

The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep

2. Quantitative digestion of carbohydrates and proteins

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1. Primary growth vegetative *Lotus pedunculatus* containing 46 and 106 g/kg dry matter (DM) of total condensed tannin and 3 and 14 g/kg DM of free condensed tannin, was cut and fed fresh at hourly intervals (750 g DM/d) to sheep fitted with permanent cannulas into the rumen and duodenum. Low- and high-tannin lotus contained respectively 41.3 and 31.6 g total nitrogen/kg DM and 132 and 152 g lignin/kg DM. The two forms of lotus were similar in carbohydrate composition.

2. Nutrient intake was recorded and faecal output measured by direct collection. Digesta flow to the duodenum was estimated by measuring dilution at the duodenum of inert ruthenium phenanthroline (Ru-P) and chromium-EDTA markers continuously infused into the rumen.

3. Effects attributable to condensed tannins were assessed by comparing the digestion of the two diets, and by comparing the digestion of each with predicted values for non-tannin-containing fresh forages fed at similar intakes.

4. Apparent digestibility of all nutrients measured was less for high- than for low-tannin lotus ($P < 0.01$). The levels of cellulose digested ruminally and post-ruminally in both forms of lotus were similar to predicted values. However, less hemicellulose and readily fermentable carbohydrate (RFC; soluble carbohydrate + pectin) was digested in the rumen in sheep given both forms of lotus than would be predicted for non-tannin-containing fresh forage diets, but this was compensated for by greater post-ruminal absorption of both nutrients.

5. Total N gains across the rumen (duodenal N flow – total N intake) were 1.8 and 10.5 g/d for low- and high-tannin lotus *v.* predicted losses of 3.7 and 2.1 g/d for non-tannin-containing fresh forages given at the same total N intakes. Post-ruminal digestion of non-ammonia-N (NAN; proportion NAN flowing at duodenum) was 0.71 and 0.67 for low- and high-tannin lotus respectively *v.* 0.76 for comparable non-tannin-containing fresh forages. Energy absorbed as amino acids from the small intestine was calculated to be 0.29 of metabolizable energy for both forms of lotus, compared with 0.17 and 0.21 for perennial ryegrass and white clover.

6. It was concluded that the presence of condensed tannins in lotus markedly increased post-ruminal NAN absorption compared with non-tannin-containing fresh forage diets, but depressed ruminal digestion of RFC and hemicellulose.

A recent review concluded that with growing and lactating ruminants consuming fresh forage diets *ad lib.*, protein absorption from the small intestine as a proportion of metabolizable energy (ME) intake was likely to be less than animal requirements (Barry, 1982). Sulphur-containing amino acids were considered to be first limiting, and a major cause of the protein deficit was the high degradation of forage proteins in the rumen (0.7). Under anaerobic conditions, condensed tannins present in certain legumes react with plant proteins by hydrogen bonding when the plant material is disintegrated, such as during chewing by animals (McLeod, 1974). Stability of the condensed tannin-protein complex is strongly pH dependent (Jones & Mangan, 1977), being insoluble and stable in the pH range 3.5–7.0 but dissociating and releasing soluble protein at pH < 3.0 and at pH 8.5. The presence of condensed tannins in fresh forages should therefore theoretically reduce plant protein degradation rates in the rumen (pH 5.8–6.8), yet allow their solubilization and release in the abomasum (pH 2.5–3.5) and small intestine (pH 7.5–8.5). John & Lancashire (1981) confirmed this for *Lotus corniculatus* cultivars containing respectively 2.5 and 14.5 g condensed tannin/kg dry matter (DM), with protein flow at the duodenum being 0.2 of total nitrogen intake higher for the legume containing the greater concentration of tannin.

However, the condensed tannin content of *Lotus pedunculatus* grown at this Centre under high and low soil fertility conditions is 46 and 106 g/kg DM (Barry & Duncan, 1984). The presence of condensed tannins in fresh *Lotus pedunculatus* at 62 g/kg DM was associated with depressions in the apparent digestibility of structural carbohydrate, in particular that of hemicellulose (Barry & Duncan, 1984). The objective of this investigation was to study the effect of condensed tannins upon the partition of protein and carbohydrate digestion between the stomach and intestines in sheep fed on fresh *Lotus pedunculatus*. This has been done through comparing the digestion of lotus containing low (46 g/kg DM) and high (106 g/kg DM) concentrations of condensed tannin, and by comparing the digestion of both with predicted values for non-tannin-containing fresh forages fed at the same intake.

EXPERIMENTAL

Diets

Lotus pedunculatus (cv. Grasslands 'Maku') containing low and high concentrations of condensed tannins was produced by growing the plant under high and low soil fertility conditions respectively (Barry & Duncan, 1984). Primary vegetative growth of both low- and high-tannin lotus was used, with no flower or bud development. Primary growth produced under high soil fertility conditions (Invermay Research Centre) was ready some 5 weeks earlier than lotus grown under low soil fertility conditions (Waiora Hill Country Farm).

Lotus was cut each day at 08.30 hours, any contaminating grasses and weed species were removed, and the lotus then chopped into 50–100 mm lengths using a revolving knife cutter (i.e. chaff cutter). Chopped lotus was then placed upon belt feeders, which delivered the day's ration in twenty-four feeds each 1 h apart, commencing at 10.00 hours. Sheep fed on low-tannin lotus were offered 700 g DM/d and those fed on high-tannin lotus were offered 775 g DM/d. All feed offered was consumed. Rapid DM determinations were performed each morning and quantities of fresh food offered so that DM intakes were maintained constant at the above levels.

Animals and marker infusion procedures

Castrated male Romney sheep approximately 14 months old and with a mean initial weight of 37 kg (SD 8.5) were used. All were fitted with a 25 mm i.d. rubber cannula into the rumen and a simple T-piece cannula in the proximal duodenum (i.d. of barrel 15 mm). After a settling in period following surgery the sheep were accustomed to the belt-feeding sequence for a pre-feeding period of 10 d, after which 50 ml of indigestible marker solution was injected into the rumen and the marker solution then continuously infused into the rumen at 250 ml/d for an 8 d period. The first 4 d were allowed for equilibration and samples were taken at intervals from the duodenal cannulas over the next 4 d. After a 2 d rest period, the sheep were equipped with harnesses and faeces collected for 7 d. Five sheep were used in the determinations with low-tannin lotus, whilst three sheep were used with high-tannin lotus.

The marker solution comprised the inert forms of ruthenium-phenanthroline (Ru-P) (Tan *et al.* 1971) and of chromium-EDTA (Binnerts *et al.* 1968). The two solutions were made up separately, mixed and the pH adjusted to 6.5, and diluted to give final theoretical concentrations of 47.7 mg Ru/l and 2000 mg Cr/l. Quantities infused were therefore 11.9 mg Ru/d and 500 mg Cr/d.

Sample collection procedures

Samples of feeds and any residue were taken daily, pooled at -20° and a pooled sample kept for each week. Duodenal samples (170 g/animal), to be used to form a matrix for the

preparation of Ru and Cr standard curves, were taken once daily from each sheep during the last 3 d of the prefeeding period with low-tannin lotus and pooled at -20° . During the last 4 d of the marker infusion period, duodenal samples were collected from each sheep at 11.00, 13.30 and 15.30 hours, with the samples being pooled for each animal and stored at 4° . Each sample was then thoroughly mixed, divided in half, and one-half designated whole digesta (WD) and stored at -20° . The remaining half was centrifuged at 1700 g for 20 min, the supernatant discarded, and the residue designated centrifuged digesta (CD) and stored at -20° . Sub-samples of collected faeces were also stored at -20° . All deep-frozen samples of food, residues, duodenal contents and faeces were then freeze-dried, ground and stored ready for laboratory analysis.

Laboratory methods

Ru and Cr concentrations in freeze-dried WD and CD were determined by X-ray fluorescence spectrometry (Evans *et al.* 1977). A Phillips PW 1410/20 AHP instrument was used, with a 3 kW tungsten tube as the X-ray source, operated at 60 kV and 30 mA. A lithium fluoride 200 analysing crystal was also used.

Standard curves for Ru and Cr were prepared by adding graded quantities of the original marker solution to duodenal digesta collected during the prefeeding period, after which these preparations were freeze-dried. Preparation of standards in this manner avoided making any adjustments in the data for changes in the mass absorption coefficient, as use of the same matrix in the standards as in the samples eliminated this problem. Duodenal digesta from sheep fed on low- and high-tannin lotus were analysed for Ru and Cr in the same batch.

Food, residues, WD, CD and faeces were analysed for carbohydrate constituents (Bailey, 1967), total N by the Kjeldahl method, gross energy by adiabatic bomb calorimetry and organic matter by ashing overnight at 550° . Ammonia in Kjeldahl digests and in water extracts of WD, CD and faeces was determined by an automated colorimetric method (Technicon, 1974). Total and free condensed tannins were determined as described by Barry & Forss (1983), free tannin being described as that present which could not be bound by finely divided mascerates of the plant.

Calculation of duodenal digesta flow and comparison with published results

Duodenal flow of nutrients was calculated using the double marker procedure of Faichney (1975a). The Cr-EDTA marker mixes with the liquid phase and the Ru-P marker is associated with particulate matter. Corrections were made for any non-ideal behaviour and for any sampling errors by mathematically reconstituting true whole digesta. The infusion rate of Cr-EDTA was reduced by 0.03 to allow for its small absorption from the reticulo-rumen (Faichney, 1975b).

Comparisons of duodenal flows, and ruminal and post-ruminal digestion, with published results for non-tannin-containing fresh forage diets were made using the equations developed by Ulyatt & Egan (1979). For nutrients where equations were not available, comparisons were made using pure white-clover diets (MacRae & Ulyatt, 1974; Ulyatt & MacRae, 1974). Further comparisons were made using the digestion of forage kale (*Brassica oleracea*), where the marker infusion and analytical procedures were identical to those used in the present paper (Barry *et al.* 1984).

Statistical methods

Comparisons in the digestion of low- and high-tannin lotus were made using one way analysis of variance. The comparison was confounded with time, which was unavoidable, but this was minimized by the analyses for individual nutrients and markers in samples from

Table 1. *Quantities of lignin eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep given Lotus pedunculatus differing in condensed tannin content, together with lignin apparent digestibility*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

Lignin flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Lignin:				
Eaten	90.9	0.56	118.9	0.72
Duodenum	83.3	3.64	118.1	4.70
Faeces	74.1	2.31	111.9	2.98
Apparent digestibility:				
Proportion of intake	0.18	0.024	0.06	0.031

sheep given low- and high-tannin lotus being performed in the same batch and at the same time.

RESULTS

Chemical composition of diet

Low- and high-tannin lotus contained respectively 46 and 106 g/kg DM of total condensed tannin and 3 and 14 g/kg DM of free condensed tannin. Total N contents were 41.3 and 31.6 g/kg DM for low- and high-tannin lotus respectively, whilst corresponding lignin contents were 132 and 152 g/kg DM. Further analyses are given in Expt 1 of Barry & Duncan (1984), but the two forms of lotus were essentially similar in carbohydrate composition.

Lignin digestion

In sheep fed on low-tannin lotus, 0.18 of the lignin ingested was digested in the whole digestive tract (Table 1) and this value was significantly greater than zero ($P < 0.01$). The mean apparent digestibility of 0.06 for the lignin present in high-tannin lotus was lower ($P < 0.01$) and not significantly different from zero ($P > 0.05$). Approximately 0.45 of the lignin digested by sheep fed on the low-tannin lotus was digested in the reticulo-rumen.

Cellulose digestion

Cellulose present in low-tannin lotus was digested to a greater extent in the whole digestive tract than cellulose present in high-tannin lotus ($P < 0.001$; Table 2). For both diets, the relative proportions of cellulose digested in the reticulo-rumen and post-ruminal regions of the digestive system were practically identical to those predicted for non-tannin-containing fresh forage diets (Table 2).

Digestion of hemicellulose and of readily fermentable carbohydrate

Hemicellulose apparent digestibility was also much lower for high-tannin than for low-tannin lotus ($P < 0.001$; Table 3). The proportion of ingested hemicellulose digested in the reticulo-rumen was much less than predicted for non-tannin-containing fresh forage diets, with the extent of depression being greater for high-tannin lotus (0.21) than for low-tannin lotus (0.14). However, for both forms of lotus the proportion of ingested hemicellulose digested in the post-ruminal region of the digestive system was considerably greater than predicted for conventional fresh forage diets (Table 3).

Table 2. *Quantities of cellulose eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on Lotus pedunculatus differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

For comparison, predicted values calculated by inserting apparent digestibility determined in the present study into the prediction equations of Ulyatt & Egan (1979), derived with non-tannin-containing fresh forage diets (n 55, r 0.96 for cellulose), are shown in parentheses

Cellulose flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Cellulose (g/d):				
Eaten	101.7	0.69	105.1	0.89
Duodenum	31.2	1.99	49.0	2.57
Faeces	22.3	0.65	39.0	0.84
Apparent digestibility:				
Proportion of intake	0.78	0.005	0.63	0.007
Ruminal digestion:				
Proportion of intake	0.69 (0.69)	0.019 (0.024)	0.53 (0.54)	0.025 (0.031)
Proportion of total digested	0.89	0.026	0.85	0.034
Post-ruminal digestion:				
Proportion of intake	0.09 (0.09)	0.019	0.10 (0.09)	0.025

Table 3. *Quantities of hemicellulose eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on Lotus pedunculatus differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

For comparison, predicted values calculated by inserting apparent digestibility determined in the present study into the prediction equations of Ulyatt & Egan (1979), derived with non-tannin-containing fresh forage diets (n 35, r 0.88 for hemicellulose), are shown in parentheses

Hemicellulose flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Hemicellulose (g/d):				
Eaten	59.1	0.34	68.8	0.44
Duodenum	32.9	2.00	54.4	2.59
Faeces	16.1	0.40	30.5	0.52
Apparent digestibility:				
Proportion of intake	0.73	0.006	0.56	0.008
Ruminal digestion:				
Proportion of intake	0.44 (0.58)	0.031 (0.033)	0.21 (0.42)	0.40 (0.044)
Proportion of total digested	0.61	0.042	0.38	0.054
Post-ruminal digestion:				
Proportion of intake	0.28 (0.15)	0.029	0.35 (0.14)	0.037

Table 4. *Quantities of readily fermentable carbohydrate eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on Lotus pedunculatus differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

For comparison, values calculated from Ulyatt & MacRae (1974) for sheep fed on fresh white clover and fresh forage kale (Barry *et al.* 1984) are shown in parentheses

Readily fermentable carbohydrate flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Readily fermentable carbohydrate (g/d):				
Eaten	127.8	0.50	143.9	0.64
Duodenum	26.1	1.47	31.8	1.90
Faeces	6.7	0.24	9.4	0.31
Apparent digestibility:				
Proportion of intake	0.95	0.002	0.93	0.002
Ruminal digestion:				
Proportion of intake	0.80 (0.93)	0.011	0.78 (0.93)	0.014
Proportion of total digested	0.84	0.011	0.83	0.015
Post-ruminal digestion:				
Proportion of intake	0.15 (0.06)	0.011	0.16 (0.06)	0.014

Readily fermentable carbohydrate (RFC), defined as water-soluble sugars + pectin, was almost completely digested in the whole digestive tract of sheep given either form of lotus (Table 4). For both diets the proportion of ingested RFC that was digested in the reticulo-rumen (mean 0.79) was less than that found for diets of fresh white clover and fresh forage kale given to sheep (0.93; Table 4), but this was almost entirely compensated for by greater post-ruminal digestion.

N digestion

Due to the higher dietary N concentration, total N intake was higher for sheep fed on low-tannin than high-tannin lotus (Table 5). Sheep fed on low-tannin lotus showed a net gain of 1.8 g total N/d (zero NAN) across the rumen (duodenal N flow – total N intake), against a predicted loss of 3.7 g total N/d for non-tannin-containing fresh forage diets fed at the same level of N intake. Sheep fed on high-tannin lotus showed a net gain of 10.5 g total N/d (8.6 g NAN/d) across the rumen, compared with a predicted loss of 2.1 g total N/d at the same level of N intake.

Post-ruminal digestion of NAN (proportion NAN flowing at duodenum per day) was slightly less for high-tannin (0.67) than for low-tannin lotus (0.71; Table 5), with both figures being slightly lower than published values for fresh white clover and forage kale diets (0.76). Consequently, despite the lower total N intake in sheep fed on high-tannin lotus, post-ruminal absorption of NAN (g/d and g/MJ ME) did not differ between sheep given the two forms of lotus ($P > 0.05$). However, when expressed as g NAN absorbed post-ruminally/g N intake, values were considerably higher for sheep fed on high-tannin than on low-tannin lotus ($P < 0.01$).

Table 5. Quantities of nitrogen eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on *Lotus pedunculatus* differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

For comparison, the following values are shown in parentheses: ^a predicted values calculated by inserting total N intake determined in the present study into the prediction equations of Ulyatt & Egan (1979), derived with non-tannin-containing fresh forage diets (n 30, r 0.91); ^b values calculated from MacRae & Ulyatt (1974) for sheep fed on fresh white clover and for sheep fed on fresh forage kale (Barry *et al.* 1984)

Nitrogen flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Nitrogen (g/d):				
Eaten: Total N	28.7	0.12	24.8	0.15
Duodenum: Total N	30.5 (25.0) ^a	0.89 (1.39) ^a	35.3 (22.7) ^a	1.15 (1.74) ^a
Duodenum: NAN*	28.7	0.82	33.4	1.06
Faeces: NAN	8.3	0.44	11.0	0.57
Apparent digestibility:				
Proportion of intake	0.71	0.017	0.56	0.021
N gain across rumen†:				
g/d	0	0.32	8.7	0.41
Proportion of N intake	-0.14	0.012	0.35	0.015
NAN apparently digested in post-ruminal region:				
g/d	20.4	0.93	22.5	1.20
Proportion of NAN arriving at duodenum	0.71 (0.76) ^b	0.017	0.67 (0.76) ^b	0.022
g/g total N intake	0.71	0.033	0.91	0.042
g/MJ metabolizable energy intake	2.7	0.12	2.8	0.15

* Non-ammonia N.

† NAN flow at duodenum - total N intake.

Digestion of organic matter and of energy

Apparent digestibility of organic matter (Table 6) and of energy (Table 7) were less ($P < 0.01$) for high- than for low-tannin lotus. For both diets, the proportion of organic matter eaten that was digested in the rumen was 0.12 less than predicted values for non-tannin-containing fresh forage diets, whilst post-ruminal digested organic matter was increased by a corresponding proportion.

DISCUSSION

The accuracy of digesta flow measurements such as those used here depend upon accurate analyses of indigestible markers and upon valid flow calculation procedures. The validity of our procedures is shown by duodenal lignin flow (Table 1) being within the very narrow difference between lignin intake and faecal excretion in sheep fed on high-tannin lotus, and that the sites of cellulose digestion were exactly as predicted from an equation derived from feeding fifty-five diets (Ulyatt & Egan, 1979). Differences in the digestion of RFC, hemicellulose and N between those observed in this investigation with lotus and those predicted for non-tannin-containing fresh forages therefore represent true differences,

Table 6. *Quantities of organic matter eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on Lotus pedunculatus differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

For comparison, predicted values calculated by inserting apparent digestibility determined in the present study into the prediction equations of Ulyatt & Egan (1979), derived with non-tannin-containing fresh forage diets (n 74; r 0.84 for organic matter), are shown in parentheses

Organic matter flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Organic matter (g/d):				
Eaten	623.2	3.13	731.4	4.04
Duodenum	417.9	11.85	523.8	15.30
Faeces	168.4	5.29	251.7	6.83
Apparent digestibility:				
Proportion of intake	0.73	0.008	0.66	0.010
Ruminal digestion:				
Proportion of intake	0.33 (0.45)	0.019 (0.022)	0.28 (0.40)	0.024 (0.028)
Proportion of total digested	0.45	0.026	0.43	0.034
Post-ruminal digestion:				
Proportion of intake	0.40 (0.28)	0.020	0.37 (0.26)	0.025

Table 7. *Energy eaten, flowing past the duodenum and excreted in the faeces (MJ/d) in sheep fed on Lotus pedunculatus differing in condensed tannin content, together with the partition of digestion into that digested in the reticulo-rumen and post-ruminal regions of the digestive tract*

(Mean values with their standard errors for five animals fed on low-tannin lotus and three animals fed on high-tannin lotus)

Energy flow and digestion	Low-tannin lotus		High-tannin lotus	
	Mean	SEM	Mean	SEM
Energy (MJ/d):				
Eaten	13.2	0.07	15.5	0.09
Duodenum	9.6	0.34	12.1	0.44
Faeces	4.0	0.13	5.8	0.17
Apparent digestibility:				
Proportion of intake	0.70	0.009	0.63	0.012
Ruminal digestion:				
Proportion of intake	0.28	0.026	0.22	0.034
Proportion of total digested	0.39	0.038	0.35	0.049
Post-ruminal digestion:				
Proportion of intake	0.43	0.027	0.41	0.035

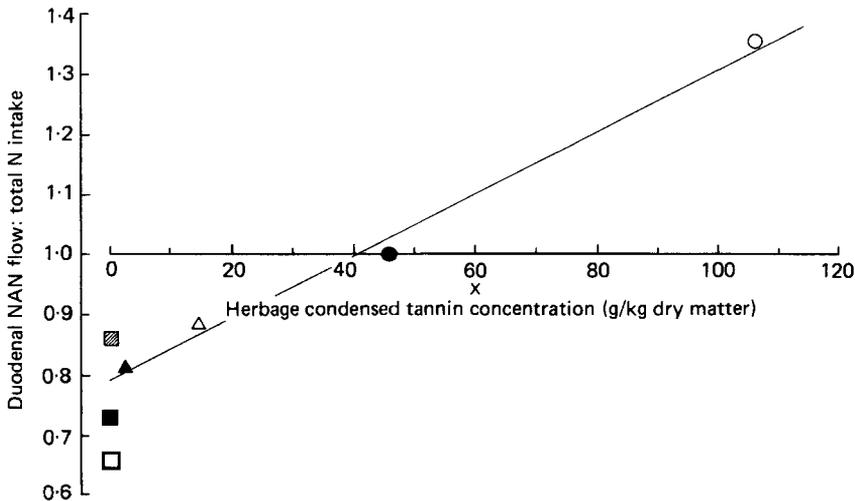


Fig. 1. Duodenal non-ammonia nitrogen (NAN) flow per unit total N intake as a function of herbage condensed tannin concentration in sheep fed on *Lotus* species. (○) high- and (●) low-tannin *Lotus pedunculatus* fed in the present investigation. (△) High- and (▲) low-tannin *Lotus corniculatus* from John & Lancashire (1981).

Results are compared with the non-tannin-containing herbage (▨) short rotation ryegrass, (□) perennial ryegrass, and (■) white clover from MacRae & Ulyatt (1974), and with (×) sainfoin from Ulyatt & Egan (1979). All results are for an N intake of 28 g/d and refer to fresh forages.

probably due to effects of condensed tannins. Results have been collated in Fig. 1 for the digestion of N in a range of fresh New Zealand forages given to sheep at similar levels of N intake. Duodenal NAN flow per unit total N intake (D) has been plotted as a function of herbage condensed tannin concentration (C). When results from the present investigation were combined with those of John & Lancashire (1981) for low- and high-tannin *Lotus corniculatus*, there was a significant linear relationship as expressed by eqn (1).

$$D = 0.79 \text{ (SE } 0.016) + 0.0052 C \text{ (SE } 0.00027) \quad r \text{ } 0.997^* \quad (1)$$

Thus each 20 g/kg DM increase in C was associated with a 0.1 increase in D , and D was predicted to be unity (i.e. no loss of N across the rumen) when the condensed tannin concentration was 40.8 g/kg DM. At a given herbage tannin concentration, D was less for sainfoin (*Onobrychis viciifolia*) than for lotus (Fig. 1). Whilst there were differences in D between the non-tannin-containing forages short rotation ryegrass, perennial ryegrass and white clover, as noted by MacRae & Ulyatt (1974), it is apparent from Fig. 1 that these effects are small compared with those produced by condensed tannins. These results suggest that condensed tannins in lotus reduce rumen degradation of fresh forage proteins, and reinforce the conclusions of Barry & Forss (1983) that high concentrations of lotus condensed tannins are necessary to reduce deamination by mixed rumen micro-organisms. The present results are most unusual for forage diets of high apparent digestibility and N content, in that gains of N across the rumen are normally only found in forages low in both these criteria (Hogan *et al.* 1970; Egan, 1974; Elliot & Armstrong, 1982).

The high digestibility of NAN in the intestines of sheep given both forms of lotus was surprising, in view of the fact that pre-treating proteins with high rates of formaldehyde to reduce rumen degradation leads to substantial reductions in post-ruminal digestion expressed as a proportion of NAN arriving at the duodenum (Beever *et al.* 1977). First, the difference may be that formaldehyde undergoes a fixed chemical bonding with ϵ -amino groups of lysine (Walker, 1964), whereas the reaction between proteins and condensed

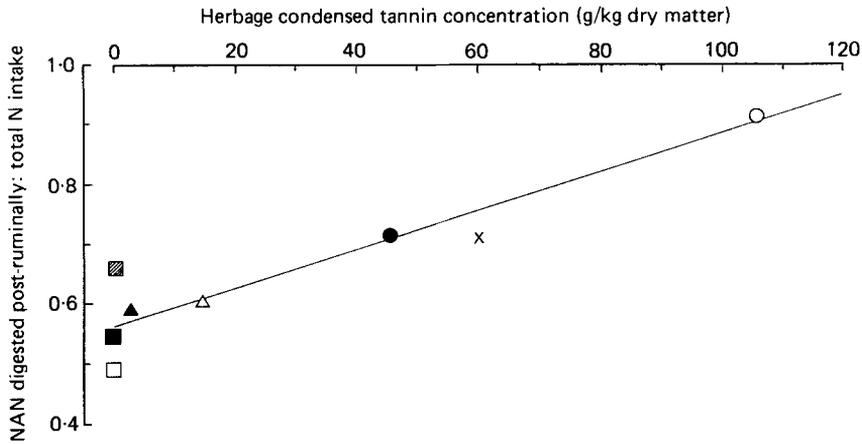


Fig. 2. Non-ammonia nitrogen (NAN) digested post-ruminally per unit total N intake as a function of herbage condensed tannin concentration in sheep fed on *Lotus* species. (○) High- and (●) low-tannin *Lotus pedunculatus* fed in the present investigation. (△) High- and (▲) low-tannin *Lotus corniculatus* from John & Lancashire (1981).

Results are compared with the non-tannin-containing herbage (▨) short rotation ryegrass, (□) perennial ryegrass and (■) white clover from MacRae & Ulyatt (1974), and with (×) sainfoin from Ulyatt & Egan (1979). All results are for an N intake of 28 g/d.

tannins under anaerobic conditions is one of loose hydrogen bonding (McLeod, 1974). Second, the insoluble complex between formaldehyde and protein could only solubilize at the low pH in the abomasum (2.5–3.5), whereas complexes between condensed tannins and protein are soluble at both low pH (< 3.5) and at high pH (8.5), giving the opportunity for dissociation and solubilization in both the abomasum and small intestine (Jones & Mangan, 1977).

Post-ruminally digested NAN per unit of N intake (*PR*) has been plotted against herbage condensed tannin concentration (*C*) in Fig. 2, using the same diets shown in Fig. 1. Again, combining the present results with the *Lotus corniculatus* results of John & Lancashire (1981) yielded the following significant ($P < 0.05$) linear relationship:

$$PR = 0.57 \text{ (SE } 0.008) + 0.0032 C \text{ (SE } 0.00015) \quad r = 0.998^* \quad (2)$$

Even after allowing for differences in post-ruminal digestibility of NAN in this manner, the condensed tannins in lotus were still more efficient than those in sainfoin for increasing amino acid supply. Also, herbage containing at least 35 g/kg DM of condensed tannins were more efficient suppliers of protein than the non-tannin-containing fresh forages, with *PR* predicted to be unity at a condensed tannin concentration of 139.4 g/kg DM. Use of the species containing condensed tannins is therefore likely to remove any constraint of protein supply upon the growth of young ruminants grazing fresh forage (Barry, 1981) and could also partially overcome the protein deficiency which occurs in lactating ruminants fed on fresh forages *ad lib.* (Barry, 1982).

The presence of condensed tannins was associated with depressed ruminal digestion of RFC and hemicellulose in the present investigation. Since dietary addition of polyethylene glycol (PEG; MW 3350) to bind the tannins increased both hemicellulose apparent digestibility and voluntary intake of *Lotus pedunculatus* (Barry & Duncan, 1984), it seems that both could have a common cause. Free tannin content is suggested as the most probable

cause, being defined as condensed tannin present in the plant above the capacity of the masticated plant to bind it. Free condensed tannins are readily water soluble, are highly reactive and are known to precipitate microbial enzymes (McLeod, 1974). It is probable that free condensed tannins depressed ruminal digestion of RFC and hemicellulose in the present study through precipitating the extra-cellular microbial enzymes that form a first step in the ruminal degradation of these moieties.

However, presence of condensed tannins in sainfoin at 60 g/kg DM did not impair rumen digestion of carbohydrates, as judged by the values being similar to those obtained for perennial ryegrass and white clover (Ulyatt & Egan, 1979). The condensed tannins present in sainfoin are of higher molecular weight (MW; 22000) and lower astringency (protein precipitated/unit weight of tannin) than condensed tannins present in *Lotus* species (MW 7700) (Jones *et al.* 1976). These differences in MW and reactivity with proteins therefore explain the differences obtained in nutritional studies, with the low MW lotus tannins being efficient at precipitating herbage proteins but also inactivating enzymes involved in rumen carbohydrate digestion. Conversely, the larger MW sainfoin tannins appear to be less efficient at precipitating herbage proteins but do not appear to inactivate rumen carbohydrate-degrading enzymes. Optimum condensed tannin concentration for the digestion of fresh legume diets by ruminants is therefore likely to be critically dependent upon the MW and reactivity of the condensed tannins present in the plant cultivar being evaluated.

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