Reduction of tannin level in a tropical legume (*Desmodium ovalifolium*) with polyethylene glycol (PEG): effects on intake and N balance, digestion and absorption by sheep

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ABSTRACT: Three feeding trials were conducted to determine the effect of reducing the concentration of condensed tannins (CT) in *Desmodium ovalifolium* Wallick ex Gagnep (CIAT 350) on voluntary feed intake, N digestion, absorption and balance in sheep. Polyethylene glycol (PEG) was used as a tannin-binding agent to reduce extractable CT. In Trial 1, 12 growing lambs were assigned at random to one of four treatments: *ad libitum* feeding of *Centrosema macrocarpum* Bentham (CIAT 5713), *C. macrocarpum* plus PEG (50 g kg^{-1} DM), *D. ovalifolium*, or *D. ovalifolium* plus PEG (50 g kg^{-1} of DM). Extractable CT values were 46 and 17 g kg^{-1} for *D. ovalifolium* and *D. ovalifolium* plus PEG, respectively. Forage of *C. macrocarpum* contained 1.7 g kg^{-1} extractable CT and was not affected by PEG addition. Voluntary DM intake did not differ ($P > 0.05$) between *C. macrocarpum* treatments, but was higher ($P < 0.10$) for *D. ovalifolium* low in CT than for *D. ovalifolium* high in CT (23 vs. 19 g kg^{-1} BW d^{-1}). When *D. ovalifolium* high in CT was fed fecal N excretion (4.4 vs 2.8 g d^{-1}) was higher and N retained (1.3 vs. 4.3 g d^{-1}) was lower ($P < 0.10$). In Trial 2, three ruminally and duodenally fistulated sheep were used in a 3 x 3 latin square design. Treatments were restricted feeding of *D. ovalifolium* (Control, 41 g kg^{-1} CT), *D. ovalifolium* plus 3.5% - PEG (17 g kg^{-1} CT) or *D. ovalifolium* plus 7.0% - PEG (16 g kg^{-1} CT). Mean rumen NH_3 - N concentrations (mg dL^{-1}) were 5.2, 12.2, and 12.7 for the control, 3.5 - PEG and 7.0 - PEG diets, respectively. Fecal N was lower ($P < 0.05$) for the low CT diets, but apparent N absorption increased ($P < 0.10$) with increasing levels of extractable CT in the diet. In Trial 3, three sheep, fistulated at the rumen, duodenum, and ileum, were used in a switch-back design. Treatments were restricted fedding of *D. ovalifolium* (Control, 46 g kg^{-1} CT) and *D. ovalifolium* plus 5.0% - PEG (18 g kg^{-1} CT). Sheep fed forage high in extractable CT had higher ($P < 0.01$) N flux to the duodenum than those fed forage low in CT (8.3 vs. 5.9 g d^{-1}). Nitrogen absorption in the small intestine was also higher ($P < 0.05$) with the diet high in CT (3.0 vs 3.8 g d^{-1}). High levels of extractable CT in this tropical legume reduced voluntary intake and protein degradation in the rumen (i.e. less rumen ammonia), but in combination with feed restriction, increased N flow and absorption in the small intestine.

Key words: Condensed tannins, intake, nitrogen retention, rumen ammonia

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Reducción del nivel de taninos en una leguminosa tropical (*Desmodium ovalifolium*) con polietileno glicol (PEG): efectos sobre el consumo, balance de N, digestión y absorción en ovinos

RESUMEN: Se realizaron tres experimentos para determinar el efecto de la reducción de concentración de taninos condensados en *Dsemodium ovalifolium* Wallick ex Gagnep (CIAT 350) sobre el consumo voluntario, digestión, absorción y balance de N en ovinos. Se utilizó polietileno glicol (PEG) como agente de enlace para reducir los taninos condensados extractables (TCE). En el Expto. 1, se aleatorizaron 12 ovinos en crecimiento en cuatro tratamienos: alimentación *ad libitum* con *Centrosema macrocarpum* Bentham (CIAT 5713), *C. macrocarpum* + PEG (50 g kg^{-1} MS), *D. Ovalifolium* o *D. ovalifolium* + PEG (50 g kg^{-1} de MS). La cantidad de TCE fue de 46 y 17 g kg^{-1} para *D. ovalifolium* y *D. ovalifolium* + PEG, respectivamente; la de *C. macrocarpum* fue de 1.7 g kg^{-1} y no se cambió al adicionar el PEG. El consumo voluntario en MS no fue di-

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ferente (P > 0.05) entre los tratamientos con *C. macrocarpum*, pero fue mayor (P < 0.10) para el tratamiento con *D. ovalifolium* + PEG que con el de *D. ovalifolium* (23 vs. 19 g kg^{-1} peso vivo d^{-1}). Al alimentar los ovinos con *D. ovalifolium* alto en TCE, la excreción de N fecal fue mayor (4.4 vs 2.8 g d^{-1}), lo cual resultó en menor (P < 0.10) retención de N (1.3 vs. 4.3 g d^{-1}) que con la adición de PEG. En el Expto. 2, se usaron tres ovinos con fístulas ruminales y duodenales en un diseño de cuadrado latino 3 x 3. Los tratamientos fueron: alimentación restringida con *D. ovalifolium* (Control, 41 g kg^{-1} TCE), *D. ovalifolium* + 3.5% - PEG (17 g kg^{-1} TCE) o *D. ovalifolium* + 7.0% - PEG (16 g kg^{-1} TCE). La concentración media de NH_3 –N ruminal (mg dL^{-1}) fue de 5.2, 12.2 y 12.7 para el Control, 3.5 - PEG y 7.0 - PEG, respectivamente. El N fecal fue menor (P < 0.05) para las dietas bajas en TCE, pero la absorción de N aumentó (P < 0.10) al aumentar el nivel diético de TCE. En el Expto. 3 se utilizaron tres ovinos fistulados de rumen, duodeno e íleo, en un diseño de 2 filas x 3 columnas. Los tratamientos fueron: alimentación restringida con *D. ovalifolium* (Control, 46 g kg^{-1} TCE) o *D. ovalifolium* + 5.0% de PEG (18 g kg^{-1} TCE). El flujo de N al duodeno fue mayor (P < 0.01) en los ovinos alimentados con niveles altos que en aquellos que recibieron niveles bajos de TCE (8.3 vs. 5.9 g d^{-1}). La absorción de N en el intestino delgado fue también mayor (P < 0.05) con dietas altas en TCE (3.0 vs 3.8 g d^{-1}). Los altos niveles de TCE en esta leguminosa tropical redujeron el consumo voluntario y la degradación de proteína en el rumen (menos amoníaco ruminal), pero en asociación con alimentación restringida se incrementó el flujo y la absorción de N en el intestino delgado.

Palabras clave**:** Amoníaco ruminal, consumo, retención de nitrógeno, taninos condensados

Introduction

Evaluation of tropical legumes in the Centro Internacional de Agricultura Tropical (CIAT) has identified legume species adapted to acid soils of low fertility, but their nutritional value may be limited due to high levels of condensed tannins (CT).

Most of the research on CT in forage plants has been done with temperate legume species such as *Lotus* spp., and results indicate that their effect on ruminants varies depending on level in the diet. Low levels of CT increase N absorption in the small intestine by reducing rumen protein degradation (Waghorn *et al.,* 1987). In contrast, high levels of CT depress intake, protein and fiber digestion (Barry and Duncan, 1984; Barry and Manley, 1984). The level of CT in tropical legumes that affects intake and N utilization by ruminants has not been defined. Therefore, to implement an effective evaluation and selection program of tropical leguminous trees and shrubs, there is a need to understand how various levels of CT affect ruminants.

The herbaceous tropical legume *Desmodium ovalifolium* Wallick ex Gagnep, which is adapted to acid soils and contains high levels of tannins (6-7% of DM), was chosen as a model plant to study the effects of reducing tannin levels with polyethylene glycol (PEG) on intake and N utilization by sheep. *Centrosema macrocarpum* Bentham, an herbaceous legume adapted to acid soil and with nil levels of extractable CT, was included as a positive control to test if PEG *per se* influenced intake, digestibility, or N utilization when fed to sheep.

Materials and Methods

The study was conducted at CIAT´s Quilichao (Cauca, Colombia) Research Station (N 3°6', W 76°31') with an annual mean temperature of 23°C, 1772 mm rainfall, and elevation 990 m.

Forages. The herbaceous tropical legumes *C. macrocarpum* Bentham (CIAT 5713), virtually free of CT, and *D. ovalifolium* (CIAT 350), with high levels of CT, were used in the study. The legumes were planted during February, 1992 and homogeneous stands were obtained in September of the same year.

Animal management treatments and markers

Trial 1. Twelve growing African type wethers (BW = 19 ± 4 kg) were blocked by weight and assigned to one of four treatments: 1) *C. macrocarpum,* 2) *C. macrocarpum* with PEG (50 g kg^{-1} of DM), 3) *D. ovalifolium*, and 4) *D. ovalifolium* with PEG (50 g kg^{-1} of DM). Animals housed in individual metabolic crates were offered forage at 10% excess of their mean daily intake, established in a 10 d adjustment period. Water and a mineral mixture were offered free choice. Lambs were fitted with fecal collection bags and rubber urinary collection funnels on day 7 of the adjustment period. Total orts, feces, and urine were collected for an additional 10 days. Two mL of 9N H_2SO_4 were added to each 100 mL of urine to avoid ammonia loss.

Legumes were cut daily during the trial (Sept-Nov) and fresh chopped forage was sieved through a 2.5 cm^2 screen to remove stems. Sieved forage of each species, was sprayed (15 mL per 100g) with either water (control) or a 100 gL^{-1} PEG (MW 8000) solution, and fed once a day (09:00).

A sample of forage offered was collected daily and oven dried at 60°C to determine dry matter content. Frozen samples of forage, orts and feces were freeze-dried and composited daily by animal. Rumen liquor (10 mL) samples from each sheep were taken 1 h before (08:00) and 5 h after (14:00) feeding by rumen puncture with a #16 gauge needLe, on d 1, 5 and 10 of the measurement period. Twenty µL of 9N H_2SO_4 were added to each mL of rumen liquor collected and approximately 5 mL were kept and frozen for NH_3 – N analysis.

Trial 2. Three growing African-type wethers (BW = 20 ± 2.8 kg) fitted with ruminal and duodenal cannulas were

allocated to one of three treatments in a 3 x 3 latin square design. Treatments were: *D. ovalifolium* (Control), *D. ovalifolium* with PEG added at 35 g kg^{-1} DM (35-PEG), and *D. ovalifolium* with PEG at 70 g kg^{-1} DM (70 - PEG). Animals, housed in individual metabolic crates were offered daily 50 g DM/kg $BW^{.75}$ in two meals, at 08:30 and 16:30. Animals were fitted with fecal collection bags on day 7 of the adjustment period. Total feces and orts were collected for an additional 5 d for a total experimental length of 12 d per period. Sheep were allowed to graze for 1 week in a pasture of *Brachiaria humidcola* between experimental periods.

Cut and chopped forage was sprayed (15 mL 100 $g^{-1}$of fresh forage) either with water, 70 gL^{-1}, or 140 gL^{-1} PEG solution, applied before feeding. DM of fresh forage was estimated to be 30%. Afternoon meals were kept in a refrigerator at 5°C.

Marker administration, sampling of digesta and estimation of digesta flow were performed following the guidelines of Faichney (1993). Liquid and particulate markers were administered during the entire experimental period. Twenty mL of a 5 gL^{-1} solution of Cr-EDTA (liquid marker) and 20 mL of a 0.5 gL^{-1} solution of $YbCl_3\,6H_2O$ (particle marker) were delivered into the rumen every hour in pulse doses. The Cr-EDTA crystals were prepared as suggested by Udén *et al.* (1980). Samples of rumen fluid from ventral sac, rumen digesta from dorsal sac, and duodenal digesta were collected every 4 h for a period of 12 h on d 8, 10, and 12. Sampling started 1 h before the morning meal. Rumen fluid (70 mL) and duodenal digesta (50 mL) were collected at each sampling time. Samples from each animal were composited by day for subsequent analysis. On d 10, additional rumen fluid (5 mL) was collected every 2 h for 24 h, and samples acidified with a 9N H_2SO_4 solution (100 µL), were used for NH_3-N analysis. Five hundred mL of rumen fluid from each sheep were collected at the end of each measurement period and used to isolate bacteria.

Forage and fecal samples were collected daily and one subsample was frozen and another oven-dried at 60°C to determine DM. Samples of forage, orts and feces were freeze-dried and composited by animal for subsequent analysis.

Trial 3. Three African-type wethers (BW = 18 ± 4 kg) fitted with ruminal, duodenal and ileal cannulas were arranged in an incomplete switch-back design (Lucas, 1974) with two treatments and three experimental periods. Treatments were *D. ovalifolium* with no PEG (Control) and *D. ovalifolium* plus PEG (50 g kg^{-1} of DM). Measurements were repeated in three periods in which sheep were on alternate treatments. Animals were accustomed to the diets for a 7-d period followed by a 5-d collection period.

Schedules were as in Trial 2, but ileal samples were also collected. Daily feed intake was restricted to 50 g DM/kg $BW^{.75}$. Forage was managed as in Trial 2, but discarding stems from the chopped legume as was described for Trial 1.

Laboratory analysis

Trial 1. Freeze-dried samples of forage, orts and feces were ground through a 1-mm screen in a Wiley mill. Samples were analyzed for ash, NDF, and ADF (Van Soest *et al.*, 1991), total phenols (Swain and Hillis, 1959), and Kjeldahl N (AOAC, 1975). Sodium sulfite was added to the NDF solution in order to partially remove tannin-protein complexes (Robbins *et al.,* 1987). Samples of rumen liquor were centrifuged and ammonia (NH_3-N) determined by the indophenol method of McCullough (1967). Urine was analyzed for Kjeldahl N (AOAC, 1975).

Condensed tannins (extractable, bound to protein and bound to fiber + PEG) in feed, orts, and feces were determined according to the butanol/HCl procedure suggested by Terrill *et al.* (1992) with some modifications. Sample size was reduced to 50 mg, and all reagents were reduced proportionally. Extractable CT were extracted with an aqueous methanol (700 mL L^{-1}), formic acid (5 mL L^{-1}), and ascorbic acid (0.5 gL^{-1}) solution (Telek, 1989). Standards were prepared as suggested by Terrill *et al.* (1992) and stock solutions were prepared from purified tannins of *D. ovalifolium.*

Purification of tannins was based on the procedure for Quebracho tannins proposed by Asquith and Butler (1985) modified by A. Hagerman (unpublished). Lyophilized leaves of *D. ovalifolium* (4 g DM) were extracted with 40 mL of an aqueous methanol (700 mL L^{-1}) solution for 1 h in a shaker. The sample was centrifuged (600 x g) and the pellet discarded. Methanol was partially evaporated by vortexing the supernatant in a water bath at 30° C and the solution was filtered and freeze-dried. Freeze-dried material was dissolved in aqueous ethanol (500 mL L^{-1}) solution, and the solution kept overnight in a cooler at 4-5° C, and then centrifuged. Tannins were separated using Sephadex LH-20 (Asquith and Butler, 1985). Purified tannins were freeze-dried and kept in a cooler in a desiccator. The capacity of tannins to bind protein (astringency) was determined by the radial diffusion method of Hagerman (1987), as modified by Lareo *et al.* (1990).

Trials 2 and 3. Duodenal and ileal samples were separated into two subsamples. One of these was separated into liquid and solid fractions by centrifugation at 600 x *g*. The solid fractions and the second subsample were freeze-dried and DM content determined. Duodenal, ileal and fecal samples were composited daily for each animal. All samples were analyzed for Kjeldahl-N (AOAC, 1975); NH_3 -N (McCullough, 1967); Cr (Costigan and Ellis, 1987); Yb (Karimi *et al*., 1986); and CT (Terrill *et al.*, 1992) as described for Trial 1. Forage offered, whole duodenal and fecal samples were analyzed for indigestible acid detergent fiber (IADF) (Waller *et al.,* 1980).

Digesta flows were estimated using Yb and IADF as particulate markers. Flows estimated with Yb were highly correlated (r = 0.95) to those estimated with IADF, so flows reported in this paper are the average of both markers. Cr - EDTA values were not used, due to low recovery of Cr in

the liquid fraction, since a large proportion of the Cr was found in the digesta particulate matter (data not shown).

Purines were determine in duodenal samples to estimate microbial N (Zinn and Owens, 1986). RNA from torula yeast type II-C from SIGMA (R-6875) was used as a standard. Ratio of N:RNA of bacteria flowing to the duodenum was estimated for each treatment from bacteria isolated form rumen fluid by differential centrifugation. Rumen fluid was centrifuged at 600 x *g* twice and the pellet discarded. The supernatant was centrifuged at 25,000 x *g* and the pellet was resuspended in saline solution (0.9% NaCl) and centrifuged at 25,000 x *g*. Finally, the supernatant was discarded and the pellet resuspended in distilled water and freeze-dried. Pelleted bacteria were analyzed for Kjeldalh-N (AOAC, 1975) and purines (Zinn and Owens, 1986). The ratio of rumen bacteria N to RNA equivalent was calculated and used to estimate the proportion of bacterial N in duodenal samples.

Data analysis

Trial 1. Data were analyzed as a 2x2 factorial arrangement in which legumes and PEG treatment were the main factors. The general linear model procedure of SAS (1990) was used and differences between means were tested using multiple way comparison where only pre-planned comparisons were considered (*C. macrocarpum vs. C. macrocarpum* + PEG, *D. ovalifolium vs D. ovalifolium* + PEG and Control vs. PEG).

Trial 2. Data were analyzed as a 3x3 latin square design and the least significant difference (LSD) procedure of SAS (1990) was used to compare treatment means. Correlation analyses between the levels of extractable tannins and N excretion (feces) and N flow to the duodenum were performed.

Trial 3. Data were analyzed as an unbalanced switch-over design (Lucas, 1974) with three experimental periods, three animal and two treatments. Sheep alternated treatments each period. In period one, only two sheep were used which resulted in an unbalance design. Due to unbalance, partial sums of squares (Type three of SAS) were used to adjust the treatment effects by the bias of the data. General lineal model (GLM) procedure of SAS (1990) was used, with the Least Significant Difference (LSD) test to compare treatment means.

Results

Trial 1

Forage quality. Crude protein content (212 vs 125 g kg^{-1}) was higher for *C. macrocarpum* than for *D. ovalifolium*, while the fiber fractions NDF and ADF were lower in *C. macrocarpum* (452 and 344 g kg^{-1}) than in *D. ovalifolium* (514 and 402 g kg^{-1}, respectively).

Effect of PEG addition on tannin level. There was no effect of PEG addition on total phenols or total CT in *C. macrocarpum* (Table 1). However, addition of PEG to *D. ovalifolium* decreased total phenols by 53% and tannin astringency by 85%. PEG treatment reduced the extractable (62%) and protein bound (30%) tannin fractions, while increasing the residual (200%) tannin fraction (that bound to both fiber and PEG).

Effect of tannins on intake and mitrogen retention. Voluntary DM intake (g kg^{-1} BW) of *C. macrocarpum* was not affected by PEG addition (Table 2). In contrast, reduction of extractable CT in *D. ovalifolium* with PEG resulted in a 21% increase ($P < 0.10$) of DM intake.

Nitrogen intake and excretion (fecal and urinary) in animals fed *C. macrocarpum* was not affected by PEG (Table 2), which suggests that PEG by itself does not alter N metabolism in sheep. However, addition of PEG to *D. ovalifolium* reduced ($P < 0.10$) fecal N excretion, but had no effect on N-urine excretion. Therefore, N retained (g d^{-1}) and N use efficiency (retained as a proportion of intake) were higher ($P < .10$ and $P < 0.01$, respectively) for sheep fed *D. ovalifolium* with a low level of extractable CT (Table 2).

Table 1. Effect of adding polyethylene glycol (PEG) to two tropical legumes fed to sheep on total phenols, condensed tannin fractions and astringency (reactivity with proteins) (Trial 1).

Item	*C. macrocarpum*		*D. ovalifolium*	
	Control	50-PEG[1]	Control	50-PEG[1]
Total phenols, g kg^{-1} DM	36	35	91	43
Condensed tannins, g kg^{-1} DM				
Extractable	1.7	1.7	45.5	17.3
Protein bound	0.8	0.6	16.6	11.6
Residual (Fiber + PEG)	2.2	2.3	5.4	16.2
Total	4.7	4.6	67.5	45.1
Astringency[2], g BSA bound kg^{-1} DM	ND[3]	ND	83.0	12.3

[1]g of PEG (MW 8000) kg^{-1} DM.
[2]Radial diffusion assay using Bovine Serum Albumin.
[3]ND = Ring not detected.

Table 2. Dy matter (DM) and nitrogen (N) intake, excretion and retention by sheep fed two tropical legumes with or without polyethylene glycol (PEG) (Trial 1).

Item	*C. macrocarpum*		*D. ovalifolium*		SEM	Significance[1]		
	Control	50-PEG[2]	Control	50-PEG[2]		Cm vs. Cm+PEG	Do vs Do+PEG	Control vs. PEG
Intake, g DM kg^{-1}BW d^{-1}	25	23	19	23	1.4	NS	+	NS
Intake, g N d^{-1}	18.1	17.3	8.6	10.2	1.1	NS	NS	NS
Feces, g N d^{-1}	4.2	4.1	4.4	2.8	0.5	NS	+	NS
Urine, g N d^{-1}	6.4	5.0	2.4	3.1	0.5	NS	NS	NS
Retained, g N d^{-1}	7.5	8.2	1.7	4.3	0.8	NS	+	+

[1]NS = not significant; + = P < 0.10.
[2]g of PEG (MW 8000) kg^{-1} DM.

Table 3. Effect of adding polyethylene glycol (PEG) to *Desmodium ovalifolium* on condensed tannin fractions and tannin astringency (reactivity with protein).

	Control	35-PEG[1]	70-PEG[1]
Trial 2			
Condensed tannins, (g kg^{-1} DM)			
Extractable	4.1 ±.58[2]	1.7 ±.21	1.6 ±.27
Protein-bound	1.5 ±.02	1.2 ±.09	1.0 ±.11
Residual[3]	0.6 ±.04	2.0 ±.41	2.4 ±.23
Total	6.2 ±.44	4.9 ±.71	5.0 ±.26
Astringency[4], (g BSA bound kg^{-1} DM)	105 ± 13	26 ± 6	ND[5]
	Control	50-PEG[1]	
Trial 3			
Condensed tannins, (g kg^{-1} DM)			
Extractable	4.6 ±.01	1.8 ±.06	
Protein-bound	1.8 ±.19	1.2 ±.06	
Residual[3]	0.6 ±.10	1.7 ±.07	
Total	7.0 ±.21	4.7 ±.14	
Astringency[4], (g BSA bound kg^{-1} DM)	8.8 ± 1.3	1.2 ± 1.1	

[1]g of PEG kg^{-1} DM.
[2]Standard Deviation estimated from CT in forage of each experimental period.
[3]Condensed tannins bound to fiber and PEG.
[4]Radial diffusion assay using Bovine Serum Albumin (BSA).
[5]Ring not detected.

Polyethylene glycol added to *C. macrocarpum* did not affect rumen NH_3 - N levels (36 vs. 37 mg dL^{-1}) but increased rumen NH_3-N when added to *D. ovalifolium* (12 vs. 4 mg dL^{-1}) particularly in the first 6 h post-feeding.

Trials 2 and 3

Forage quality. Chemical characteristics of *D. ovalifolium* forage offered to sheep were similar in Trials 2 and 3. However, higher CP (120 vs. 112 g kg^{-1} DM) and slightly lower NDF (530 vs. 540 g kg^{-1} DM) and ADF (400 vs. 420 g kg^{-1} DM) content were observed in Trial 3, when stems were removed from the forage. Nitrogen bound to NDF accounted for 17% and 15%, while nitrogen in ADF (unavailable N) accounted for 10 and 8 % of the total plant N in Trials 2 and 3, respectively.

Effects of PEG addition on tannin level. The addition of PEG to *D. ovalifolium* forage decreased the level of extractable CT and increased the residual (fiber + PEG) bound CT fraction in Trials 2 and 3, but there was no effect of PEG on protein bound CT (Table 3).

Nitrogen flow and absorption. In Trial 3, total N and non-ammonia non-microbial N (NANMic - N) flow to duodenum and fecal N excretion were lower (P < 0.05) with lower levels of extractable CT in the diet (Table 4). Apparent efficiency of N absorption (g N absorbed/g N duode-

Table 4. Effect of adding polyethylene glycol (PEG) to *Desmodium ovalifolium* on nitrogen (N) intake, flow, and apparent absorption by sheep (g N d^{-1}).

Item	Control	35-PEG[1]	70-PEG[1]	SEM
Trial 2				
Intake	8.9	8.8	8.9	0.02
Duodenal flow	9.1[a2]	6.2[b]	5.1[b]	0.40
Microbial flow	3.8	3.4	3.4	0.03
NANMic-N[3] flow	5.2[a]	2.7[b]	1.6[b]	0.40
Fecal excretion	4.4[a]	2.7[b]	2.4[b]	0.10
Absorbed	4.7[a]	3.4[b]	2.7[b]	0.40
	Control	50-PEG[1]	SEM	
Trial 3				
Intake	7.9	7.6	0.5	
Duodenal flow	8.3[a]	5.9[b]	0.3	
Microbial flow	3.6	3.4	0.3	
NANMic-N[3] flow	4.6[a]	2.5[b]	0.1	
Ileal flow	4.5[a]	3.0[b]	0.2	
Absorbed	3.8[a]	3.0[b]	0.2	

[1]g of PEG kg^{-1} DM.
[2]Means within a row with different superscripts are different ($P < 0.05$).
[3]NANMic-N = Non-ammonia non-microbial nitrogen.

num) did not differ ($P = 0.67$) among treatments (data not shown). Rumen NH3 - N concentrations varied with time and among animals but were consistently lower ($P < 0.001$) in the forage high in extractable CT than in the forage low in this fraction (Figure 1A). Mean rumen NH_3 - N concentration across sampling times were 5.2, 12.2 and 12.7 mg dL^{-1} in the control, 3.5-PEG and 7.0-PEG treatments, respectively.

In Trial 3, flows of total N and of NANMic-N to the duodenum were lower ($P < 0.05$) when the concentration of extractable CT was reduced by adding PEG to the legume (Table 4). However, microbial N flow did not differ among treatments. Nitrogen flow to the ileum and N absorbed in the small intestine were greater ($P < 0.05$) for *D. ovalifolium* with higher levels of extractable CT (Table 4). As in Trial 2, mean rumen NH_3 - N concentration was lower ($P < 0.001$) for the *D. ovalifolium* high-extractable-CT control diet (Figure 1B).

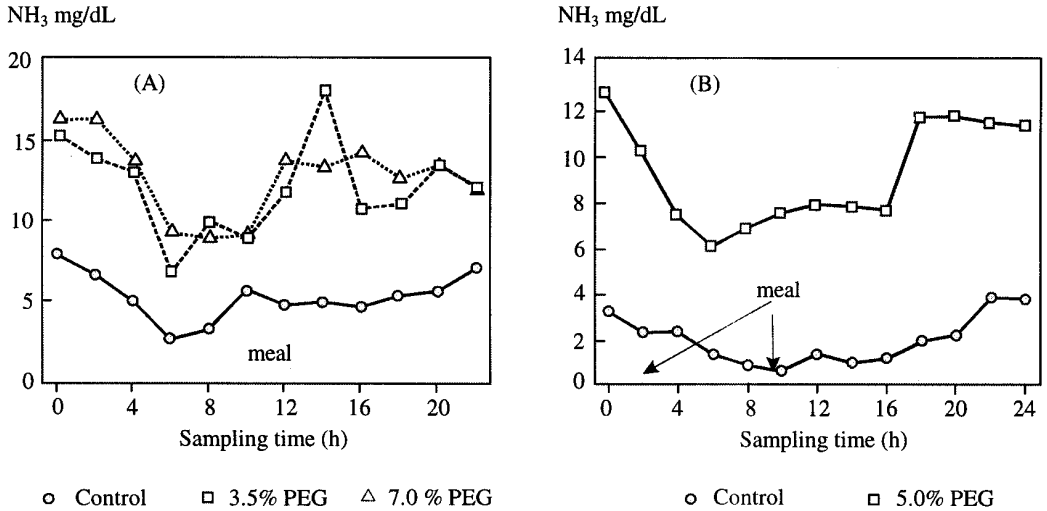

Figure 1. Rumen ammonia concentrations (mg dl^{-1}) in sheep fed *D. ovalifolium* with and without PEG. (A) Trial 1: Control (○), PEG 35 g kg^{-1} DM (□) and PEG 70 g kg^{-1} DM (△). (B) Trial 2: Control (○) and PEG 50 g kg^{-1} DM (□).

Discussion

In these trials, PEG was used as a tannin binding agent to reduce the levels of extractable CT present in *D. ovalifolium*; also the effects of reducing CT on N utilization by sheep were studied. In all cases, extractable CT were reduced by the addition of PEG to *D. ovalifolium*. The addition of PEG to forage *C. macrcarpum* did not influence DM intake, or N balance in the control legume. Therefore, it can be assumed that differences in response variables between *D. ovalifolium* treated or not with PEG are due mainly to the reduction of extractable CT in the forage fed to sheep.

The accuracy of digesta flow measurements depends on recovery of indigestible markers and valid flow calculation procedures for the liquid and solid phases of digesta. Unfortunately, in our study the liquid marker (Cr-EDTA) did not perform to expectations due to incomplete recovery in the liquid phase. Therefore, digesta flow values were estimated using the particulate markers (Yb and IADF) as the only reference point for flow calculation; as a result, flow values presented in Table 4 may be inaccurate. However, the flow data may still be useful because relative differences between treatments may indicate important biological effects.

Lower rumen NH_3 - N was observed in sheep fed *D. ovalifolium* with high extractable CT (Figure 1). This could be explained by the capacity of extractable CT to bind protein and form insoluble complexes resistant to microbial degradation at rumen pH (Jones and Mangan, 1977). Lower ammonia concentrations observed with the legume forage high in extractable CT indicate that less protein was degraded in the rumen. This in turn resulted in increased flow of plant protein to the duodenum, as indicated by the higher NANMIC-N flows in the high-tannin control treatments in Trials 2 and 3 (Table 4). On the other hand, microbial growth could have been limited due to the low concentrations of rumen NH_3-N observed with the high tannin forage diets, especially so in Trial 3 when NH_3 - N levels in the post-feeding period were below the suggested levels for maximal microbial growth (Satter and Slyter, 1974). However, there were no statistical differences between treatments in microbial flow to the duodenum (Table 4).

Total N flow to the duodenum and N absorbed in the small intestine were higher for *D. ovalifolium* high in extractable CT. However, fecal N excretion increased as the level of extractable CT increased in the diet ($r = 0.86$, $P < 0.001$) and this increase was related to N flow to duodenum ($r = 0.96$, $P < 0.0001$). Other studies with temperate legumes have also shown that tannins protect protein from being degraded in the rumen and thereby increase protein flow to the small intestine and amino acid absorption (Barry and Manley, 1984; Waghorn *et al.*,1987). Increased N flow to the lower GI tract has also been associated with increased fecal N when temperate legumes high in tannins are fed (Egan and Ulyatt, 1980; Barry *et al.*, 1986; Terrill *et al.*, 1989), as was observed in the present studies.

Interestingly, when *D. ovalifolium* with low extractable CT was fed *ad libitum* (Trial 1), there was greater N retention in sheep. This finding is not in agreement with the lower N absorption in the small intestine when intake of *D. ovalifolium* was restricted (Trials 2 and 3). Low tannin content in temperate legumes offered *ad libitum* (i.e. grazing) has been associated with increased animal performance. For example, Barry (1985) and Pritchard *et al.* (1988) reported greater liveweight gains and wool growth with sheep offered temperate legumes with low levels as compared with high levels of CT. Therefore, we suggest that the benefits of higher N absorption that were observed with *D. ovalifolium* high in extractable CT (Trials 2 and 3) could be offset by reduced DM intake (Trial 1). When the level of extractable CT in *D. ovalifolium* was reduced with PEG, there was a 21% increase in DM intake by sheep offered the legume *ad libitum* (Trial 1).

Conclusions

High levels of CT in *D. ovalifolium* in combination with restricted intake increased N flow and absorption from the small intestine of sheep, as has been shown to occur with temperate legumes. However, high levels of extractable CT also result in lower rumen ammonia levels and lower *ad libitum* DM intake. On balance this may have negative implications in tropical livestock feeding systems based on roughage diets low in protein and high in fiber and where legumes high in tannins are used as protein supplements.

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