HPLC Identification of Toxic Non-Protein Amino Acids in the Tropical Leguminous Tree *Acacia angustissima*

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The leguminous tropical multipurpose tree *Acacia angustissima* is a potential source of protein supplement for ruminants fed roughage diets. *A. angustissima* is well adapted to free draining infertile acidic soils, shows drought tolerance by retaining green leaf during dry seasons (Gutteridge, 1994), has high leaf biomass yields and seeds profusely. However there have been reports of toxicity associated with feeding of *A. angustissima* (Odenyo *et al*., 1997) to ruminants and preliminary evidence has shown that the plant contains toxic non-protein amino acids (Shah *et al*., 1992). We have developed an HPLC method to identify the amino acid components of this species.

A. angustissima (Oxford Forestry Institute, Accession number 38/88) was planted at CSIRO Lansdown Research Station, Townsville, Queensland. The first five fully expanded leaves were collected from each branch and freeze dried. Ground leaf (1 mm screen, 0.1 g) plus BIORAD anion exchange resin (0.1 g; BIO-RAD AG 1-X2, 200-400 mesh, chloride form) was extracted overnight with 0.01M HCl (10 ml) on a rotary mixer. The supernatant was purified by centrifugation through a 5,000 MWCO membrane (Sartorius Centrisart C4) then derivitised using the Waters AccQ-Fluor system (Reagent Kit Cat: WAT052880). The amino acids were separated by HPLC using a Shimadzu LC-10 HPLC at 1.2 ml min^{-1} flow rate with fluorescence (Ex 250 Em 395nm) and UV-VIS (248nm) detection, a Waters C18 column (Nova-Pack 3.9 × 300mm), and a gradient elution protocol (Table 1). Buffer A contained 133mM sodium acetate, 3mM triethylamine and 1 ppm calcium di-sodium EDTA, adjusted to pH 6.43 with phosphoric acid.

Non-protein amino acid standards used were DAP, DAB, beta ADAP, beta ODAP, gamma ODAB, and albizziin, where A=acetyl, O = oxalyl, DA= diamino, P = propionic and B = butyric. The protein hydrolysate standard contained the following amino acids; aspartate, serine, glutamate, glycine, histidine, arginine, threonine, alanine, proline, cystine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine. Concentration of non-protein amino acids was calculated from the peak area of the internal standard alpha amino butyric acid.

Table 1. HPLC gradient profile

	Buffer	Acetonitrile	Water
Time (min.)	%A	%B	%C
0	100	0	0
35	98	2	0
60	97	3	0
76	96	4	0
85	91	9	0
101	88	12	0
112	85	15	0
120	80	20	0
122	0	60	40
126	100	0	0
145	100	0	0

This accession (38/88) contained all the amino acids present in the hydrolysate standard as well as 10 putative non-protein amino acids. Three of the non-protein amino acids were present (1.0 - 4.4 g/kg DM), one of which co-eluted with the authentic standard beta ADAP while the other two have not been identified. Three minor peaks corresponded with beta ODAP, gamma ODAB and albizzin. The compounds DAP and DAB were not present in this accession.

Two of the non-protein amino acids that were present at lower concentration (ODAB and ODAP) are known neurotoxins (Rosenthal and Bell, 1979). DAP is also a potential neurotoxin but only the acetyl derivative occurred in this plant and the potential for toxicity is unknown.

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