EFFECT OF CONDENSED TANNIN *ON* PROTEIN DIGESTION AND NUTRITIVE VALUE OF FRESH HERBAGE

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SUMMARY

Condensed tannins (CT) are polymeric flavanols that occur in a variety of plants including lotus, sainfoin, dock and mulga. CT are not toxic to ruminants, and when the concentration is below about 4% of dry matter (DM), they improve the nutritive value of herbage by binding to plant proteins and protecting them from excessive degradation in the rumen. The percentage absorption from the intestine is reduced, but the net effect of CT is to increase the amount of essential amino acids (AA) absorbed by 20-50% compared to plants without *CT.* Sheep fed herbage containing CT have an increased absorption of sulphur-AA which should increase wool production, have leaner carcasses, and CT prevent bloat in cattle.

INTRODUCTION

Condensed tannins occur in several plants commonly eaten by ruminants. Examples include *Lotus corniculatus* (Birdsfoot trefoil), *Lotus pedunculatus* (lotus major), Sainfoin *(Onobrychis viciifolia,* Scop.), dock *(Rumex obtusifolius*) and Mulga *(Acacia aneura,* Bonth). *Common* plants having low CT concentrations include *Lotus corniculatus, Lotus pedunculatus* grown under high fertility conditions and sainfoin, but none of these species persist under high fertility pastoral regimes. When concentrations of CT are high, exceeding about 6% of the DM, they have a detrimental effect on nutritive value for ruminants by reducing intakes and digestibility (Barry and Duncan 1984; Pritchard *et al.* 1988). In contrast, lower concentrations of CT, under about 4% of DM appear to have very considerable benefits to ruminant digestion.

This *paper* summarises some effects of CT in low concentrations *on* protein digestion and nutritive value of herbages and presents preliminary results from recent experiments with *Lotus pedunculatus.*

CONDENSED TANNINS

Condensed tannins (polymeric flavanols) are derived by carbon-carbon polymerisation of leucoanthrocyanidins and catechins. Molecular weights range from about 6000 in lotus to 28000 in sainfoin but *CT* account for only a proportion of the total phenol content of the plants. Large molecular weight CT are less able to align themselves between plant proteins in rumen digesta, and are therefore less astringent and reactive towards proteins (Mangan 1988).

CT are released during mastication and rapidly bind to plant proteins forming an insoluble CT-protein complex which is stable at rumen pH, but is able to dissociate at pH below 3.5 (Jones and Mangan 1977). Less plant protein is lost as ammonia during rumen digestion and therefore more plant protein passes to the intestines for enzymic digestion, In addition, the precipitation of soluble proteins effectively removes one of the primary factors involved in foam stabilisation, so that bloat does not occur when CT are present in the diet (Jones and Waghorn unpublished data),

A property which has proved most useful in studies evaluating the effectiveness of CT (e.g. Barry and Duncan 1984; Waghorn *et al.* 1987b), and which may have field applications (Pritchard *et al.* 1988) is the preferential binding between

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CT and polyethylene glycol (PEG). When PEG is applied to forages containing CT, given orally, or infused into the rumen with digesta containing CT it binds the CT so that the CT-protein complex does not form (Jones and Mangan 1977). The forage retains its nutritive characteristics but is essentially 'CT-free'. This binding affinity between CT and PEG has been used to evaluate the effects of CT on digestion when feeding lotus to sheep by giving half of them PEG, so that in effect a 'CT-free' lotus is compared with the control.

NITROGEN DIGESTION

Sheep fed herbages containing low concentrations of CT have more non-ammonia nitrogen (NAN) reaching the small intestine than similar herbages without CT (Waghorn *et al.* 1987a) resulting in an improved essential AA: energy ratio compared to that with other fresh forage diets. Results from 10 metabolic studies with lotus and sainfoin, all containing low concentrations of CT (Waghorn *et al.* 1990), showed an improved performance (N retention, AA absorption, protein: energy absorption) when compared to equivalent diets without CT. The nutritional benefits were due primarily to the protection of plant proteins from microbial degradation in the rumen and the resultant increase in protein flow to the intestine, and not to increased N digestion.

Table 1 Effect of condensed tannins on digestion when *given* to sheep fed fresh lotus (first cut) with and without an intra-ruminal infusion of PEG

	Lotus corniculatus (2.2% CT)			*Lotus pedunculatus* (5.5% CT)		
	Control	PEG	Sig.	Control	PEG	Sig.
Number of sheep	4	4		8	6	
DM intake (g/d)	1400	1461	n.s.	1168	1312	*
DM digestibility (%)	69	71	n.s.	68	70	n.s.
N intake (g/d)	37.8	39.4	n.s.	43.5	47.6	*
Rumen NH3 (mg N/l)	367	504	***	175	458	***
Nitrogen digestion (% of intake)						
Total	70	78	***	66	81	***
Rumen	12	21	*	12	26	*
Small intestine	52	56	n.s.	53	55	n.s.
Essential amino acid fluxes (g/day)						
Intake	112	117	n.s.	104	117	*
Abomasal flux	95	66	**	123	106	**
Absorption	69	46	**	78	83	n.s.

PEG, polyethylene glycol (m wt. 3300) given at about 1.5 g/g CT; DM, dry matter; N, nitrogen; n.s., not significant; *, P<0.05; **, P<0.01; ***, P<0.001.

In addition to the increased quantity of protein reaching the small intestine (Table 1), CT affected protein quality. This has been demonstrated in sheep fed *Lotus corniculatus* containing 2.2% CT in the DM, with or without an intra-ruminal infusion of PEG (Waghorn et *al.* 1987b). Control sheep had a 50% higher flux and a 62% greater absorption of essential AA compared to those infused with PEG (ie. 'CT-free'). Non essential AA were largely unaffected by CT (Table 1). *More* recently we found that sheep fed *Lotus pedunculatus (5.5%* CT in the DM) had a 30% increase in flux of essential AA including sulphur AA, (W.C. McNabb, unpublished data) and a 25% increase in non essential AA flux to the intestine compared to those given the same diet and an intra-ruminal infusion of 100 g PEG/day. This comparison is based on equal levels of intake, but 5.5% CT resulted in a 12% reduction in DM intake after 3 weeks of feeding so that the gains achieved through protection from rumen degradation were considerably reduced. Quantities of essential AA absorbed from the intestine were similar for both groups (Table 1), but control sheep retained 42% more N (P<0.05) than those infused with PEG.

In both trials protein was protected from degradation in the rumen by CT, but CT also reduced the apparent absorption of AA from the intestine, especially with 5.5% CT in the feed (Table 1). Reduced absorption negates some of the advantage gained from protection of plant proteins from degradation in the rumen. The cause of the reduced AA absorption may be *a* rapid rise *in* intestinal pH enabling the CT-proteincomplexes to re-form (Jones and Mangan 1977) and reduce enzymic digestion of the protein. Alternatively, the CT may also bind endogenous proteases and reduce their effectiveness for digestion.

DIGESTION OF NON PROTEIN CONSTITUENTS

Low concentrations of CT (2.2% of DM) did not affect fibre digestion when *Lotus corniculatus* was fed to sheep (Waghorn *et al.* 1987b) but when *Lotus pedunculatus* containing 5.5% CT in the DM was given there was a 13% increase in cellulose digestion in the rumen, possibly as a consequence of a longer retention time. Intakes of control sheep given *Lotus pedunculatus* declined from 1320 g DM/day during the first two weeks of feeding to 1220 g DM/day over the following 10 days ($P<0.05$). At slaughter these sheep had a larger rumen volume (484 g DM) than those receiving intra-ruminal PEG (420 g DM; NS) despite DM intakes for the 24 h prior to slaughter of 1128 and 1334 g respectively ($P<0.05$). The concentration of volatile fatty acids (VFA) in rumen digesta of control sheep was only 67% of that in sheep infused with PEG ($P<0.001$), suggesting a slower rate of fermentation in the control group.

Both trials with lotus (2.2 and 5.5% CT) showed a significant ($P<0.01$) reduction in apparent absorption of sulphur from the rumen in controls compared to PEG sheep (0.29 v 0.94 g/day with 2.2% *CT,* and 0.53 v 1.13 g/day with 5.5% CT lotus). Phosphorus absorption was affected when CT was 5.5% of DM with a higher ($P<0.01$) apparent absorption in the control sheep (0.41 g/day) then those infused with PEG (0.31 g/day). This may have been a consequence of the higher N retention by the control sheep,

EFFECT OF CT CONCENTRATION IN FORAGE DM

When CT concentrations exceed about 4% of herbage DM the beneficial effects begin to diminish, however at present the lower limit below which CT become ineffective is not known, John and Lancashire (1981) showed lotus with 1.45% CT in the DM improved N retention relative to lotus with only 0.25% CT in the DM. However, in a trial with dock Waghorn and Jones (1989) showed CT concentrations of only 0.17% in the herbage DM affected rumen function. In that trial docks (with 1.7% CT in the DM) were fed as 10% of intake to cows, the remainder of the diet *being* predominantly lucerne. The *CT* lowered the concentration of soluble protein in rumen contents ($P<0.05$) and in this instance also prevented bloat- Thus some benefits may result from very low concentrations of CT in the diet.

High concentrations CT do not appear to affect palatability. With *Lotus pedunculatus* (5.5% CT) the sheep prefered leaves containing 8.5% CT in their DM to the stems with 2.2% CT in the DM. This has important implications, because CT from one plant species have been shown to bind proteins from another species (Waghorn and Jones 1989) so that plants containing high concentrations of CT in their foliage may have an important place as a minor pasture component. When consumed with other pasture *components* by the grazing animal, this would result in a diet having an overall low concentration of CT, and evidence to date -suggests that this diet would have an improved nutrition value.

Do CT ALWAYS WORK?

In the majority of sheep CT protect proteins from degradation in the rumen, however we have observed anomalous behaviour by a few sheep in two trials. When *Lotus pedunculatus (5.5%* CT) was fed, the protection of proteins from rumen degradation was minimal in three of the eight sheep in the control group. The CT did reduce the percentage of AA apparently absorbed from the intestine in these sheep, so that the CT in fact reduced the net apparent absorption of AA. In the remaining sheep CT increased the essential AA flux from the rumen by 42% over that for sheep infused with PEG. In a separate trial, again involving *Lotus pedunculatus*, one of six control sheep had rumen ammonia concentrations three times that of the others, again suggesting an extensive protein degradation in the rumen of that sheep despite the presence of CT.

These rather important observations should not be ignored. It appears that CT do not always reduce microbial degradation of plant proteins in the rumen, in which case the question must be asked, do some sheep have a microbiota able to degrade CT? If so, this would have serious implications for any program designed to genetically engineer clovers to express *CT* in their foliage.

A further problem which should be addressed concerns the reduction in apparent absorption of AA from the intestine in the presence of CT. If CT could be engineered so that binding with proteins occurred at a higher pH, above 5.5 for example, then the absorption of AA from proteins which had been protected from rumen degradation would be much greater.

CONCLUSIONS

Incorporating CT into forage diets at a concentration between 1 and 4% of DM is likely to result in significant advantages in terms of ruminant feed efficiency and productivity. Efficiency will be achieved by improving the ratio of protein:energy absorbed. Productivity will be improved by increasing the net absorption of protein. Although there have been comparatively few field trials, lotus fed lambs are leaner than those fed white clover (Purchas and Keogh 1984). The increased absorption of sulphur AA with CT, (McNabb unpublished) may result in a higher wool production, (Reis *et al.)* and in addition bloat does not occur in cattle fed forages containing CT.

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