangladesh, livestock are mainly reared at a subsistence level to supplement farm income. A scarcity of livestock feed is the major limitation to the success of such systems. Commonly, various tree leaves, flowers, pods and twigs are offered to livestock to augment the feed available in conventional grazing and straw feeding systems. It is estimated that the land allocated to crops, which comprise 60% of the total land area, provides about 87% of the feed offered to livestock and is mainly available as byproducts such as straw and crop residues. The remaining 13% comes from leaves of multipurpose trees, shrubs and herbs (Alam, 1998).

To supplement crop byproducts, foliage from leguminous trees is being increasingly recognized as a high quality feed resource (Evitayani et al, 2004). However, it remains underutilized. This is in part due to the presence of anti-nutritive factors, such as tannins, often found at excessive levels in the foliage of such trees. The presence of tannins at high levels can significantly restrict intake and the utilization of nutrients, particularly nitrogen (Mangan, 1988; Lawry, 1990; Reed et al., 1990; Pritchard et al., 1992; Kumar, 1992; McNeill et al., 1998; Barry et al., 2001). On the other hand, low levels of tannins in foliage can increase the utilization of crude protein by protecting it from digestion in the rumen thereby increasing the flow of essential amino acids to the small intestine for absorption (Waghorn, 1990; Barry et al., 2001). The increasing importance of various legume tree and shrub foliages in ruminant feeding systems in Bangladesh necessitates the

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need for further studies to define the nutritive values of the common foliages on offer and to understand the limitations imposed by their tannin contents. The present experiment was conducted to investigate the nutritive values and the effect of tannins on dry matter and N utilization and microbial N yield in foliage from *Acacia nilotica* (Acacia), *Albizia procera* (Albizia) and *Sesbania acculeata* (Sesbania).

MATERIALS AND METHODS

Collection and preparation of foliages

Leaves and pods were harvested from Acacia and Albizia that were approximately 6 years of age and 6 to7 meters high, and with approximately 2 months of regrowth. Sesbania was harvested 3 months after sowing. Branches were lopped at 1 meter in length from the growing points and the leaves and pods were manually separated. The bulk of the foliages were dried in shade for the feeding trial and another batch of fresh foliage from each tree species was collected and immediately frozen until freeze-drying. This was to minimize the loss of tannins normally caused by drying. Both samples were ground through a 0.5 mm sieve, bottled and covered with aluminum foil and kept in desiccators for determination of chemical composition. Shade dried samples were also ground through a 1 mm sieve for fiber analyses, *in vitro* digestibility and *in sacco* degradability studies.

Analytical methods

The proximate composition of shade and freeze-dried samples, for DM (Official method 934.01), OM (Official method 942.05), and N (Official method 984.13), was determined according to the AOAC (1990). Ash free neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were estimated using sodium sulphite according to the method of Faichney and White (1983). Total phenols (TP) were estimated by the Folin-Ciocaltau reagent method and Total tannin (TT) was determined by subtracting the values of polyvinylpolypyrrolidone bound tannins from TP and expressed as tannic acid equivalents (Makkar et al., 1993). Condensed tannin (CT) was determined by oxidation of CT in butanol-HCl reagent in the presence of iron and expressed as leucocyanidin equivalents (Porter et al., 1986). Gallotannin content was determined by hydrolysis to gallic acid with rhodanine and expressed as gallic acid equivalents (Inoue and Hagerman, 1988). Protein precipitatable phenolics were estimated by formation of protein complexes with BSA using the Ferric chloride assay (Makkar et al., 1988). Protein-binding capacity was measured according to the formation of protein-tannin complexes on filter paper by the filter paper-protein Ponceau S dye assay (Dawra et al., 1988). Tannin content was also assayed by formation of protein tannin complex formed on agarose gel with bovine serum albumen (BSA). The diameter of ring formed was used to indicate the protein precipitation/binding capacity of tannins (Hagerman, 1987). ^{125}I was used to label BSA for the Radio-labeled BSA precipitation method and the BSA precipitated was determined from counts and plotted/unit forage DM (Hagerman et al., 1998).

In sacco degradability

Three matured fistulated bulls were fed *ad libitum* rice straw, 1.5 kg each of grass and tree legume foliages and 1 kg wheat bran per day during the trial. Approximately 3 g of each shade dried forage sample in decron bags was incubated in triplicate in the rumen for up to 96 hrs and the samples were withdrawn at 4, 8, 16, 24, 36, 48, 72 and 96 h. Immediately after withdrawal the bags were kept in cool water, and to remove adhered feed particles they were washed in cool water in a domestic washing machine for 20 minutes and dried in an oven for about 30 h at 65°C to constant weight. The degradability of DM was calculated using "NAWAY" program developed at the Rowett Research Institute according to Bhargava and Orskov (1987).

In vitro digestibility

Rumen fluid from 2 adult bulls was used. The bulls were fed on rice straw *ad libitum*, and 2 kg each of a concentrate mixture, grass hay and tree foliages per day. Rumen buffer solution was prepared by the addition of bicarbonate buffer solution, macro and micro mineral solutions to rumen fluid according to the recipe given by (Manke et al., 1979). Approximately 375 mg of ground shade dried foliage, with or without 750 mg of PEG added, were put into 100 ml glass syringes, in triplicate, and incubated in the rumen fermentation buffer solution for 24 h at 39°C. Gas volume was recorded at 0, 3, 9, 12 and 24 h and the effect of tannins on fermentation was determined by the difference between the net gas produced from the syringes with or without PEG, after a correction for gas produced from the incubation of a standard hay (Makkar et al., 1995). After 24 h of fermentation the contents of syringes were collected in tubes, centrifuge for 30 min (20,000 g at 4°C), washed and centrifuged again. The pellets were freeze-dried and ground in pestle and mortar and microbial mass was estimated from the concentration of ribonucleic acid in the pellets, according to Makkar and Becker (1999).

In vivo digestibility

A short term *in vivo* feeding trial was conducted to

Table 1. Composition of the foliages and the other dietary ingredients (% DM)

	Acacia	Albizia	Sesbania	Hay	Conc.
Shade dried					
OM	79.8	78.3	76.9	77.2	81.6
N	2.9	3.1	3.7	1.4	4.9
NDF	15.4	58.8	21.0	60.9	-
ADF	12.8	43.2	14.8	36.4	-
Lignin	4.5	20.9	4.3	23.9	-
Freeze dried					
OM	86.3	86.8	83.4		
N	4.1	3.9	5.8		
NDF	14.7	60.3	18.5		
ADF	13.1	41.7	12.1		
Lignin	4.1	18.5	5.9		

OM = Organic matter; N = Nitrogen.
NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

determine the effect of each shade dried tanniniferous foliage species on digestibility, N balance, and microbial protein yield by purine derivatives method (Chen et al., 1990). Twelve adult sheep with a mean live weight of 12.5 (±2.15) kg were used in the experiment. The animals were randomly allocated on the basis of live weight to 4 groups with 3 sheep in each and were housed in individual metabolic crates. Water was available at all times. The sheep were offered the shade dried foliages over 3 feeding periods, with a set level of foliage offered at each period. The amounts of foliage offered were calculated to provide 15, 10 and 5 g of N/d for the first, second and third feeding periods respectively. In addition to a basal amount of grass hay (95 g DM/d), at each feeding the levels of foliage fed were: Acacia 495, 330 and 165 g/d; Albizia, 330, 220 and 110 g/d; Sesbania 410, 270 and 135 g/d. Across the successive feeding periods the control group was offered a concentrate mixture of wheat bran, oil cake and salt fed at 430, 220 and 140 g/d respectively, in addition to the basal 95 g/d of grass hay. Each trial comprised 7 d preliminary, 7 d collection and 10 d rest periods. The sheep were re-randomized to treatments after each trial.

During each feeding trial, feed intake, faces and urine voided were measured daily. Faces were bulked in a freezer, sub-sampled after the collection period, freeze-dried and ground for chemical analysis. Urine was collected in 10% sulphuric acid daily, measured, processed and kept frozen for each sheep prior to the determination of purine derivatives and microbial N yield according to IAEA-TECDOC-945 (1997).

Statistical analysis

The data of *in vitro* and *in vivo* parameters were analyzed in 3×2 and 4×3 factorial arrangements using randomized block design. *In sacco* degradability was determined by regression analysis using the General Linear Model Procedures. Pearson's correlation coefficient was used to estimate associations between tannin contents determined by each of the assays used. General Linear Models Procedure of SAS version 6.03 (1988) was used to analyze the data and means were separated by Duncan's multiple range test.

RESULTS

Chemical composition

The chemical composition of foliages, grass hay and

Table 2. Contents of tannins in the foliages as defined by a variety of tannin assays before and after shade drying

	Acacia		Albizia		Sesbania	
	FD	SD	FD	SD	FD	SD
Total phenols (mg/dg DM)[a]	20.08±0.08	11.94±0.08	13.90±0.07	5.92±0.05	4.93±0.08	4.99±0.03
Tannin (mg TA/dg DM)[a]	13.61±0.03	9.16±0.04	6.62±0.02	3.91±0.02	2.60±0.02	2.65±0.04
Condensed tannin (mg TA/dg DM)[b]	1.18±0.00	0.81±0.00	0.84±0.00	0.51±0.00	0.43±0.00	0.27±0.00
Gallotannins (mg GA/dg DM)[c]	4.43±0.01	3.73±0.02	1.92±0.00	1.25±0.00	0.05±0.00	0.04±0.00
Protein precipitable phenolics (mg TA/dg DM)[a]	14.7±0.06	13.2±0.05	19.5±0.03	6.8±0.03	5.3±0.02	3.8±0.04
Filter paper assay						
(mg BSA pptd/dg DM)	17.8±0.07	18.9±0.05	19.2±0.08	11.2±0.07	1.9±0.00	1.9±0.00
(mg TA/dg DM)[a]	13.9±0.05	15.1±0.05	15.1±0.06	11.2±0.03	1.1±0.00	1.1±0.00
Radial diffusion assay (mg TA/dg DM)[a]	14.8±0.03	13.4±0.04	3.6±0.01	2.5±0.00	0.9±0.00	0.5±0.00
Radio-labeled protein precipitable phenolics (mg BSA pptd/dg DM)	14.2±0.02	13.9±0.03	15.7±0.06	13.2±0.03	1.9±0.00	2.0±0.00

FD = Freeze dried; SD = Shade dried; dg, decigram; DM = Dry matter; TA = Tannic acid; GA = Gallic acid; BSA = Bovine serum albumin.
[a] Tannic acid equivalent; [b] Leucocynadin equivalent; [c] Gallic acid equivalent.

Table 3. Mean *in sacco* degradability (%) parameters of the shade dried foliages

Foliages	a	b	a+b	c	RSD
Acacia	25.13	59.55	84.68	0.052	1.893
Albizia	20.10	31.54	51.64	0.033	1.828
Sesbania	23.58	62.97	86.55	0.038	1.631

a = Solubility of dry matter; b = Extent of digestion in the rumen.
a+b = Potential digestibility; c = Rate of digestion.

concentrate mixture is shown in Table 1. Each of foliages, shade dried, were rich in N and contained on average 2.3 times more N than the grass hay. The concentrations of NDF, ADF, and lignin in the Albizia and the grass hay were similar and high compared to those found in the Acacia and Sesbania foliage. The shade dried Albizia foliage contained approximately 1.7, 2.6, and 5.4 times more NDF, ADF, and lignin than the average of each for the Sesbania and Acacia foliage.

Tannin contents

All of the tannin assays showed high values for Acacia and Albizia and low for Sesbania (Table 2). Drying of all the foliages in shade reduced the concentration of tannins, according to almost all of the assays, by an average of 55%, as indicated from the differences of the values between freeze and shade dried samples. The reduction was most notable for the TP, TT, and CT assays for the Acacia and Albizia.

***In sacco* degradability of foliages**

The potential digestibility of DM was similarly high in the Acacia and Sesbania foliage (84.68 vs. 86.55%) compared to the relatively poor degradability of the Albizia foliage, at 51.64% (Table 3). This was largely due to the poor extent of digestion in the rumen for the Albizia, which was approximately half that observed for the Acacia and Sesbania. The highest rate of digestion was observed for the Acacia foliage and lowest for the Albizia.

Gas production following *in vitro* digestion

The addition of PEG to the incubation increased gas production and the production of microbial RNA for all foliages (Table 4). The increases were highest for the Acacia, compared to the responses for the Albizia and Sesbania which were similarly low. Gas production was 3.6 times higher and microbial RNA increases 1.4 times higher for the Acacia compared the average of the Albizia and Sesbania.

Correlation between tannin concentrations and *in vitro* gas production

The correlation coefficient between the tannin concentrations or activities as measured by each of the assays used and the increase in gas production following the addition of PEG is shown in Table 5. All assays for tannin content and activity were positively correlated with gas production increase ($p<0.05$), with the exception of CT ($R = 0.76$).

Intake and *in vivo* digestibility

Within each level of N offered, intakes of DM were statistically similar across treatments (Table 6). However, the apparent digestibility of DM, OM, and N were consistently lower for the treatments containing the shade dried foliage compared to the hay+concentrate control diet ($p<0.05$). Of the shade dried foliages DM and OM digestibility tended to be lower for Albizia whilst N digestibility tended to be lowest for the Acacia ($p<0.05$).

Utilisation of N

At the highest level of N intake, N balance was depressed in the sheep fed the Acacia and Albizia whereas

Table 4. Gas production and the associated production of microbial RNA following *in vitro* digestion of the foliages before and after the addition of PEG

	Total gas production (ml)		Increase in gas production (%)	Level of sig.	Rumen microbes (mg RNA equi/dg)		Increase in microbes (mg RNA equi/dg)	Level of sig.
	-PEG	+PEG			-PEG	+PEG		
Acacia	15.44	24.58	59.21	**	0.30	0.39	0.09	*
Albizia	4.95	5.80	17.17	*	0.13	0.19	0.06	*
Sesbania	17.72	20.58	16.14	*	0.13	0.19	0.06	*

DM = Dry matter; PEG = Polyethylene glycol; RNA = Ribonucleic acid; dg = Decigram.
Significant differences ** $p<0.01$; * $p<0.05$.

Table 5. Correlation coefficients between the concentrations of tannins determinations by each of the tannin assays including the gas production assay

	Total phenols	Total tannins	Condensed tannins	Gallotannins	Gas production
Total phenols		0.99*	0.83*	0.98*	0.99*
Total tannins			0.86*	0.99*	0.98*
Condensed tannins				0.92*	0.76
Gallotannins					0.95*

* Significant differences ($p<0.05$).

Table 6. Intake and *in vivo* digestibility (%) of diets supplemented with the foliages as compared to the concentrate supplemented diet, offered at 3 levels of N intake

N intake level (g/d)	Parameters	Hay+concentrate	Hay+Acacia	Hay+Albizia	Hay+Sesbania	SEM
15	DMI (g/LW/d)	28.6	25.5	29.1	35.2	0.69
	Digestibility:					
	DM	75.8^{a}	51.3^{b}	41.5^{c}	51.1^{b}	0.04
	OM	78.7^{a}	53.0^{b}	44.6^{b}	53.9^{b}	0.04
	N	77.5^{a}	51.9^{b}	45.9^{b}	61.4^{bc}	0.03
10	DMI (g/LW/d)	16.0	20.8	25.7	17.6	0.24
	Digestibility:					
	DM	69.2^{a}	60.4^{ac}	45.5^{b}	53.5^{c}	0.03
	OM	72.4^{a}	63.4^{ac}	48.2^{b}	57.5^{bc}	0.02
	N	64.5^{a}	39.9^{b}	45.0^{c}	49.4^{c}	0.03
5	DMI (g/LW/d)	17.2	19.1	13.6	15.1	0.23
	Digestibility:					
	DM	63.7^{a}	51.9^{b}	47.1^{b}	51.2^{b}	0.03
	OM	68.8^{a}	51.8^{bc}	43.5^{b}	55.8^{bc}	0.04
	N	67.4^{a}	38.0^{b}	41.7^{b}	52.7^{c}	0.04

DM = Dry matter; OM = Organic matter; N = Nitrogen; SEM = Standard error of mean, LW = Live weight.
Different superscripts within rows denote significant differences ($p<0.05$).

Table 7. Retention of nitrogen (mg/kg LW/d) by sheep offered diets supplemented with the foliages as compared to the concentrate supplemented diet, at 3 levels of N intake

N intake level (g/d)	Parameters	Hay+concentrate	Hay+Acacia	Hay+Albizia	Hay+Sesbania	SEM
15	N intake	1,193^{a}	686^{b}	853^{b}	1,143^{a}	75.5
	Faecal N	272^{a}	325^{ab}	459^{b}	443^{b}	30.8
	Urinery N	449^{a}	106^{b}	197^{b}	241^{b}	45.2
	N balance	472^{a}	254^{b}	197^{b}	459^{a}	43.6
	Net N retained (g N/g NI)	0.40	0.37	0.23	0.40	
10	N intake	622	470	631	541	28.9
	Faecal N	223	254	349	273	18.9
	Urinery N	209	129	220	166	16.1
	N balance	190	59	62	102	26.1
	Net N retained (g N/g NI)	0.31	0.13	0.10	0.19	
5	N intake	539	435	320	408	31.7
	Faecal N	176	277	205	194	20.4
	Urinery N	183^{a}	93^{a}	43^{b}	62^{a}	18.6
	N balance	180	65	65	152	18.7
	Net N retained (g N/g NI)	0.33	0.15	0.23	0.37	

LW = Live weight; N = Nitrogen; NI = Nitrogen intake; SEM = Standard error of mean.
Different superscripts within rows denote significant differences ($p<0.01$).

N balance in the sheep fed the Sesbania remained as high as that for the sheep fed the control diet containing the concentrate ($p<0.05$). At the other levels of N a similar pattern emerged but did not reach statistical significance.

Yield of purine derivatives and microbial N by sheep

Only at the highest level of N offered were significant treatment effects observed, with microbial N flows highest for the sheep fed the control diet containing concentrate and the sheep fed Acacia compared to Albizia and Sesbania ($p<0.05$, Table 8). The comparatively high production of microbial N in the sheep fed Acacia was despite them tending to have a lower intake of DM and N than the other treatments ($p>0.05$, Tables 6 and 7).

DISCUSSION

The tanniniferous foliages compared in this study were grown under similar climatic and edaphic conditions and were harvested at approximately the same stage of regrowth. Therefore, the nutritional differences observed between them may be considered genetic rather than environmental.

Table 8. Excretion of purine derivative (PDe) and estimated microbial N yield by sheep offered diets supplemented with the foliages as compared to the concentrate supplemented diet, at 3 levels of N intake

N intake level (g/d)	Parameters	Hay+concentrate	Hay+Acacia	Hay+Albizia	Hay+Sesbania	SEM
15	Total PDe (mmol/d)	8.50[a]	8.12[a]	6.44[b]	6.78[b]	0.38
	Allatoin (mmol/d)	6.25	5.39	4.78	4.95	0.32
	Xan+Hypoxan (mmol/d)	1.05	1.22	0.43	0.69	0.13
	Uric acid (mmol/d)	1.21	1.51	1.23	1.14	0.12
	Microbial N (g/d)	6.84[a]	6.07[a]	4.72[b]	5.11[b]	0.32
10	Total PDe (mmol/d)	7.09	6.45	6.13	5.94	0.33
	Allatoin (mmol/d)	5.24	4.63	4.49	4.23	0.30
	Xan+Hypoxan (mmol/d)	0.67	3.76	0.85	0.57	0.84
	Uric acid (mmol/d)	1.18	1.16	1.06	1.16	0.06
	Microbial N (g/d)	5.08	4.64	4.49	4.09	0.27
5	Total PDe (mmol/d)	4.86	4.25	4.00	3.95	0.19
	Allatoin (mmol/d)	3.82	3.51	3.46	3.41	0.15
	Xan+Hypoxan (mmol/d)	0.84	0.55	0.34	0.34	0.08
	Uric acid (mmol/d)	0.20	0.20	0.20	0.20	0.00
	Microbial N (g/d)	3.51	2.88	2.54	2.59	0.17

PDe = Purine derivative excretion. N = Nitrogen. Xan+Hypoxan = Xanthene and hyposanthine. SEM = Standard error of mean.
Different superscripts within rows denote significant differences ($p<0.05$).

The shade dried Acacia and Albizia foliage contained consistently more tannin than the Sesbania foliage and most of that tannin appeared to be of the hydrolysable rather than condensed type. The hydrolysable tannin (HT) content of the Acacia and Albizia, in terms of the gallotannin assay, was 4.6 to 2.5 times more concentrated than that estimated for CT using the Butanol-HCl assay. The protein precipitation capacity of forage is particularly dependent on its content of HT (Makkar et al., 1991) and consistent with this the Acacia and Albizia foliage reacted strongly with the Protein precipitable phenolics assay. Values obtained by Filter paper and Radio-labeled methods are comparable for Acacia and Albizia, while the radial diffusion method was less sensitive for Albizia.

According to almost all of the tannin assays, shade drying reduced the concentration of tannins from 2 to 134% of the original concentrations, for Acacia and Albizia. A similarly dramatic decline in the concentration of extractable free and total CT has been shown in Calliandra (Perez-Maldonado and Norton, 1996; Wina et al., 1999) and Gliricidia (Ahn et al., 1989) foliage following drying. It is assumed that drying causes tannins to bind to cell wall in the plant DM rendering it unavailable for extraction. Whether these declines in tannin content translate into improved animal performance requires testing *in vivo* before any clear recommendations on the benefits of drying to feeding value can be made.

The *in sacco* degradability of the foliages appeared more related to assayable fiber content than to tannin content. Tannins can reduce the digestibility of DM in forages, although the effect is inconsistent (Barry et al., 2001; Makkar, 2003). Yet in the current study, the foliage with the highest tannin content, Acacia, also had the highest extent and rate of digestion in the rumen. By contrast, the extent of digestibility of the Albizia in the rumen was less than half that for the Acacia. The Albizia did contain a moderate level of tannin but had nearly twice the level of NDF and more than 5 times the level of lignin than the Acacia. Fiber and particularly lignin content are key factors influencing the digestibility of DM and consequently the availability of energy in forage (Van Soest, 1994). However, any assessment of the extent to which fiber content impedes energy availability in tanniniferous forages is complicated by the ability of tannins to interfere with current fiber assays. Tannins tend to inflate the results of fiber assays and the extent of this interference is unpredictable and so cannot be corrected for (Van Soest, 1994). Hence, the question of whether the digestibility of tanniferous forages is more influenced by cell wall content than tannin content awaits the development of an improved assay for the determination of fiber in them.

The increase in gas production and rate of synthesis of microbial mass following the addition of PEG provides a measure of the effects of tannin on the rate of fermentation of forage that is independent of fiber content (Makkar et al., 1995; Makkar and Becker, 1996). The increase in gas production when PEG was added in the current study showed that the tannins were inhibiting fermentation in all 3 of the foliages tested, and especially so for the tannin-rich Acacia. However, consistent with a higher fiber level being a more important limiter of fermentation rate than tannin

level, with or without PEG, the rate of fermentation and synthesis of microbial protein in the Albizia was less than a third and half respectively compared to the Acacia and the Sesbania. Compared to such a difference, the increase in gas production and microbial RNA synthesis in response to the addition of PEG was similar and relatively small (16-17%) for the Albizia and Sesbania. The effects of tannin on fermentation rate may therefore be only of importance at higher concentrations (Osuga, et al., 2005) such as those seen in the Acacia.

The short term *in vivo* trial was designed to offer 3 levels of tannins and 15, 10 or 5 g of N intake per day across 3 feeding periods. However, throughout the trial the sheep offered the tanniniferous foliage showed a preference for grass hay. This was likely a consequence of the sheep having had no experience with the foliages prior to the trial. Consequently, the desired intake of N per day in the foliage supplemented groups was not achieved during the first 2 levels of feeding. Although the high variance in feed intake meant a significant effect of treatment on feed intake was not found it appeared that the least preferred foliage was Acacia, followed by Albizia and Sesbania. The variation in feed intake may be related to differences in odor between the forages, as observed in other foliages (Abdulrazak et al., 1996; Stewart et al., 1998). High concentrations of tannins can depress intake. Forages containing 5.5%-10.6% CT could depress voluntary feed intake from 12% and 27% (Barry and Duncan, 1984; Waghorn et al., 1994), but levels of 3.4-4.4% of CT appear unimportant (Wang et al., 1996). The foliages in the current trial had an even lower content of CT (<1% DM), although TP did reach 12% of DM in the shade dried Acacia indicative that tannins other than CT could still be part of the intake problem.

Of the 3 forages, the availability of nitrogen in terms of apparent digestibility and N balance also tended to be lowest in the tannin-rich Acacia, and highest in the Sesbania, which contained minimal tannin. However, this effect only reached significance at the highest level of tannin and N intake. Tannins consistently reduce the digestibility of protein in forages (McNeill et al., 1998, Barry et al., 2001) and these data fit that general hypothesis. Yet, surprisingly, the yield of microbial N, as defined by the urinary excretion of purines technique, was higher in the Acacia compared to both the Albizia and Sesbania treatments. Remembering too that rate of synthesis of microbial RNA *in vitro* was also higher for Acacia than for the other 2 foliages, despite its higher tannin content, it seems the types of tannins or tannin-like substances in the Acacia may not bear deleterious to rumen microbes as expected. The relatively poorer utilization of N in the Acacia treatment could instead be a consequence of a post-ruminal inhibition of digestion and or metabolism. Before the effects of CT on protein digestibility, cellulolytic activity and post ruminal digestibility become apparent, concentrations need to be within the range of 2-10.6% (Barry and Manley, 1984; Waghorn, 1990; Kumar, 1992; Pritchard et al., 1992; Perez-Maldonado and Norton, 1996; Norton and Ahn, 1997; Norton, 1999). The concentrations of CT in the current study were below this range but the data suggests that the other types of tannins in the Acacia, with their high protein precipitating capacities, were sufficient to allow dietary protein to escape ruminal fermentation, as evident from the trend of high faecal N excretion, but impede post ruminal digestion, as evidenced by depressed excretions of urinary N.

Sesbania ranked as the most nutritionally promising of the 3 foliages tested. N balance in the sheep fed the Sesbania was similar to that in the sheep fed the concentrate, and was superior to that achieved with the other foliages. *In vivo* and *in sacco* digestibility of DM and OM for Sesbania was similar to the Acacia and better than Albizia, as was gas production *in vitro*. It was only in terms of the ability to support the synthesis of microbial protein that Sesbania appeared less effective than Acacia, and since it appeared to provide sufficient N, the limiting factor that sheep supplemented with Sesbania may respond to most is more rumen fermentable energy.

CONCLUSION

Sesbania contained minimal amounts of tannins, compared to Albizia, and Acacia. Acacia was particularly rich in tannins, of the non-condensed type. Drying reduced the content of tannins in Acacia and Albizia according to almost all of the tannin assays used and the addition of PEG improved the ability of microbes to ferment the foliages. Of the 3 foliages, Sesbania showed the most potential as a protein supplement, whilst Albizia was most limited by it lower availability of digestible energy and protein.

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