The effects of condensed tannin in *Lotus corniculatus* upon nutrient metabolism and upon body and wool growth in grazing sheep

Y. WANG, G.C. WAGHORN[1], G.B. DOUGLAS[1], T.N. BARRY AND G.F. WILSON

Department of Animal Science, Massey University, Palmerston North, New Zealand.

ABSTRACT

Lotus corniculatus containing 23 g/kg DM of extractable condensed tannin (CT) and 35 g total CT/kg DM was fed to sheep in two experiments. In both experiments half the sheep received supplementation with polyethylene glycol (PEG), which binds and inactivates CT, enabling the effects of CT to be quantified by comparing control sheep (CT operating) with PEG sheep (CT inactivated). Experiment 1 was conducted indoors, with sheep held in metabolism crates and fed hourly to determine the effects of CT upon apparent digestibility and upon plasma irreversible loss (IRL) rates of methionine, cystine and inorganic sulphate, using ^{35}S labelling. Experiment 2 was a field trial with weaned lambs given a restricted allowance of either *lotus corniculatus* (CT-containing) or lucerne (non CT-containing), with or without PEG supplementation. In Experiment 1, CT slightly lowered the apparent digestibility of organic matter (0.77 vs 0.80 ; $P < 0.1$), hemicellulose (0.61 vs 0.67 ; $P = 0.118$) and markedly reduced nitrogen apparent digestibility (0.72 vs 0.80 ; $P < 0.01$), but had no effect upon cellulose digestibility. CT increased the IRL of plasma cystine (13.1 vs 7.0 μmol/min ; $P < 0.05$) and reduced the IRL of plasma inorganic sulphate (36.8 vs 48.1 μmol/min ; $P < 0.01$) but had no effect upon plasma methionine IRL. In Experiment 2, live weight gain and wool growth were similar for sheep grazing lucerne (with and without PEG supplementation) and for sheep grazing lotus with PEG supplementation. In sheep grazing lotus, action of CT increased wool production (12.1 vs 10.9 g/d ; $P < 0.05$) and slightly increased live weight gain (203 vs 188 g/d; $P = 0.07$). These experiments showed that CT in *Lotus corniculatus* increased the amount of cystine available for body synthetic reactions and increased wool growth.

Keywords: condensed tannin; sulphur amino acids; sheep; digestibility & wool growth.

INTRODUCTION

Condensed tannins (CT) are plant secondary compounds that occur in the leaves and stems of specialized plants, such as *Lotus pedunculatus, Lotus corniculatus, Onobrychis viciifolia* (Sainfoin), *Hedysarum coronarium* (sulla) (Jones *et al.*, 1976). Nutritional effects of CT in ruminant animals depend upon the concentration in the forage. High concentrations of CT (50-100 g extractable/kg DM) have depressed voluntary feed intake (VFI) and reduced rumen digestibility of DM, OM and fibre (Barry and Duncan, 1984). However, for the Lotus species, levels of extractable CT in the range 20-40 g/kg DM are thought to be beneficial (Barry, 1989; Waghorn *et al.*, 1987a). CT can react by hydrogen bonding with plant protein to form CT-protein complexes which are stable and insoluble at pH 3.5-7.0, but dissociate and release protein at pH <3.5 (Jones and Mangan, 1977). Thus forages containing CT reduce dietary protein degradation in the rumen and may increase amino acid (AA) supply for absorption in the small intestine. In *Lotus corniculatus*, CT has been demonstrated to reduce dietary protein degradation in the rumen, and to increase AA, especially essential amino acids (EAA), absorption from small intestine (Waghorn *et al.*, 1987b). Objectives of the present study were to evaluate the effects of CT in *Lotus corniculatus* upon nutrient digestion and sulphur amino acid (SAA) metabolism in plasma, and to assess the effect of a low level of CT upon body and wool growth in grazing sheep.

MATERIAL AND METHODS

Experiment 1

Twelve sheep (48.4 kg live weight; SE 1.53) with rumen cannula were held in metabolism crates. Polyethylene glycol (PEG; MW 3,500) was intraruminally infused (50g/day in 240 ml water) to six animals, from day 12-34 of the experimental period. The PEG binds with CT, preventing the CT from binding with protein (Jones and Mangan, 1977) so that the *Lotus corniculatus* (Birdsfoot trefoil ; *cv.* Grasslands Goldie) was essentially CT-free. The remaining six animals did not receive an intraruminal infusion and were the control group.

Fresh lotus was fed hourly from overhead feeders at about 90% of *ad libitum* intake. Refusals were collected at 0800 hours and daily DM intakes measured throughout the experimental period.

Six control sheep and six PEG sheep had catheters installed in both jugular veins on day 15 of the experimental period to infuse ^{35}S - methionine (day 18), ^{35}S-cysteine (day 21) and $^{35}SO_4$ (day 24). Each isotope was infused for 30 hours and blood was sampled from the opposite catheter prior to infusion (background) and after 24, 26, 28, 30 hours of infusion. Plasma samples were used to determine the specific radioactivity (SA) of methionine, cystine and inorganic sulphate using procedures similar to those described by McNabb *et al.*, (1993).

Following the isotope infusions, harnesses were put onto the sheep to collect faeces (day 27-34). Faeces were collected,

[1] AgResearch Grasslands, Palmerston North, New Zealand.

weighed and a sample (10% by weight) was bulked over the 7 day collection period and kept at -20 °C for analysis, and apparent digestibility calculated for the above period.

Experiment 2

Romney male lambs aged 8-10 weeks were used. Ten lambs with live weight of 22.4 kg (SE, 0.36) were weighed, shorn and slaughtered at the beginning of the experiment as the initial slaughter group. The 80 selected lambs with initial live weight of 22.8 kg (SE, 0.50) were divided randomly into two groups of 40 lambs, one to graze lotus and one to graze lucerne swards. Half (20) of the lambs that grazed each forage were selected randomly for PEG treatment (CT inactivated) and the remainder acted as control lambs (CT operating). PEG (MW 3,500) was administrated orally twice daily for 22 weeks, at 08.00 hours and at 18.00 hours, initially at 40 g PEG/lamb daily and gradually increasing to 67 g PEG/lamb at the end of the trial.

Pure swards of *Lotus corniculatus* (Birdsfoot trefoil; *cv.* Grasslands Goldie) and lucerne (*cv.* Grasslands Oranga) were grazed by lambs in the experiment. The feed allowance for both groups of lambs was 2 kg green DM per lamb daily when the trial commenced and this was gradually increased to 2.5-3 kg green DM. Each sward was partitioned into breaks using electric fences and each break was grazed for 2-3 days. Control and PEG lambs grazed together on each sward. Contaminating species were controlled by grubbing and spraying with selective herbicides. Herbage mass and botanical composition were determined weekly. Herbage samples for determination of pre-grazing herbage quality (diet offered) were collected weekly by cutting to ground level. Herbage samples, which were taken corresponding to what animals were observed to be eating and are hence referred to as diet selected, were collected weekly from two caged areas per break immediately after grazing and pooled over two week intervals for chemical composition.

VFI was measured using slow releasing chromium capsules, as described by Parker *et al.*, (1989). Chromium release rates were measured by grazing 6 sheep (3 for control, 3 for PEG drench) with rumen cannula on each sward, and measuring chromium disappearance from capsules suspended in the rumen.

Live weight gain was measured fortnightly. Lambs were shorn 3 weeks prior to the trial conclusion and the growth of clean wool over the experiment period was estimated. The lambs were slaughtered at the end of experiment and hot carcass weight and carcass GR measurement recorded.

Analytical

All analyses of feeds, refusals and faeces were conducted on freeze dried material. Carbohydrate fractions were determined by sequential detergent extraction (Van Soest 1983) and total nitrogen (N) was determined by Kjeldahl digestion. CT were determined as extractable, protein-bound and fibre-bound fractions using the modified butanol-HCl procedure (Terrill *et al.*, 1992a).

The concentrations of methionine, cysteine and cystine were determined by HPLC (Waters Associates, USA), using a reverse phase Pico.Tag column for free amino acids and using the Pico.Tag analytical method for physiological samples (Cohen *et al.*, 1989). The ^{35}S radioactivity in methionine, cysteine and cystine and the concentration and radioactivity of inorganic sulphate were determined as described by McNabb *et al.*, (1993).

Calculation of the data and statistical analysis

The calculation of SA and irreversible loss rate (IRL) in Experiment 1 used methods described by McNabb *et al.*, (1993). DM and OM digestibility were calculated on a PEG free basis, by deducting PEG administrated from faeces output. In Experiment 2 regression equations were established of carcass weight and clean wool weight on live weight using data from the initial slaughter group. The predicted initial carcass and clean wool weights of the 80 experimental animals were then calculated from the regression equations. Both carcass weight gain and wool growth were calculated by deducting predicted initial carcass and clean wool weight from the final ones. VFI was calculated as faecal OM output divided by (1-OMD), where OMD was the *in vitro* OM digestibility of feed selected.

Statistical analysis was done using analysis of variance. Means are presented with their standard error (SEM).

RESULTS

Experiment 1

The DM content, and total N, cellulose, hemicellulose and total CT in DM were 15.7±0.96 (SE), 3.5±0.03, 14.2±0.62, 7.8±0.13 and 3.5±0.18% respectively for the lotus fed. Mean DMI for control and PEG sheep was 987±79.3(SE) and 983±88.2 g DM/d.

PEG intraruminal infusion did not affect methionine IRL ($P>0.05$), but markedly reduced cystine IRL ($P< 0.05$) and increased inorganic sulphate IRL ($P<0.01$; Table 1). The control sheep had lower apparent digestibilities of OM ($P < 0.1$) and total N ($P < 0.01$) than PEG sheep. Hemicellulose digestibility also tended to be lower in control sheep than in PEG sheep ($P = 0.118$). However, there were no treatment effects on the digestibility of cellulose and minerals.

TABLE 1: Experiment 1. The irreversible loss rate (IRL; µmol/min) of cystine, methionine and inorganic sulphate from blood plasma and apparent digestibility of feed components in sheep fed fresh *Lotus corniculatus*, with and without intraruminal infusion of polyethylene glycol (PEG).

	Control sheep	PEG sheep	SEM	Significance of difference
Irreversible loss rate (µmol/min)				
Methionine	16.9	17.0	1.48	NS
Cystine	13.1	7.0	1.44	*
Sulphate	36.8	48.1	2.36	**
Apparent digestibility				
Organic matter	77.4	79.7	0.84	(*)
Total N	72.1	79.5	1.24	**
Hemicellulose	60.5	66.6	2.56	NS
Cellulose	75.6	76.3	1.51	NS
Minerals	69.2	68.4	0.97	NS

NS $P > 0.1$; (*) $P < 0.1$; * $P < 0.05$; ~* $P < 0.01$.

Experiment 2

Over the 22 week experimental period, lotus and lucerne swards had similar amounts of green DM (5.3 vs 5.2 tonne/ha, SEM 0.42; $P > 0.05$) and dead matter (17.2 vs 13.5%; $P > 0.05$). Lotus had a lower leaf/stem ratio (39.9%) than lucerne (47.0%; $P < 0.05$). No contaminating species were detected in either sward. Leaf was absent from post-grazing herbage samples for both swards. Total CT in lotus diet selected was 3.4% of DM, whilst lucerne was essentially CT-free. Lotus contained less N than lucerne (Table 2), but both forages had similar *in vitro* OM digestibility ($P>0.05$).

Lambs grazing lotus had higher live weight gain ($P<0.05$), carcass gain ($P<0.001$) and wool growth ($P < 0.05$) than lambs grazing lucerne (Table 3). PEG supplementation substantially reduced wool growth ($P < 0.05$) and slightly reduced live weight gain ($P=0.07$) without affecting carcass weight gain ($P > 0.05$) in lambs grazing lotus, whilst it had no effect upon any of these measurements in lambs grazing lucerne ($P > 0.05$). Lambs grazing lucerne had a slightly higher VFI than those grazing lotus ($P < 0.05$), but PEG supplementation had no effect on VFI for both swards ($P > 0.05$).

TABLE 2: Experiment 2. Chemical composition of *Lotus corniculatus* and lucerne apparently selected by grazing sheep.

	Lotus	Lucerne	Significance of difference
In vitro OM digestibility (%)	73.5 ± 1.47	76.5 ± 1.41	NS
Total N (%DM)	3.14 ± 0.137	4.18 ± 0.128	**
Total CT (%DM)	3.40 ± 0.215	0.03 ± 0.003	***

NS $P > 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3: Experiment 2. Voluntary feed intake, body growth and wool growth of sheep grazing *Lotus comiculatus* and lucerne with or without twice daily oral PEG administration.

	LOTUS		LUCERNE		SEM
	Control (20)	PEG (20)	Control (20)	PEG (20)	
OMI (kg/day)	1.19	1.20	1.32	1.30	0.056
Live weight gain (g/day)	203^{a}	188^{ab}	185^{b}	178^{b}	5.8
Carcass gain (g/day)	78.7^{a}	75.2^{ab}	67.7^{bc}	62.9^{c}	2.87
Wool growth (g/day)	12.1^{a}	10.9^{b}	10.8^{b}	10.2^{b}	0.39

Data along a row followed by the same letter do not differ at 5% significance level.

DISCUSSION

The most significant finding in this study was that CT in lotus increased plasma cystine IRL and increased wool growth in grazing sheep. The increased cystine IRL in plasma was probably a consequence of CT reducing the breakdown of methionine and cystine in the rumen, as indicated by the reduced plasma IRL of inorganic sulphate in control sheep, and an increased transulphuration of methionine to cystine (McNabb *et al.*, 1993). Cystine is a major component of wool protein and it is a limiting AA for wool growth (Reis, 1979). Because CT increased IRL of cystine in plasma in this study and increased cystine leaving the plasma cystine pool to be used for body synthetic reactions (McNabb *et al.*, 1993), forages containing low concentrations of CT should be beneficial for increasing wool growth. The field trial confirmed this hypotheses, and supports observations of increased wool growth due to CT in sheep grazing sulla (*Hedysarum coronarium ;* Terrill *et al.*, 1992b).

Effect of PEG on nutrient digestion suggested that the CT level in the present study (3.5% DM) markedly reduced protein apparent digestibility in the whole digestive tract. Although there is evidence that CT reduced protein degradation in rumen, the effect of CT on protein digestion in the post-rumen tract is unknown. Research is needed in this area. Experiments with a lower level of CT should be conducted to search for the optimum level of CT in forage to improve the efficiency of protein digestion, and further experiments are needed with grazing sheep in other physiological states to further define production responses to dietary CT.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R.W. Purchas for measuring animal carcass, Miss R. A. Watson, Mrs. M. Hendriks, Mr. J. A. Anderson (Department of Animal Science, Massey University) and Mr. H. B. Toes (Department of Soil Science, Massey University) for their skilled technical assistance, Mr. S. Rutherfurd and Mr. H. Voon of the Monogastric Research Centre (Massey University) for their guidance and advice with HPLC procedures, Assoc. prof. R. W. Tillman (Soil Science Department, Massey University) for his help and advice with scintillation counting, Mr. G. S. Purchas for his help with harvesting lotus plants, Mr. I.D. Shelton and Mr.A.G. Foote (AgResearch, Grasslands) for their help in management of animals and pasture.

REFERENCES

Barry, T.N. and Duncan, S.J. 1984, The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. *British Journal of Nutrition.* **51:** 484 - 491.

Barry, T.N. 1989, Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecology. *In* The role of protozoa & fungi in ruminant digestion. Edited by J.V. Nolan, R.A. Leng & D.I. Deneyer. pp 153 -169. Armidale, Australia. Pernambul Books.

Cohen, S.A., Meys, M. and Tarvin, T.L. 1989, *The pico. tag method. A manule of advanced techniques for amino acid analysis.* Millipore Corporation, USA.

Jones, W.T. and Mangan, J.L. 1977, Complexes of the condensed tannins of Sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture.* **28:** 126 -136.

Jones, W.T., Broadhurst, R.B. and Lyttleton, J.W. 1976, The condensed tannins of pasture legume species. *Phytochemistry.* **15:** 1047 - 1049.

McNabb, W.C., Waghorn, G.C., Barry, T.N. and Shelton, I.D. 1993, The effect of condensed tannins in *Lotus pedunculatus* upon the digestion and metabolism of methionine, cystine and inorganic sulphate in sheep. *British Journal of Nutrition.* **70:** 647 - 661.

Parker, W.J., McCutcheon, S.N. and Carr, D.H. 1989, Effect of herbage type and level of intake on the release of chromic oxide from intra

- ruminal controlled release capsules in sheep. *New Zealand Journal of Agricultural Research.* **32:** 537 - 546.

Reis, P.J. 1979, Effects of amino acids on the growth and properties of wool. *In Physiological and environmental limitations to wool growth.* P 223 - 242. Edited J.L. Black and P.J.Reis. Australia, University of New England Publishing Unit.

Terrill, T.H., Rowan,A.M., Douglas, G.B. and Barry, T.N. 1992a, Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of the Science of Food and Agriculture.* **58:** 321 - 329.

Terrill, T.H., Douglas, G.B., Foote, A.G., Purchas, R.W., Wilson, G.F. and Barry, T.N. 1992b, The effect of condensed tannins upon body growth, wool growth & rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *Journal of Agricultural Science,* Cambridge. **119:** 265-273.

Van Soest, P.J. 1983, *Nutritional Ecology of the Ruminant.* O & B Books, Inc.

Waghorn, G.C., John, A., Jones, W.T. and Shelton, I.D. 1987a, Nutritive value of *Lotus corniculatus L.* containing low and medium concentrations of condensed tannins for sheep. *Proceedings of the New Zealand Society of Animal Production.* **47:** 25 - 30.

Waghorn, G.C., Ulyatt, M.J., John, A. and Fisher, M.T. 1987b, The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus. British Journal of Nutrition.* **57:** 115 - 126.