Intake, digestibility and nitrogen utilization by sheep fed with provenances of *Calliandra calothyrsus* Meissner with different tannin structure

C. Lascano¹, P. Avila¹ and J. Stewart²

**ABSTRACT:** A trial with sheep (housed in metabolic cages, and fitted with ruminal and duodenal cannulas) fed sun-dried forage of two *Calliandra calothyrsus* provenances (San Ramon — CIAT 22310 and Patulul CIAT—22316) harvested in sites with contrasting soil fertility was carried out to further define the biological significance of the different chemical structure in the tannin of *Calliandra* provenances. Six African-type sheep were assigned to one of four treatments (T1: San Ramón grown in Quilichao with infertile soils, T2: Patulul grown in Quilichao with infertile soils, T3: San Ramón grown in Palmira with fertile soils, and T4: Patulul grown in Palmira with fertile soils) arranged in an Unbalanced Simple Crossover Design. Results showed that extractable tannins in Patulul comprised mainly procyanidin subunits, whereas the tannin fraction in San Ramón was composed largely of prodelphinidin subunits. The DM intake and digestibility of *Calliandra* were greater (P<0.05) with San Ramón than with Patulul. Intake was also greater (P<0.05) with provenances harvested in the site with fertile soil than in the site with acid soils. Absolute and relative values of ruminal escape dietary N in sheep were greater (P<0.05) with San Ramón than with Patulul, which is consistent with laboratory results on tannin astringency. Thus for the first time we have evidence suggesting that the chemical structure of condensed tannins in tropical legumes can have an effect on N utilization by ruminants. The fact that in vivo results on ruminal escape N were in close agreement with results on astringency of extractable condensed tannins from *Calliandra* provenances validates the use of laboratory astringency tests for screening tropical legumes with tannins for quality traits.

Key words: Woody legumes, astringency, ruminal escape N, delphinidin, cyanidin, and sheep


Consumo, digestibilidad y utilización de nitrógeno por ovinos alimentados con procedencias de *Calliandra calothyrsus* Meissner on taninos de diferente estructura química

**RESUMEN:** Para poder definir mejor el significado biológico de la diferente estructura química de los taninos de dos procedencias (San Ramon - CIAT 22310 y Patulul - CIAT 22316) de *Calliandra calothyrsus* se realizó un ensayo de alimentación con ovinos fistulados en el rumen y duodeno, alojados en jaula metabólica y alimentados con forraje seco al sol de las dos procedencias de *Calliandra* cosechadas en sitios con suelos de fertilidad contrastante. Seis ovinos de pelo tipo Africano se asignaron al azar a 4 tratamientos (T1: -San Ramón cosechada en Quilichao con suelos de baja fertilidad, T2: -Patulul cosechada en Quilichao con suelos de baja fertilidad, T3: -San Ramón cosechada en Palmira con suelos fértiles, y T4: -Patulul cosechada en Palmira con suelos fértiles) dispuestos en un diseño Reversible Simple no Balanceado. Los resultados mostraron que el consumo de MS y la digestibilidad de *Calliandra* fueron mayores (P<0.05) con la procedencia San Ramón que con la procedencia Patulul, y que el consumo también fue mayor (P<0.05) con el forraje cosechado en el sitio con suelos más fértiles. Los valores absolutos y relativos de proteína del forraje que escapo degradación en el rumen (proteína de escape o sobrepasante) fueron mayores (P<0.05) con la procedencia San Ramón que con Patulul, lo cual es consistente con resultados de astringencia de los taninos de las dos procedencias medida en el laboratorio. Por lo tanto, por primera vez tenemos evidencia que sugiere que la estructura química de los taninos de leguminosas tropicales puede tener un efecto en la utilización de nitrógeno por rumiantes. El hecho de que los resultados de proteína sobrepasante medidos in vivo estuvieran de acuerdo con los resultados de astringencia de taninos de las procedencias de *Calliandra* determinadas en el laboratorio, valida el uso de estos métodos de astringencia para la selección de leguminosas tropicales por calidad nutritiva.

Palabras clave: Leguminosas arbustivas, astringencia, proteína sobrepasante, delphinidina, cyanidina

¹Centro Internacional de Agricultura Tropical (CIAT), Tropical Forage Project, Cali, Colombia
²Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, Oxford, UK
Introduction

The evaluation and selection of shrub legumes as a feed resource for livestock is an area of interest in the tropics, because of the direct contribution that fodder from trees and shrubs can make to the livelihoods of resource-poor farmers. Nitrogen-fixing leguminous species, in particular, are generally high in protein and provide a valuable supplementary feed, particularly during the dry season when basal feeds such as grasses and crop residues are of extremely low nutritive value. Improved production from dual-purpose cattle (milk and meat) is a direct result of better animal nutrition, and this in turn has a direct impact on livelihoods, through both income generation and improved family nutrition.

For these reasons, research on fodder shrubs is receiving high priority in CIAT’s Tropical Forages Project. Over the last three years we have been evaluating the nutritional characteristics and feed value of two contrasting provenances of Calliandra calothyrsus Meisner (Calliandra) as part of a collaborative study between the Oxford Forestry Institute (OFI) and the Centro Internacional de Agricultura Tropical (CIAT).

It is well documented that Calliandra, shrub native to Central America, is adapted to acid, low-fertility soils with high levels of Al saturation and produces high yields of edible biomass rich in protein (Palmer et al, 1994). However, one limitation of Calliandra as a source of fodder is the high concentration of condensed tannins (CT) in the edible forage, which have been associated with low palatability and digestibility (Jackson et al, 1996; Larbi et al, 1998).

To address some of the questions related to the feed value of Calliandra, together with OFI we carried out a detailed characterization of the chemical composition of the edible forage of two Calliandra provenances, San Ramón (CIAT 22310) and Patulul (CIAT 22316), harvested in a location with acid, low-fertility (CIAT’s Quilichao Research Station) and in a location with fertile and neutral soils (CIAT’s Palmira Research Station) and in a location with fertile and neutral soils (CIAT’s Palmira Research Station) in the Cauca Valley, Colombia. An interesting finding was that the proanthocyanidin (extractable condensed tannin fraction) structure varied between proveniences when measured using high performance liquid chromatography, regardless of location (Lascano et al, unpublished). The extractable condensed tannins (ECT) in Patulul were comprised mainly of catechin / epicatechin subunits (producing procyanidin on treatment with butanol / HCl), whereas in San Ramón the ECT were mainly gallo-catechin / epigallocatechin (producing prodelpphinidin with butanol / HCl) (Lascano et al, unpublished). Similar results were found earlier with samples of the same provenances grown in a greenhouse at the Plant Environment Laboratory, Department of Agriculture, University of Reading, UK (Stewart et al, 2000).

A feeding trial was carried out with sheep housed in metabolism crates and fed with forage harvested in two sites with contrasting soil fertility to determine the biological significance of the different chemical structure found in the ECT fraction of two Calliandra proveniences.

Materials and Methods

The feeding experiment was conducted in Colombia, South America at the CIAT-Quilichao Research Station. This station is located at lat 3° 6' N, long 76° 31' W, at 990 m above sea level.

Forage and Animal Management. The two Calliandra calothyrsus provenances (San Ramón - CIAT 22310 and Patulul - CIAT 22316) selected in OFI were grown in CIAT’s Palmira Research Station with fertile soils (Vertisol: pH 8.2, MO 4.4%, P 55 ppm, Ca 20.5 meq 100 g^-1, Mg 8.03 meq g^-1 and K 0.61 meq g^-1) and in CIAT’s Quilichao Research Station with acid, low-fertility soils (Ultisol: pH 3.8, Al 4.3 meq g^-1, MO 8.0%, P 5.8 ppm, Ca 0.44 meq g^-1, Mg 0.05 meq g^-1). Plants were cut after 6 months and edible material (leaf + non-lignified stem) was collected from each provenance at each site and sun-dried for 48 h. Previous results (Lascano et al, unpublished) from a short term intake trial had shown no differences in intake between dry and fresh Calliandra forage. The sun-dried material was placed in paper bags and stored at room temperature in a ventilated room prior to feeding to sheep housed in metabolism crates.

Six African-type sheep (BW 30 ± 4 kg) were fitted with flexible ruminal and intestinal cannulas from Akon (distributed by Bar Diamond Inc., Parma, ID). The surgical procedures used were those described by Balch and Cowie (1962) and by Streeter et al (1991) for rumen and intestinal cannulation, respectively. Animals were housed in metabolism crates with head gates and assigned to one of the following four treatments:

- T1: San Ramón (CIAT 22310) grown in Quilichao,
- T2: Patulul (CIAT 22316) grown in Quilichao,
- T3: San Ramón (CIAT 22310) grown in Palmira,
- T4: Patulul (CIAT 22316) grown in Palmira.

Sheep were offered daily 50 g DM per kg BW^{0.75} (1.7 % of body weight) of sun-dried edible (leaves + fine stems) Calliandra forage divided between two meals (08:00 and 15:00). The six animals used in the trial were distributed across the four treatments (T) in an unbalanced simple crossover design with four experimental periods, so that each animal received all four treatments over the course of the experiment. Each experimental period lasted for 17 days (7 days for adjustment and 10 days for collections). After each experimental period, the sheep were allowed to graze Brachiaria humidicola.
pasture for one week.

All animals were intraruminally supplemented with 4 g kg of BW⁻¹ d⁻¹ of starch-extracted cassava meal as a constant source of readily fermentable carbohydrates. Animals were also offered water 4 times a day and had ad libitum access to a mineral mix (NaCl, 40%; Ca, 10.8%; P, 10%; K0.1%; Mg, 0.3%; S, 3%; Cu, 0.15%; Zn, 0.6%; I, 0.03%; and Co, 0.05%) ad libitum.

Fecal collection bags were placed on the animals on day 5 of the adjustment period. Total feces to determine digestibility of DM, NDF and N were collected for 10 days and weighed daily. On each sampling day, a subsample (100 g) was dried in a forced-air oven at 60°C for DM determination and another subsample (100 g) was frozen at −20°C for subsequent freeze drying and chemical analysis. Samples of ruminal fluid (20 mL) and duodenal digesta (100 mL) were collected at 6-hour intervals during days 8 and 9 of the collection period. On the last day (day 10) of the collection period ruminal samples (500 mL) were collected to isolate ruminal bacteria. Ruminal and duodenal digesta samples were frozen at −20°C for subsequent analysis. The ruminal fluid was acidified with 18.6 N H₂SO₄ (0.02 mL per mL of ruminal fluid) to prevent ammonia volatilization.

Forage offered to and refused by each animal was weighed daily during the collection period and subsamples were taken for DM determination and subsequent analysis. Orts were removed at 8:00 for weighing. Forage offered was combined across days into a single composite sample for each period, whereas forage refused and feces were combined across days within each period for each animal separately. Ruminal fluid samples collected in each period for bacterial isolation by differential centrifugation were combined across animal within the same treatment.

Laboratory Analysis. Freeze-dried samples of forage, orts and feces were ground through a 1 mm screen in a Wiley mill. Extractable condensed tannins (ECT) in the forage offered were analyzed using the Butanol HCl method (Terrill et al., 1992) with modifications by Carulla et al. (2001). Samples size was reduced to 10 mg, and all reagents were reduced proportionally. The relative proportions of cyanidin and delphinidin in the two provenances of Calliandra harvested in Palmira and Quilichao were measured by high-performance liquid chromatography (HPLC) using the method developed at CIAT and described by Stewart et al. (2000). The peaks were identified using delphinidin chloride and cyanidin chloride, which had retention times of 10.0 and 11.3 minutes, respectively. There is general agreement that anthocyanins with minor differences in structure do not vary appreciably in absorbance (Jurd, 1962). Thus the ratio of the peak heights can be assumed to give the procyanidin: prodelphinidin ratio directly. Astringency of tannins (ability to bind protein) was estimated by the radial diffusion method of Hagerman (1987) using BSA (Bovine Serum Albumin) as the protein source.

Forage (offered and refused) and fecal samples were analyzed for Kjeldahl N (AOAC, 1995), in vitro DM digestibility (IVDMD; Tilley and Terry, 1963), and fiber (NDF and ADF) using the method of Van Soest et al. (1991). Concentration of indigestible acid detergent fiber (IADF) was measured in the forage offered, refused and feces according to Waller et al. (1980) and was used as an internal marker to estimate flows of the solid phase of digesta to the duodenum.

Purines measured in bacterial isolates from ruminal and duodenal digesta were used to estimate bacterial N flow to the duodenum (Zinn and Owens, 1986). The ratio of N: RNA of bacteria flowing to the duodenum was estimated for each treatment from bacteria isolated from the ruminal fluid by differential centrifugation. The ratio of ruminal bacteria N: RNA equivalent was used to estimate the proportion of bacterial N in duodenal samples. Escape N was estimated as duodenal N flow minus microbial N flow and endogenous N flow, which was considered to be proportional to DM intake (2.2 g N/ kg DM intake; Hart and Leibholz, 1990).

Statistical Analysis. Data were analyzed by ANOVA for an unbalanced simple crossover design with four periods using the General Linear Models Procedure of SAS (1990). Carry-over effects from previous treatments were found to be not significant (P>0.05). Duncan's multiple range test was used to separate means.

Results and Discussion

Chemical composition-Provenance effects. Results on chemical composition of the forage of Calliandra offered are shown in Table 1. The concentration of crude protein (CP) did not differ (P>0.05) between provenances. In contrast, fiber (NDF and ADF) concentration differed between Calliandra provenances, being higher (P<0.05) in San Ramón than in Patulul, regardless of site.

In other studies carried out in CIAT (Lascano et al., unpublished), fiber content measured as NDF was also higher in San Ramón than in Patulul, and was not affected by drying method or by location, which is in agreement with results found at the University of Reading with the same provenances (Stewart et al., 2000). The higher fiber concentration in San Ramón was associated with lower IVDMD as compared with Patulul, which again is consistent with results recorded in plants grown in a green house at the University of Reading (Stewart et al., 2000). However, the absolute IVDMD values were considerably lower (P<0.05) in samples
Boyazoglu y Nardone

Table 1. Chemical characterization of Calliandra calothyrsus provenances fed to sheep housed in metabolism crates

<table>
<thead>
<tr>
<th>Calliandra (Provenances and Site)</th>
<th>Crude Protein (% of DM)</th>
<th>In Vitro DM Digestibility (%)</th>
<th>NDF (% of DM)</th>
<th>ADF (% of DM)</th>
<th>Condensed Tannins Extractable (% of DM)</th>
<th>Condensed Tannins Insoluble (% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Ramón — Quilichao</td>
<td>15.5 b</td>
<td>20.1 c</td>
<td>35.0 a</td>
<td>32.1 a</td>
<td>28.3 b</td>
<td>6.3 a</td>
</tr>
<tr>
<td>San Ramón — Palmira</td>
<td>17.0 a</td>
<td>32.2 b</td>
<td>36.7 a</td>
<td>29.6 a</td>
<td>18.0 c</td>
<td>4.4 b</td>
</tr>
<tr>
<td>Patulul — Quilichao</td>
<td>13.5 c</td>
<td>32.8 b</td>
<td>27.4 b</td>
<td>24.0 b</td>
<td>35.4 a</td>
<td>4.0 a</td>
</tr>
<tr>
<td>Patulul — Palmira</td>
<td>18.0 a</td>
<td>39.9 a</td>
<td>31.1 b</td>
<td>24.9 b</td>
<td>33.6 a</td>
<td>3.6 a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>0.9</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Significance (p) of effects of Provenance: NS 0.0001 0.0001 0.0001 0.0001 0.0001
Significance (p) of Site: NS 0.0001 0.01 0.0004 0.003
Significance (p) of Provenance x Site: NS 0.0036 0.004 0.003 0.003

a, b, c Values in the same column with the same letters are not different (P < 0.05)

The concentration of ECT in the forage offered to sheep was higher (P<0.05) in Patulul as compared to San Ramón, but the insoluble ECT fraction did not change due to provenance (Table 1), which again is in agreement with previous findings of CIAT and OFI (Stewart et al., 2000). Although the ECT fraction was higher (P<0.05) in Patulul, IVDMD was also higher (P<0.05) with this provenance possibly as a result of lower fiber concentration. Similar results were recorded in other feeding trials where the two Calliandra provenances were used as supplements to sheep fed a low quality grass diet (Lascano et al., unpublished). These results confirm previous findings in CIAT (Lascano et al., unpublished) that suggested that digestibility of Calliandra forage may be more related to fiber content than to the concentration of ECT.

Chemical composition - Site effects. For the two provenances of Calliandra, the protein content and IVDMD were both lower (P<0.05) in the edible forage harvested from the site with acid soils (Quilichao) than from the site with fertile soil (Palmira), but these differences due to site were not explained by differences in fiber content (Table 1). In addition, results showed that ECT concentration in San Ramón was lower (P<0.05) in the edible forage harvested in Palmira as compared to Quilichao, which is a clear indication of an effect of soil fertility on forage quality. A similar trend was observed with Patulul, but differences due to site were less pronounced than with CIAT San Ramón, and did not achieve statistical significance.

In New Zealand there has been an interest in defining how soil fertility and fertilizer applications influence tannin levels in legumes such as Lotus pedunculatus and Lotus corniculatus when grown in acid soils (Barry and Forss, 1983; Lowther et al., 1987). In field studies it was shown that the level of CT in L. pedunculatus cv. Grasslands Maku declined significantly (8-11% of DM to 2-3% of DM) when the legume was grown in a high fertility soil as opposed to an acid soil with no fertilizer application (Barry and Forss, 1983). However, it was also observed that application of combined P and S fertilizer to L. pedunculatus grown in acid soils reduced tannin concentration to 4-5% of DM and increased biomass yield.

With tropical legumes there have been limited research efforts towards defining how environmental factors influence the concentration of ECT and other quality parameters. In the early 1980’s research carried out by CIAT showed that cattle grazing a pure stand of Desmodium ovalifolium CIAT 350 in the Llanos of Colombia had a marked preference for forage that had relatively large quantities of fertilizer. This observation led to the design of a field experiment to compare quality parameters and acceptability of forage by cattle as a function of the application of different elements alone and in combination (P + Ca; P + Ca + K; P + Ca + K + S; and a control). Results showed small difference in forage yield with the combination of P, Ca and K in the fertilizer, but almost a 2-fold increase when S was added to the mixture (Lascano and Salinas, 1982; Lasinas and Lascano, 1983). Increased biomass production with the combined fertilizer was associated with a 9-percentage unit reduction in tannin concentration and 0.5 percentage units increase in N in the leaf tissue.

In plants grown at the University of Reading under greenhouse conditions (high nutrient soil and no environmental stress) forage from the two provenances of Calliandra had lower ECT and higher IVDMD than forage grown in the two fields sites in Colombia (Stewart et al., 2000). These contrasting results reinforce the hy-
The relationship between environment and animal production

Hypothesis that edaphic and climatic stresses influence forage quality of tropical legumes with tannins.

Tannin structure and function - Provenance and Site effects. Tannin structure in the two Calliandra provenances expressed as ratio of procyanidin: prodelphininid showed that in San Ramón the ratio due to site was not significant (P> 0.05) and varied between 37: 63 and 30: 70 in the Palmira and Quilichao sites, respectively. In Patulul the procyanidin: prodelphininid ratios was not affected by site and varied between 9: 73 and 9: 19 for samples harvested in Palmira and Quilichao, respectively (data not shown). These results agree with previous results from CIAT and with results recorded in the University of Reading, which indicated that ECT in Patulul comprised mainly procyanidin subunits, whereas in San Ramón samples comprised largely prodelphininid subunits (Stewart et al 2000).

Astringency (g of protein precipitated per g of ECT) was not influenced by site (P> 0.05) but varied between Calliandra provenances (Table 2). The ECT from San Ramón with more delphinidin units were more (P<0.05) astringent than the ECT from Patulul with more cyanidin units, which again is in agreement with results obtained in Calliandra grown in a greenhouse in the University of Reading (Stewart et al., 2000). These findings are also in agreement with results reported with temperate legume species. The ECT fraction in L. corniculatus is mostly composed of cyanidin units, whereas the ECT in L. pedunculatus is mostly composed of delphinidin units, resulting in tannins from L. pedunculatus being more reactive with Rubisco than tannins from L. corniculatus (McNabb et al., 1997).

Intake and digestibility- Site Effects. Intake of Calliandra (Table 3) harvested in Palmira (fertile soil) was 58% higher (P<0.05) than intake from Calliandra harvested in Quilichao (infertile soil), confirming the positive effect of soil fertility on the feed value of tropical legumes with tannins (Lascano and Salinas, 1982; Salinas and Lascano, 1983). The higher intake of Calliandra provenances grown in the fertile site as opposed to the site with the acid, low-fertility soils was associated with lower (P<0.05) concentration of ECT as shown in Table 1. Previous results from CIAT had shown that reducing the concentration of tannins by spraying PEG (polyethylene glycol) to the forage of tropical legumes (Desmodium ovalifolium and Flemingia macrophylla) was associated with increased intake by sheep (Barahona et al., 1997), but these increases were accompanied by increases in fiber digestibility, which was not the case in this study. Thus we hypothesize that the higher intake of Calliandra provenances grown in the site with fertile soils is a direct consequence of having lower concentration of tannins.

Intake and digestibility-Provenance Effects: Results on intake and digestibility are presented in Table 3 for main effects given that the interaction of site x provenance was not significant (P>0.05) for the variables measured. Intake of the two Calliandra provenances was very low when expressed as the proportion of body weight (range 0.5 to 1.2 kg DM 100 kg of BW⁻¹). The amount of Calliandra consumed represented 41% and 63% of the amount offered daily of Patulul and CIAT San Ramón, respectively. More specifically, DM intake of San Ramón was 30% higher (P<0.05) than intake of Patulul, which was an unexpected result. In previous feeding trials with sheep (Lascano et al., unpublished) we had observed that intake of Patulul was higher than San Ramón when fed in combination with a poor quality grass, and this was related to the lower fiber content in Patulul. However, it should be noted that intake of digestible DM was not affected (P>0.05) by provenance, because the digestibility of Patulul was higher (P<0.05) than San Ramón. This finding is consistent with the lower fiber content and higher IVDMD of Patulul shown in Table 1.

Nitrogen utilization by sheep

The main objective of running this feeding trial was to test the hypothesis that the higher proportion of delphinidin relative to cyanidin found in the ECT fraction of San Ramón would result in greater protection of protein in the rumen (by-pass protein) as compared with Patulul with ECT comprised mainly by procyanidin subunits.

Table 2. Astringency of extractable condensed tannins (ECT) of Calliandra calothyrsus provenances grown in two sites with contrasting soil fertility

<table>
<thead>
<tr>
<th>Provenances</th>
<th>Quilichao (infertile soil)</th>
<th>Palmira (fertile soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Ramón</td>
<td>0.90 a</td>
<td>0.97 a</td>
</tr>
<tr>
<td>Patulul</td>
<td>0.59 b</td>
<td>0.57 b</td>
</tr>
</tbody>
</table>

* Protein used: Bovine Serum Albumin – pH 5.0
a, b Values with the same letters are not different (P< 0.05)
Results shown in Table 4 are for main effects, given that we did not find a significant (P>0.05) provenance x site interaction for any of the response variables measured. Total N intake was 30 % higher (P<0.05) with San Ramón than with Patulul. In addition, N intake was 85% higher (P<0.05) when Calliandra harvested in the site with fertile soil (Palmira) was fed. These differences are the result of the higher DM intake of San Ramón and of the higher CP level in the forage harvested in the fertile soils of Palmira (see Table 1).

Flow of different N fractions in the solid phase to the lower gastrointestinal tract was affected by provenance fed and by site (Table 4). A higher amount (P<0.05) of total N in the solid phase reached the duodenum when San Ramón and when Calliandra harvested in the site with fertile soil (Palmira) was fed. These differences are the result of the higher DM intake of San Ramón and of the higher CP level in the forage harvested in the fertile soils of Palmira (see Table 1).

Table 3. Intake and digestibility of Calliandra calothyrsus fed to sheep housed in metabolism crates and supplemented with extracted cassava meal

<table>
<thead>
<tr>
<th>Item</th>
<th>Site Effect</th>
<th>Provenance Effect</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quilichao (infertile soil)</td>
<td>Palmira (fertile soil)</td>
<td></td>
</tr>
<tr>
<td>Intake of DM (g kg of BW⁻¹ d⁻¹)</td>
<td>6.7 b</td>
<td>10.6 a</td>
<td>10.2 a</td>
</tr>
<tr>
<td>Digestibility of DM (%)</td>
<td>57.8 a</td>
<td>53.7 a</td>
<td>51.7 b</td>
</tr>
<tr>
<td>Digestibility of NDF (%)</td>
<td>50.1 a</td>
<td>50.9 a</td>
<td>45.5 b</td>
</tr>
<tr>
<td>Intake of Digestible DM (g kg of BW⁻¹ d⁻¹)</td>
<td>3.6 b</td>
<td>5.4 a</td>
<td>5.1a</td>
</tr>
</tbody>
</table>

a, b, c for each main effect, values in the same row with the same letters are not different (P<0.05)

14 g kg of BW⁻¹ d⁻¹ of extracted cassava meal was fed via rumen cannula to each sheep.

Table 4. Nitrogen (N) utilization by sheep housed in metabolic crates and fed two provenances of Calliandra calothyrsus grown in contrasting sites

<table>
<thead>
<tr>
<th>Item</th>
<th>Site Effect</th>
<th>Provenance Effect</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quilichao (infertile soil)</td>
<td>Palmira (fertile soil)</td>
<td></td>
</tr>
<tr>
<td>N intake, g d⁻¹</td>
<td>6.7 b</td>
<td>12.4 a</td>
<td>10.8 a</td>
</tr>
<tr>
<td>Duodenal N, g d⁻¹</td>
<td>12.5 b</td>
<td>18.6 a</td>
<td>18.6 a</td>
</tr>
<tr>
<td>Fecal N, g d⁻¹</td>
<td>5.7 b</td>
<td>9.7 a</td>
<td>9.1 a</td>
</tr>
<tr>
<td>Apparently absorbed N, g d⁻¹</td>
<td>6.9 b</td>
<td>8.9 a</td>
<td>9.5 a</td>
</tr>
<tr>
<td>Ruminal escape dietary N, g d⁻¹</td>
<td>5.5 b</td>
<td>9.5 a</td>
<td>11.0a</td>
</tr>
<tr>
<td>Ruminal escape N, % of N intake</td>
<td>77.2 a</td>
<td>79.0 a</td>
<td>99.0 a</td>
</tr>
</tbody>
</table>

14 g kg BW⁻¹ d⁻¹ of extracted cassava meal was fed via rumen cannula to each sheep.

a, b for each main effect, values in the same row with the same letter are not different (P<0.05)

2Duodenal N – Fecal N
3Ruminal escape dietary N = N flow to the duodenum – (Bacterial N flow + Endogenous N) where: Endogenous N = 2.2 g N per kg of DM intake
consumed (Barahona et al., 1997) or no effect on N consumed (Carulla et al., 2001), but in all cases less N reached the duodenum, which translated in less N escaping ruminal degradation. Other studies with temperate legumes also showed that tannins protect protein from being degraded in the rumen and by so doing increase protein flow to the small intestine and amino acid absorption (Barry and Manley, 1984; Waghorn et al., 1987). Total N flow to the duodenum and N apparently absorbed in the small intestine were higher with San Ramón than with Patulul (see Table 4). This finding indicates that differences in N utilization by sheep fed the two provenances were related to tannin structure rather than to tannin concentration, given that the ECT was higher in Patulul than in San Ramón (see Table 1).

The most significant finding of this study is that for the first time it has been shown that the structure of tannins in a tropical legume can have an effect on the utilization of N by ruminants. The fact that our in vivo results were in close agreement with laboratory results on astringency of extractable condensed tannins in the two Calliandra provenances evaluated is also a major finding, since they validate the utility of the astringency assay for screening tropical legumes for quality traits.

Conclusions

Results from this study indicate that Patulul (CIAT 22316) was superior to San Ramón (CIAT 22310) in terms of having lower fiber (NDF and ADF), higher digestibility, and higher DM intake. However, Patulul also contained higher concentration of extractable tannins than San Ramón, although the insoluble tannins did not differ between provenances. Thus differences in fiber content between the two Calliandra provenances may have a greater effect on digestibility than level of extractable condensed tannins, as has been the general belief. The higher DM intake found with San Ramón resulted in greater N intake and apparent absorption of N in the small intestine, but not in greater intake of digestible DM given the higher digestibility of Patulul. Thus it is not possible to conclusively infer that the feed value of San Ramón is higher than that of Patulul. However, these results do indicate that differences in tannin structure of the two Calliandra provenances fed to sheep had an effect on ruminal escape of N and thus on protein utilization. The effect on animal production of these differences between Calliandra provenances is an effect of tannins for quality traits.

Acknowledgements

The help of the staff who work in CIAT’s Quilichao Research Station and in CIAT’s Forage Quality Laboratory is gratefully acknowledged. Special thanks are due to Gerardo Ramirez, Biometrician of CIAT’s Forage Project for assistance in the statistical analysis of results. This publication is an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID, CIAT or OFI. Project R6549, Forestry Research Program in collaboration with CIAT.

Literature Cited


