



The digestion of dietary protein bound by condensed tannins in the gastro-intestinal tract of sheep

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Abstract

The digestion of dietary protein bound by condensed tannins (CTs) in ruminants was investigated by determining the extent of dissociation of insoluble ¹²⁵I-BSA + CT complexes administered to abomasally and intestinally fistulated sheep. The extent of dissociation was registered as the true digestibility of iodinated bovine serum albumin (¹²⁵I-BSA). The true digestibility of ¹²⁵I-BSA originally bound to *Leucaena pallida* CT (0.721) was lower ($P < 0.05$) than that of ¹²⁵I-BSA originally bound to *L. leucocephala* CT (0.880) between the abomasum and terminal ileum. These results indicate that differences in the ability of CT to inhibit ¹²⁵I-BSA digestion *in vivo* matched the relative abilities of the same CT to bind BSA *in vitro*, indicating that the *in vitro* BSA-binding assay for ranking CT behaviour was biologically relevant *in vivo*. Furthermore, the true digestibility of CT-bound ¹²⁵I-BSA between the mouth and faeces permitted the prediction of the quantitative contribution that CT-bound dietary proteins make to improved nitrogen supply to the small intestines.

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Abbreviations: CT, condensed tannins; ¹²⁵I-BSA, iodinated bovine serum albumin; ⁵¹Cr-EDTA, chromium EDTA; GIT, gastro-intestinal tract.

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1. Introduction

Both the total amount and types of condensed tannins (CTs) in a forage is likely to influence its nutritive value. In a review of *in vitro* CT assays, Schofield et al. (2001) noted the relatively weak reactivity of Quebracho CT, when compared with CT from forages such as *Gliricidia sepium* and *Desmodium ovalifolium*. Similarly, within the *Leucaena* genus, CT from *L. leucocephala* are particularly weak by comparison with CT isolated from other *Leucaena* species (Osborne and McNeill, 2001). CT characteristics that mostly affect digestion in ruminants are the type (astringency, molecular structure), form (free, protein-bound, fibre-bound) and concentration of CT in the ingesta (plant material), and the extent to which the CT releases proteins for digestion post-ruminally (Perez-Maldonado and Norton, 1996). The most valued CT has an ability to bind soluble proteins during passage through the rumen, and to release these proteins for digestion in the small intestine (McNeill et al., 1998; Barry et al., 2001). However, few workers (e.g. Andrabi et al., 2005) have directly examined whether the differences in CT reactivity (binding affinity) seen *in vitro* translate to biological differences *in vivo*. There is also little known about the relative binding and release rates of proteins from tannins during transit through the gut, nor it is clear whether the protein bound with CT in faeces is of plant or endogenous origin.

The following paper investigates whether CT-binding affinity (mg CT/0.375 mg BSA at pH 5) rankings defined *in vitro* are reflected *in vivo* in relation to the true digestibility of feed protein bound by CT in the gastro-intestinal tract of ruminants. Abomasally and intestinally fistulated sheep were used to determine the relative abilities of CT (isolated from different sources) to release protein from ^{125}I -BSA + CT complexes (CT–protein complex stability) between the rumen, abomasum, small intestines, and excretion in faeces. Results from this study were expected to provide information on the extent of tannin–protein complex breakdown at different sites in the digestive tract, and whether CT-binding affinity for BSA *in vitro* is a useful measure for prediction of protein release from CT during digestion.

2. Materials and methods

2.1. CT sources and purification

Shoot material (Dalzell and Shelton, 1997) and mature leaves (Bassala et al., 1991; Dalzell and Shelton, 1997) were harvested from *Leucaena pallida* K748, *L. pallida* K748 \times *L. leucocephala* K636 F1 hybrid (KX2), and *L. leucocephala* K636 cv. Tarramba trees growing at the University of Queensland's Mt. Cotton research farm, Queensland, Australia (latitude 27°37'S; longitude 153°14'E; altitude 25 m). The harvested material was immediately frozen on dry ice, freeze-dried, and stored at 4°C in air-tight containers (Dalzell and Shelton, 1997). The CT was isolated and purified using the procedure in Perez-Maldonado et al. (1995) with some slight modifications (Kariuki, 2004). CT from *Calliandra calothyrsus*, *Acacia aneura*, and *Desmodium intortum* were extracted and purified by Dr. Perez-Maldonado following the procedure in Perez-Maldonado et al. (1995). Com-

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