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The effect of mixtures of sun-dried tropical shrub legumes on intake and nitrogen balance by sheep

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Abstract

A feeding trial with sheep was carried out at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia to investigate the concept of using mixtures of sun-dried tropical shrub legumes to dilute tannin levels in the diet and improve nitrogen (N) utilisation by ruminants. Intake, digestibility and N retention were measured with animals consuming: low quality grass (*Brachiaria dictyoneura*) alone; low quality grass supplemented with *Cratylia argentea* alone (49:51); and grass plus *C. argentea* with high tannin *Flemingia macrophylla* at 2 levels (53:37:10; 55:26:19). The 2 diets with *F. macrophylla* averaged 0.5 and 0.9% extractable condensed tannins. Intake and N retention were not significantly affected by legume supplementation, even though apparent N digestibility was higher when legumes were added. Urinary nitrogen was reduced as *F. macrophylla* increased in the legume mixture, suggesting less rumen ammonia losses due to protein protection by condensed tannins. However, with more *F. macrophylla* in the mixture, there was a reduction in dry matter and fibre digestion and an increase in faecal N and faecal N-ADF. These results suggest that, in formulating legume mixtures to supplement low quality forages, it is important to consider not only tannin levels but also the digestibility of the legumes used.

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Introduction

A common feature in many woody tropical legumes is the presence of high levels of condensed tannins, which are known to depress intake (Barry and Duncan 1984), and reduce digestibility (Lyford *et al.* 1967) and bodyweight gains (Barry 1985). However, low levels of condensed tannins in temperate legumes are believed to be beneficial in ruminant diets, since they reduce soluble protein degradation in the rumen and increase protein flow to the small intestine (Barry *et al.* 1986; Waghorn *et al.* 1987). It follows that mixtures of shrub legumes with and without tannins could have practical implications in smallholder feeding systems, which involve supplementation of low quality grasses or agricultural by-products with fodder from woody legume species.

The Tropical Forages Program of CIAT (Centro Internacional de Agricultura Tropical) identified *Cratylia argentea* and *Flemingia macrophylla* as promising shrub legumes for acid infertile soils. However, the value of *F. macrophylla* as a fodder could be limited by high levels of condensed tannins (CIAT, unpublished data). *C. argentea* is free of tannins, but has high protein degradability in the rumen (Aroeira and Xavier 1990). Therefore, it could be expected that dilution of tannins through mixtures of *C. argentea* and *F. macrophylla* could increase rumen escape protein and improve nitrogen utilisation by ruminants, when fed as a supplement to low quality forages. This paper reports the results of a feeding trial designed to measure intake and nitrogen retention by sheep fed a low quality grass supplemented with sun-dried *C. argentea* alone or in mixture with sun-dried *F. macrophylla*.

Materials and methods

The feeding trial was conducted at CIAT's research station in Santander de Quilichao,

Departamento de Cauca, Colombia using 4 groups of 3 African hair sheep, with an average initial weight of 18 kg. Animals were randomly assigned to one of the following 4 treatments: (1) grass basal diet, 100% *Brachiaria dictyoneura*; (2) grass basal diet (60%), supplemented with 40% *C. argentea*; (3) grass basal diet (60%), supplemented with 32% *C. argentea* and low (8%) *F. macrophylla*; and (4) grass basal diet (60%), supplemented with 24% *C. argentea* and high (16%) *F. macrophylla* levels. The grass fed was mature with approximately 60% dead material and was fed unchopped, whereas *C. argentea* and *F. macrophylla* (10–12 weeks regrowth) were sun-dried, chopped and screened to separate leaves from thick stems.

Animals housed in metabolism crates were offered 70 g DM/$W^{.75}$/d (3.5% of body weight) in 2 daily allotments. To minimise selectivity by the animals, the morning (800 h) forage allowance contained half of the grass allowance and the entire legume supplement, whereas the ration offered in the afternoon (1500 h) consisted of grass only. During the entire trial, animals had free access to a mineral salt mix and were offered water individually 3 or 4 times daily.

Measurements

After a 10-day adjustment period, forage refused and faecal and urine excretion were measured daily during 10 consecutive days. Forage refused by each animal was hand-separated into grass and legume for weighing and subsequent chemical analysis. Forage samples, offered and refused, and faeces were analysed for total Kjeldahl nitrogen (N) (AOAC 1980), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Van Soest 1963), and nitrogen in acid detergent fibre (N-ADF) (Goering *et al.* 1972). Extractable condensed tannin levels (ECT) were analysed in freeze-dried samples of *F. macrophylla* using Butanol-HCl (Porter *et al.* 1986) and purified tannins of *F. macrophylla* as the standard. Urine samples were analysed for N by the Micro-Kjeldahl method.

Apparent digestibility coefficients for DM, NDF, ADF and N were calculated from dietary intake of each constituent and amounts recovered in faeces. Nitrogen retention was calculated using N data from forage offered, forage refused, faeces and urine.

Analysis of results

Animal response variables were analysed for a completely randomised design with 3 replications, using the ANOVA-2 procedure of MSTAT-C (1991). Correlation analysis between some animal response variables and forage quality attributes was also performed.

Results

Chemical analysis of forage offered

The chemical composition of the grass and sun-dried legumes offered to sheep is shown in Table 1. As expected, the CP concentrations of the 2 shrub legumes were 3–4 times higher than that of the grass. In contrast, NDF values of the 2 legumes were lower than in the grass fed. Levels of ADF and N-ADF were higher in *F. macrophylla* than in *C. argentea*. The nitrogen fraction bound to ADF was 32 and 50% of the total N in *C. argentea* and *F. macrophylla*, respectively. The level of ECT in *F. macrophylla* was 5%, which is in the range expected for sun-dried leaves (Cano 1993). The *in vitro* dry matter digestibility (IVDMD) of the forage of *C. argentea* and *F. macrophylla* used in the feeding trial averaged 42 and 31%, respectively. These digestibility values are considerably lower than the range (52–72%) found in a number of tropical legume species grown under similar conditions (Abaunza *et al.* 1991).

Table 1. Chemical composition of forages fed to sheep housed in metabolism crates.

Forage	CP[1]	NDF	ADF	N-ADF
		(%)		
B. dictyoneura	5.1	76.4	43.4	0.2
C. argentea	25.6	59.8	35.6	1.3
F. macrophylla[2]	18.8	58.7	47.7	1.5

[1]CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; N-ADF = nitrogen content in the acid detergent fibre.
[2]Extractable condensed tannin concentration = 5% (Butanol-HCl).

Intake and digestibility

Intake and apparent digestibility of dry matter (DM) and fibre (NDF, ADF) for sheep fed the grass alone and in mixture with the 2 legumes are presented in Table 2. Sheep consumed on

average 77% of the DM offered (2.5–2.6% of body weight) and clearly selected for legumes. As a result, legume comprised 45–51% of the forage consumed. Intake of DM, digestible DM, NDF and ADF did not differ ($P>0.05$) among treatments. Daily intakes of ECT for the mixtures with *F. macrophylla* were 2.4 and 4.7 g for the low and high *F. macrophylla* mixtures, respectively, which corresponded to 0.5 and 0.9% extractable condensed tannins in the diet.

Apparent DM digestibility was not affected ($P>0.05$) by legume supplementation. However, NDF and ADF digestibility coefficients were lower ($P<0.05$) in legume-supplemented diets than in straight grass diets.

Nitrogen intake and excretion

Results on N intake and excretion by sheep fed the grass with and without legume supplementation are shown in Table 3. Nitrogen intake and excretion in faeces and urine were higher ($P<0.05$) in animals supplemented with legumes than in animals fed the grass basal diet. There was a tendency for slightly positive N retention in animals supplemented with legume mixtures, while N retention of animals on grass alone or supplemented with *C. argentea* was negative, even though these differences were not statistically significant.

Nitrogen intake was similar ($P>0.05$) for the 3 legume-supplemented diets. However, urinary N was lower ($P<0.05$) when animals were fed the legume mixtures with low and high proportions of *F. macrophylla*. In contrast, faecal N was higher ($P<0.05$) in animals fed the legume mixture high in *F. macrophylla* than in animals fed the tannin-free legume (*C. argentea*) supplement. Excretion of faecal N associated with the ADF fraction (N-ADF) was lower in the grass-only diet than in the legume- supplemented diets and increased ($P<0.05$) when mixtures of *F.*

Table 2. Intake and digestibility of dry matter and fibre components by sheep fed grass alone (G) or supplemented with *Cratylia argentea* (C.a.) alone or in mixtures with *Flemingia macrophylla* (F.m.).

Measurement	Grass	Grass + Legumes			
	(G)[1]	C.a. (GC)	C.a. + low F.m. (GCLF)	C.a. + high F.m. (GCHF)	s.e.m.
Dry matter					
Intake (g/d)	457	476	510	511	19
Digestibility (%)	44.7	43.8	45.2	40.9	1.1
Neutral detergent fibre					
Intake (g/d)	348	311	347	351	13
Digestibility (%)	52.3 a[2]	49.0 b	50.2 ab	45.4 c	0.9
Acid detergent fibre					
Intake (g/d)	196	179	205	213	7
Digestibility (%)	47.4 a	34.4 b	35.6 b	32.0 b	1.2

[1]Forage consumed: G = 100% grass; GC = grass (49%) + C.a. (51%); GCLF = grass (53%) + C.a. (37%) + F.m. (10%); GCHF = grass (55%) + C.a. (26%) + F.m. (19%).
[2]Means in the same row followed by different letters are significantly different ($P<0.05$).

Table 3. Nitrogen (N) intake, excretion and retention by sheep fed grass alone (G) or supplemented with *Cratylia argentea* (C.a.) alone or in mixtures with *Flemingia macrophylla* (F.m.).

Measurement	Grass	Grass + Legumes			
	(G)[1]	C.a. (GC)	C.a. + low F.m. (GCLF)	C.a. + high F.m. (GCHF)	s.e.m.
N intake (g/d)	3.9 b[2]	12.1 a	11.5 a	10.8 a	0.5
N in faecal DM (g/d)	2.7 c	4.4 b	4.8 ab	5.3 a	0.2
N in faecal ADF (g/d)	1.4 c	2.4 b	3.2 a	3.6 a	0.2
N in urine (g/d)	1.7 d	7.8 a	6.5 b	5.1 c	0.3
N retained (g/d)	−0.5	−0.1	0.2	0.4	0.4

[1]Forage consumed: G = 100% grass; GC = grass (49%) + C.a. (51%); GCLF = grass (53%) + C.a. (37%) + F.m. (10%); GCHF = grass (55%) + C.a. (26%) + F.m. (19%).
[2]Means in the same row followed by different letters are significantly different ($P<0.05$).

macrophylla were fed (Table 3). The N-ADF as a proportion of total faecal N was 67% in the mixtures with *F. macrophylla* and 55% in the treatment with only *C. argentea*.

In general, the results of this study showed that intake of *F. macrophylla* was negatively correlated with digestibility of DM ($r = -0.89$, $P<0.03$); NDF ($r = -0.9$, $P<0.006$); ADF ($r = -0.9$, $P<0.005$); and N ($r = -0.9$, $P<0.006$).

Discussion

In this study, supplementation of a low quality grass diet with legume mixtures of variable tannin and low digestibility level did not improve total intake. It could be argued that this occurred because the entire legume supplement was offered in one meal. However, Lascano and Palacios (1993) observed that, when a mature grass (*Andropogon gayanus*) was supplemented with *Desmodium ovalifolium* high in tannins and of low digestibility, total DM intake did not increase. Large increases in total dry matter intake were found when the same grass was supplemented with a tannin-free legume (*Stylosanthes capitata*) of high digestibility. In this study, when the low quality grass was supplemented with *C. argentea*, with no tannins but of relatively low digestibility, total dry matter intake did not increase. These contrasting results suggest that, in formulating legume mixtures to supplement low quality forages, it is important to consider not only tannin level but also the digestibility of the legumes used.

The 2 legumes used in the feeding trial had similar N-ADF values (1.3 and 1.5%), which were above those (0.3%) normally associated with heat-damaged protein (Goering 1976). However, inclusion of *F. macrophylla* in the legume mixture resulted in more faecal N excretion than when only *C. argentea* was fed, with 67% of the faecal N being in the ADF fraction. These results could be explained by the high proportion of condensed tannins that are bound to protein and fibre in *F. macrophylla* (Cano 1993). It has been suggested that protein-tannin complexes can escape degradation in the rumen, but can be dissociated in the low pH environment of the abomasum (McLeod 1974). However, our results suggest that protein bound to tannins in *F. macrophylla* did not dissociate in the lower gastro-intestinal tract, probably related to irreversible covalent links formed during sun-drying of the forage. Alternatively, protein-tannin complexes that are naturally present in the plant could be stable at low pH. In sheep fed fresh *D. ovalifolium*, duodenal flow of protein bound to condensed tannins was similar to the intake of this form of tannins (Carulla 1994). High levels of faecal N-ADF (55% of total faecal N) observed with supplementation of *C. argentea* can not be attributed to tannin-protein complexes, but rather to other phenolic-protein linkages (Goering *et al.* 1972). In Brazil, sheep fed only *C. argentea* exhibited negative nitrogen balance in spite of high protein intake (Aroeira and Xavier 1990).

Recently, it has been suggested that low levels of condensed tannins (1–2% of DM) in temperate legumes of relatively high digestibility (i.e. *Lotus* spp.) may be nutritionally beneficial in circumstances where protein requirements of the animal are not met with basal forage diets (Terrill *et al.* 1992). In this study, when the legume mixture high in *F. macrophylla* was fed, the level of extractable condensed tannins in the diet was in the order of 1%. This low level of tannins seemed to be beneficial to the animal's nutrition, since it was associated with reduced excretion of N in urine, probably as a result of protein escaping ruminal degradation. However, increasing *F. macrophylla* in the legume mixture also resulted in reduced fibre digestibility, which could be attributed to condensed tannins as has been observed with temperate legumes (Barry and Duncan 1984; Barry *et al.* 1986; Terrill *et al.* 1989) and to non-astringent polyphenols. In a range of tropical shrub legumes, only 20–60% of the variation in *in vitro* dry matter digestibility within species could be explained by level of extractable or bound condensed tannins (CIAT, unpublished data). Therefore, we propose that future research should determine the relative impact of condensed tannins and other phenolics on cell wall digestibility of tropical shrub legumes.

Conclusions

Dilution of tannins through mixtures of sun-dried shrub legumes did not improve significantly the feed intake or nitrogen retention by sheep fed a low quality grass basal diet. Nitrogen excreted in urine was lower in animals supplemented with

the legume mixtures, suggesting that tannins from *F. macrophylla* partially protected ingested protein from being degraded in the rumen. However, this otherwise positive effect was offset by an increase in faecal N, and reduction of dry matter and fibre digestibility as *F. macrophylla* increased in the legume mixture. Future studies with legume mixtures should involve feeding fresh forage and measuring not only tannin levels in the legumes, but also their digestibility. Supplementation of mature tropical grasses or agricultural by-products with mixtures of tannin-free, highly digestible legumes and tannin-containing legumes could possibly combine the positive effects of tannins on protecting protein from ruminal degradation with the increased energy availability due to higher digestibility.

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