Content of soluble and bound condensed tannins of three tropical herbaceous forage legumes

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Abstract

The soluble, total-bound and fibre-bound condensed tannins (CT) in \textit{Cassia rotundifolia} (cassia), \textit{Lablab purpureus} (lablab) and \textit{Macroptilium atropurpureum} (siratro) grown in tropical and subtropical regions were determined by the butanol–HCl method. Cassia had the highest total CT content of 29.5 g/kg DM than that of lablab, 16.9 g/kg DM which was intermediate while that of siratro, 12.4 g/kg DM, was the least. The legumes had most of their CT in the protein-bound fraction that constituted 54.5, 74.0 and 86.2\% of the total CT in cassia, lablab and siratro, respectively. The fibre-bound CT content of the legumes was small and constituted 1.75, 3.71 and 1.41\% of total CT in cassia, lablab and siratro, respectively. The low CT content of lablab and siratro may not be high enough to confer beneficial effects on legume nitrogen utilisation in ruminants as reported in other studies. However, the high CT content of cassia may cause astrigency leading to low intake. The presence of condensed tannins in these legumes may influence their utilisation as protein supplements to low quality roughages given to ruminants during the dry season in tropical regions. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Tannins are found in dicotyledenous forbs, shrubs and trees (Haslam, 1989) and are polyphenolic compounds which are classified based on their structural types as either hydrolysable or condensed tannins (Makkar, 1995). The amount and structure of tannins present determines their nutritional effects. Condensed tannins (CT) or proanthocyanidins in forages have been associated with a reduction of voluntary intake (Barry and Duncan, 1984; Chiquette et al., 1989) and apparent digestibility of dry matter, organic matter, crude protein and fibre (Waghorn et al., 1990; Muhammed et al., 1994).

Forage legumes are utilized in tropical and sub-tropical regions as protein supplements to ruminants grazing poor quality roughages especially during the dry season. The presence of tannins in these legumes may result in reduced availability of nitrogen and amino acids required for rumen microbial growth since tannins form strong complexes with proteins (Barry and Duncan, 1984; Reed, 1995). Tropical herbaceous legumes, *Arachis pintoi*, *Centrosema spp.* and *Desmodium spp.* have been reported to contain CT levels ranging from 22.2 to 237.5 g/kg DM (Jackson et al., 1996). However, little is known about the condensed tannin content of some herbaceous forage legumes commonly grown in Zimbabwe. The condensed tannin level in a plant sample can be determined either in comparative amounts by the use of a commercial standard (external standard) or in actual amounts by using purified extracts of tannins from the plant under study (Giner-Chavez et al., 1997).

The objective of this study therefore was to determine the soluble, bound and total CT content of three legumes *Cassia rotundifolia*, *Lablab purpureus* and *Macroptilium atropurpureum* using mimosa tannin as an external standard in the acid butanol assay.

2. Materials and methods

2.1. Sample source and preparations

The three tropical legumes, namely *Cassia rotundifolia* (Cassia), *Lablab purpureus* (Lablab) and *Macroptilium atropurpureum* (Siratro) were established in 1994/1995 growing season on sandy loam soil at Marirangwe, Zimbabwe (1500 m asl; average annual rainfall 950 mm; mean annual temperature is 20°C; pH 5.5 CaCl₂ scale). Samples were collected from forages cut at 10 cm above the ground at the end of 1995/1996 growing season. The harvested samples, whole plant material, were air dried and milled through a 1 mm screen and bagged pending laboratory analysis.

2.2. Extraction and determination of tannins

2.2.1. Soluble condensed tannins

Soluble condensed tannins were extracted from finely ground (1.0 mm mesh) samples (200 mg) of each legume species in triplicate with 10 ml of aqueous acetone (70 : 30; v/v) in Erlenmeyer flasks for 24 h at room temperature with occasional mixing by swirling as described by Makkar (1995). After extraction, the supernatant of each sample
was decanted into a conical flask and mixed thoroughly. A 1 ml aliquot of the supernatant from each sample was added to 6 ml of butanol–HCl reagent in duplicate test tubes. The tubes were capped and transferred to a dry block heater (Grant Instruments, Cambridge, UK) that had been pre-heated and maintained at 100°C. The tubes were heated for 1 h, removed and cooled before decanting samples into vials and absorbance read at 550 nm using a Cecil CE 2030 single beam spectrophotometer (Cecil Instruments, England).

2.2.2. Total bound condensed tannins

The residues after extraction of soluble tannins was washed twice with 70% aqueous acetone (Makkar, 1995). The residue was freeze dried and 10 mg weighed into duplicate tubes and 6 ml of butanol–HCl reagent added. The tubes were then placed into a pre-heated, 100°C, dry block heater, Techne Ori-Block DB-3, and heated for 1 h after which they were removed, cooled and centrifuged at 3000 × g for 10 min (Makkar, 1995). The supernatant was decanted into vials and absorbance read at 550 nm using a Cecil CE 2030 single beam spectrophotometer (Cecil Instruments, England). Blank samples containing the reagent were only included in the measurements.

2.2.3. Fibre-bound condensed tannin

Fibre-bound condensed tannins were determined in the dried neutral detergent fibre fraction of the forage legumes after extraction of NDF (Goering and Van Soest, 1970). NDF samples of 100–200 mg were weighed into each of duplicate screw cap test tubes and 5 ml of butanol–HCl reagent added to each tube. A similar procedure of extraction as described for bound tannins was followed. The protein-bound tannins were calculated as the difference between the total bound-PA and the NDF-bound PA. Mimosa tannin (MT; Hodgson, England) was used as an external standard. The concentration of CT in the legume samples were converted to g MT equivalent/kg DM from the mimosa tannin regression equation: \( Y = 0.038 + 0.97X \) \( (R^2 = 0.99) \).

3. Statistical analysis

Analysis of variance was carried out using the General Linear Model Procedure of SAS (SAS, 1990). The difference between means was compared using Tukey Studentised range test of SAS (1990).

4. Results

The condensed tannin concentration (analysed by the butanol–HCl method) in the three legumes are presented in Table 1. All the legumes contained CT in all the fractions tested. The amount and proportion of soluble CT was higher \( (p < 0.001) \) for cassia than in the other two legumes with lablab being intermediate and siratro having the least. The total bound CT fraction was significantly \( (p < 0.05) \) greater in cassia and lablab than in siratro.
The fibre-bound CT constituted a small fraction of total CT being 1.75% for cassia, 3.71% for lablab and 1.41% for siratro. However, cassia and lablab had significantly \( (p < 0.05) \) more fibre-bound CT than siratro. The amount of protein-bound CT fraction was numerically higher \( (p < 0.05) \) in cassia than that in siratro with lablab being intermediate. However, the proportion of CT which was bound to protein was lowest \( (p < 0.05) \) for cassia compared to lablab and siratro. The total CT concentration of the legumes ranged from 12.4 to 29.5 g mimosa tannin equivalent/kg DM. Cassia had a greater \( (p < 0.01) \) total CT concentration than either lablab or siratro which did not differ significantly \( (p > 0.05) \).

5. Discussion

The legumes contained 12–44% of total CT in the soluble fraction. These values are lower than those reported by Jackson et al. (1996) for tropical legumes *Arachis pintoi*, *Centrosema latidens* and *Desmodium ovalifolium* that had 70–95% of total CT in the soluble fraction using the butanol–HCl method. The differences may be attribute to variation in environmental conditions, plant nutrition and stage of plant growth (Makkar and Singh, 1991). In addition, the variations in tannin content may be due to different reactions of tannins or other compounds present in the forage legumes (Dr. T. Acamovic, personal communication). Other reasons for differences in the soluble tannin content values obtained in this study and published values may be due to different procedures followed in sample preparation such as heat treatment. Different drying procedures have been reported to affect measured tannin content of legumes (Mahyuddin et al., 1988). The high soluble CT content of cassia may cause astrigency resulting in low voluntary intake and this may partly explain the low intake of cassia by grazing cattle which has been reported in the literature (Clements, 1989).

The fibre-bound CT constituted a small proportion of the total CT content of the three legumes. Similar results have been reported in previous studies (Terrill et al., 1992). A high proportion of bound CT has been reported to reduce rumen fibre digestion possibly due to a reduction in cell wall fermentation caused by inactivation of hemicellulase and cellulase enzymes secreted by rumen microbes or inhibition of the attachment of rumen microbes (Muhammed et al., 1994). Tannins also inhibit microbial endoglucanase

### Table 1

The concentration of condensed tannins (g/kg DM) in tropical herbaceous forage legumes determined by the butanol–HCl method

<table>
<thead>
<tr>
<th></th>
<th>Cassia(^a)</th>
<th>Lablab(^b)</th>
<th>Siratro(^a)</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble CT</td>
<td>12.9 a</td>
<td>3.7 b</td>
<td>1.5 c</td>
<td>0.36</td>
</tr>
<tr>
<td>Total-bound CT</td>
<td>16.6 a</td>
<td>13.2 ab</td>
<td>10.9 b</td>
<td>1.50</td>
</tr>
<tr>
<td>NDF-bound CT</td>
<td>0.5 a</td>
<td>0.6 a</td>
<td>0.2 b</td>
<td>0.10</td>
</tr>
<tr>
<td>Protein-bound CT</td>
<td>16.1 a</td>
<td>12.6 ab</td>
<td>10.7 b</td>
<td>0.37</td>
</tr>
<tr>
<td>Total CT</td>
<td>29.5 a</td>
<td>16.9 b</td>
<td>12.4 b</td>
<td>1.86</td>
</tr>
</tbody>
</table>

\(^a\) Means in the same row with different letters differ significantly \( (p < 0.001) \).
activity and the attachment of fungi to cellulose in vitro (Muhammed et al., 1994) resulting in reduced degradation of cellulose. However, other plant anti-nutrients may also contribute to a reduction in fibre degradation in the rumen (Salawu, 1997).

The forage legumes analysed in this study contained 54.5–86.2% of total CT in the protein-bound form which is contrary to results reported in other studies (Jackson et al., 1996). These authors reported low concentrations of protein-bound CT as a proportion of total CT with A. pintoi having a content of protein-bound CT of 1.2 g/kg DM, C. latidens with 3.7 g/kg DM and D. ovalifolium with 15.6 g/kg DM. However, values obtained in this study of 10.7–16.1 g/kg DM are comparable to that of C. latidens Accession 15 092 which had a concentration of 17.3 g/kg DM.

The total CT content of cassia is comparable to that of C. latidens and A. pintoi of 22.2 and 33.6 g/kg DM, respectively, but lower than that of D. ovalifolium which was high and ranged from 105 to 238 g/kg DM (Jackson et al., 1996). The presence of high concentrations of tannins is reported to reduce N degradation in the rumen through formation of tannin–protein complexes which are stable at rumen pH but do cleave at the low gastric pH (2.5–3.5) of the abomasum and the relatively high pH (8–9) of the distal small intestines (Mangan, 1988; Salawu et al., 1997). Ruminant nutrition studies with Lotus species have indicated an optimal CT content in forage of 22 g CT/kg DM, while a range of 60–100 g CT/kg DM depresses intake and growth (Barry and Duncan, 1984; Waghorn et al., 1987). Thus, the CT content of legumes in this study should be beneficial when included in ruminant diets.

Although relatively little literature has been published regarding the CT content of tropical herbaceous forage legumes, the present study has shown that these legumes do contain condensed tannins and possibly other types of anti-nutritional factors. The lack of standard condensed tannin as well as the variation in the types of tannin and their interaction makes it difficult to assess the real quantities of tannin present in forage legumes and also to compare data with other published work. The paucity of such information also makes it difficult to predict the effects of CT on animals and on the nutritional value of the legumes that are used as protein supplements to low quality roughages in the tropical region.

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References


