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

## *4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration

BY T. N. BARRY†, T. R. MANLEY AND S. J. DUNCAN

*Invermay Agricultural Research Centre, Mosgiel, New Zealand*

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1. Vegetative secondary growth *Lotus pedunculatus* was cut daily, and fed fresh at hourly intervals (600 g dry matter (DM)/d) to three groups each of three sheep fitted with permanent cannulas into the rumen and duodenum. Lotus fed to two of the groups was sprayed with low and high rates of polyethylene glycol (PEG; molecular weight 3350), which specifically binds the condensed tannins (CT). Nutrient intake and faecal excretion were measured directly, duodenal flows estimated from continuous intraruminal infusion of inert ruthenium phenanthroline (Ru-P) and CrEDTA markers, and rumen pool sizes measured at slaughter.

2. Dietary concentrations of total reactive CT (i.e. that not bound to PEG) were 95, 45 and 14 g/kg DM, whilst the corresponding values for free CT were 15, 5 and 2 g/kg DM.

3. Increasing dietary reactive CT concentration linearly increased duodenal flows of non-ammonia nitrogen, but linearly decreased the apparent digestibility of energy and organic matter, and rumen digestion of hemicellulose but not of cellulose. Rumen digestion as a proportion of total digestion was increased by the higher PEG rate for organic matter, energy, pectin and lignin.

4. High dietary CT concentration was associated with increased N retention. Rumen ammonia concentration and pool size showed only a slight decline on this diet, indicating that there must have been increased recycling of N into the rumen.

5. Increasing dietary reactive CT concentration had no effect on the rate at which carbohydrate constituents were degraded in the rumen per unit time (FDR), but increased the rate at which their undegraded residues (FOR) left the rumen per unit time. The latter appeared to be the principal mechanism by which rumen digestion as a proportion of total digestion was reduced at high dietary CT concentrations. From a comparison of FDR and FOR of carbohydrate components in lotus and *Brassica oleracea* diets, it was concluded that hemicellulose digestion was rate-limiting for rumen cell-wall digestion, probably due to bonding with lignin. However, the considerable post-rumen digestion of hemicellulose was not associated with post-rumen lignin digestion.

6. It was concluded that a desired concentration of CT in *Lotus* sp. should represent a balance between the positive effect of CT in improving the efficiency of N digestion and their negative effect in depressing rumen carbohydrate digestion. A recommended concentration is 30–40 g/kg DM.

From measurements of duodenal non-ammonia nitrogen (NAN) flow it has been estimated that the absorption of essential amino acids was limiting the output of high producing ruminants consuming fresh forages *ad lib.*, and this has been verified through post-rumen supplementation studies with protein (Barry 1981, 1982; Beever & Siddons, 1986). From a review of New Zealand (NZ) literature, Barry & Reid (1986) concluded that the presence of condensed tannins (CT) uniformly distributed throughout leaf and stem tissue in forage plants would increase amino acid supply through CT reacting with plant proteins by reversible hydrogen bonding; the protein–CT complex was insoluble at rumen pH, but was soluble and released protein at the low pH of the abomasum and the high pH of the small intestine. Such reduction in plant protein solubility in the rumen would also eliminate the disorder 'frothy bloat'.

Barry & Manley (1984) concluded that CT in *Lotus* sp. (molecular weight 7700) increased

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† Present address: Department of Animal Science, Massey University, Palmerston North, New Zealand.

amino acid supply in a dose-dependent manner over the range 3–106 g/kg dry matter (DM). However, high CT concentrations also depressed rumen carbohydrate (CHO) digestion, and were associated with depressions in voluntary intake (Barry & Duncan, 1984). The objective of the present investigation was therefore to define a concentration of CT in *Lotus* sp. that would reduce degradation of protein in the rumen, whilst causing little or no reduction in rumen CHO digestion. This was investigated through feeding *Lotus pedunculatus* high in CT, and progressively reducing reactive CT concentration through spraying the forage with polyethylene glycol (PEG), using the principle that CT will bind to PEG in preference to protein (Barry & Forss, 1983), with the CT–PEG complex assumed to be inert and to be indigestible in the animal.

## EXPERIMENTAL

*Lotus pedunculatus* containing three concentrations of reactive CT was fed under steady state conditions to groups of three sheep, and intake, rumen pool size, duodenal flow and faecal excretion of nutrients measured, together with N retention.

### *Diets*

*Lotus pedunculatus* (*cv* Grasslands 'Maku') containing a high concentration of CT (95 g/kg DM) was produced by growing the plant in a low fertility acid soil, as described by Barry & Duncan (1984). Vegetative secondary growth was used, with no flower or bud formation.

Lotus was cut each day at 08.30 hours, any contaminating grasses and weed species removed, and the lotus then chopped into 50–100 mm lengths using a revolving knife cutter. Each day's feed was then divided into three equal portions, which were spread out on a clean concrete floor and evenly sprayed at 77 ml/kg fresh weight with tap water containing respectively 0, 75 and 300 g PEG/l (MW 3350; Sigma Chemical Co., St Louis, Missouri). The diets so produced will hence be referred to as control, low-PEG and high-PEG diets respectively. Chopped and sprayed 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 were offered 600 g lotus DM/d (excluding PEG), practically all of which was consumed. Rapid DM determinations were performed each morning, and quantities of fresh food offered so that DM intakes were maintained constant at the previously-mentioned level.

### *Animals and marker infusion procedures*

Castrated male Romney sheep approximately 18 months old and with a mean initial weight of 58·4 kg (SE 2·92) were used. All were drenched with an anthelmintic (Thibenzole; Merck, Sharpe & Dohme (NZ) Ltd) plus 5 mg selenium as sodium selenate when brought into the animal house. The sheep were then fitted with a 25 mm i.d. cannula into the rumen and a simple 'T'-piece cannula into the proximal duodenum (i.d. 15 mm). After a settling in period following surgery, all sheep were given the control diet once daily for 1 week, after which the three diets were fed for a pre-experimental period of 10 d. The sheep were then equipped with harnesses and faeces collected for 6 d; urine was collected at the same time into 100 ml 6 M-hydrochloric acid.

A 60 ml portion of a solution containing indigestible markers was then injected into the rumen, and the marker solution then continuously infused into the rumen at 170 ml/d for 10 d. The sheep were then anaesthetized with intravenous sodium pentabarbitone (May & Baker, NZ), the jugular veins and carotid arteries severed and drained, and the rumen then ligated at the oesophagus and reticulo-omasal orifice and removed.

The marker solution comprised the inert forms of ruthenium phenanthroline (Ru-P) (Tan *et al.* 1971) and of CrEDTA (Binnerts *et al.* 1968). The two solutions were made up separately, mixed, and the pH adjusted to 6·5. Final theoretical concentrations were 59·8 mg Ru/l and 2000 mg Cr/l, with the quantities infused therefore being 10·12 mg Ru/d and 340·0 mg Cr/d.

*Sample collection procedures*

Duplicate samples of each feed and any residue were taken daily, pooled at −20°, and a pooled sample kept for the weeks that faeces and urine were being collected and when duodenal samples were being collected. Duodenal samples (170 g), to be used to form a matrix for the preparation of Ru and Cr standard curves, were collected from each animal at 11.30 and 14.00 hours during the last 2 d of the pre-experimental period, and the samples pooled for sheep given each diet and stored at −20°.

During the faeces and urine collection period, sub-samples of faeces and 50 g/kg of the daily urine output were also stored at −20°. After 4 d of marker infusion, samples of duodenal contents (170 g) were taken from each sheep at 11.30, 13.30 and 15.30 hours on four consecutive days, with the samples being pooled for each animal and stored at 4°. Each digesta sample was then thoroughly mixed, divided in half, and half designated whole digesta and stored at −20°. The remaining half was centrifuged at 1700 ***g*** for 20 min, the supernatant fraction discarded, and the residue designated centrifuged digesta and stored at −20°. Total rumen contents at slaughter were weighed, and sub-samples of entire contents and of deproteinized fluid stored at −20°. All deep-frozen samples of feeds, residues, rumen and duodenal contents and faeces were then freeze-dried, ground and stored ready for laboratory analyses.

*Laboratory methods*

Contents of total and free CT in feeds were determined as described by Barry & Forss (1983). As total CT was obviously not altered in the PEG-treated feeds, that portion reacting with the vanillin-HCl reagent, and therefore not bound by PEG, has been defined as total reactive CT. Free reactive CT was defined as that present in plant mascerates which was not precipitated by high-speed centrifugation (20000 ***g***, 30 min), and had therefore exceeded the binding capacity of plant proteins.

All other analyses, including the determination of Ru and Cr by X-ray fluorescence spectrometry, were carried out as described by Barry & Manley (1984). Standard curves prepared by adding graded quantities of the original marker solution to duodenal digesta collected during the pre-experimental period, followed by freeze-drying, showed no difference in slope for either Ru or Cr between sheep given the control or either of the PEG-treated diets. Thus dietary PEG addition did not affect the mass absorption coefficient, and thus did not introduce any bias into the determination of Ru and Cr in samples of whole digesta and centrifuged digesta collected during the period of marker infusion.

*Calculation of duodenal digesta flow*

Duodenal digesta flow was calculated using a double marker procedure (Faichney, 1975; eqns (1)–(3)). PEG consumed as part of the low- and high-PEG-treated diets (25 and 108 g/d) was assumed to be completely indigestible. Consequently, the amounts of organic matter (OM) and gross energy supplied per d by the PEG were deducted from the intakes, duodenal flows and faecal excretion of these components. Fractional disappearance rate (FDPR) and fractional degradation rate (FDR) of each nutrient in the rumen and its fractional outflow rate (FOR) were calculated as:

$$\text{FDPR} = \frac{\text{intake (g/h)}}{\text{rumen pool size (g)}}, \quad (1)$$

$$\text{FDR} = \frac{\text{intake (g/h)} - \text{duodenal flow (g/h)}}{\text{rumen pool size (g)}}, \quad (2)$$

$$\text{FOR} = \frac{\text{duodenal flow (g/h)}}{\text{rumen pool size (g)}}. \quad (3)$$

FDPR is the sum of FDR and FOR. FOR values for the CrEDTA and Ru-P markers were calculated from their pool sizes in the rumen at slaughter, using the reciprocal of eqn (7) of Faichney (1975).

Comparisons of FDR and FOR 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; Barry & Manley, 1985).

### *Statistical methods*

Comparisons between the three diets were made using one way analysis of variance, with trends being established by examining linear and quadratic contrasts of nutrient digestion as a function of dietary total reactive CT concentration.

## RESULTS

### *Chemical composition of the diet*

Control lotus contained high concentrations of total reactive CT, with about 16% as free reactive CT, and also contained high concentrations of lignin (Table 1); the overall composition was similar to that found by Barry & Duncan (1984) for the same cultivar grown in low-fertility acid soil.

Low and high rates of PEG addition reduced total reactive CT concentration to 47 and 15% of control values, with the reductions being as predicted from the amount of PEG applied/unit total CT (i.e. that of the control diet) in the relations of Barry & Forss (1983). Free reactive CT remained relatively constant at 11–16% of total reactive CT. PEG addition increased OM concentration and heat of combustion, but reduced the concentration of most other constituents due to its dilution effect; when expressed as g/kg non-PEG OM, concentrations of N, pectin, hemicellulose, cellulose, phosphorus and sulphur were practically identical for each diet. However, PEG addition caused concentrations of soluble CHO and of lignin in the non-PEG OM to rise. Each 10 g/kg decrease in total reactive CT concentration in the non-PEG OM was associated with a 1·2 (SE 0·14) g/kg rise in soluble CHO concentration and a 4·2 (SE 0·01) g/kg rise in lignin concentration.

### *OM and energy digestion*

Increasing concentration of dietary reactive CT linearly depressed the apparent digestibility of OM (Table 2) and of energy (Table 3). Relations between apparent energy digestibility (ADE; proportion intake), apparent OM digestibility (ADOM; proportion intake) and dietary CT concentration (g/kg DM) were described by the following equations:

$$\text{ADE} = 0{\cdot}73\ (\text{SE}\ 0{\cdot}017) - 0{\cdot}00089\ (\text{SE}\ 0{\cdot}000284)\ \text{CT} \quad r\ 0{\cdot}764;\ P<0{\cdot}05;\ \text{RSD}\ 0{\cdot}0285, \quad (4)$$

$$\text{ADOM} = 0{\cdot}75\ (\text{SE}\ 0{\cdot}016) - 0{\cdot}00082\ (\text{SE}\ 0{\cdot}000256)\ \text{CT} \quad r\ 0{\cdot}769;\ P<0{\cdot}05;\ \text{RSD}\ 0{\cdot}0257. \quad (5)$$

The quantity of OM and energy digested in the entire gastrointestinal tract that was apparently digested in the rumen was similar for the control and low-PEG diets, but was markedly increased by further reducing dietary reactive CT concentration with the high rate of PEG addition ($P < 0{\cdot}01$).

Table 1. *Chemical composition (g/kg dry matter) of vegetative secondary growth* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350)*

| | Control lotus | Low-PEG lotus | High-PEG lotus | Proportion non-PEG organic matter* (mean value for all diets) |
|---|---|---|---|---|
| PEG | 0 | 48·1 (0·53)† | 187·8 (2·44)† | |
| Total reactive condensed tannin | 94·7 (103·9)* | 45·1 (51·9)* | 14·2 (19·2)* | |
| Free reactive condensed tannin | 15·1 (0·159)‡ | 5·1 (0·112)‡ | 2·3 (0·162)‡ | |
| Organic matter | 911·2 | 916·1 | 928·4 | |
| Heat of combustion (MJ/kg) | 19·64 | 20·06 | 20·89 | |
| Total nitrogen | 30·8 | 29·7 | 26·9 | 34·78 |
| Soluble CHO | 79·8 (87·6)* | 82·3 (94·8)* | 72·3 (97·6)* | |
| Total pectins | 51·5 | 48·2 | 45·3 | 57·7 |
| Hemicellulose | 95·0 | 86·5 | 79·5 | 103·8 |
| Cellulose | 156·5 | 143·0 | 123·8 | 167·9 |
| Lignin | 178·8 (196·2)* | 189·3 (218·1)* | 171·8 (231·9)* | |
| Total phosphorus | 2·5 | 2·3 | 2·1 | 2·7* |
| Total sulphur | 2·2 | 2·1 | 2·0 | 2·5* |

* Calculated as g/kg non-PEG organic matter.
† PEG applied (g)/g total condensed tannin, calculated using total tannin concentration in the control diet.
‡ Ratio, free:total condensed tannin.

Table 2. *Quantities of organic matter eaten, flowing past the duodenum and excreted in the faeces (g/d) in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Organic matter flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Organic matter (g/d) | | | | |
| Eaten | 494·9 | 451·9 | 497·2 | 22·92 |
| Duodenum | 284·3 | 266·7 | 201·0 | 11·54 |
| Faeces | 162·5 | 128·5 | 132·9 | 11·19 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·67 | 0·72 | 0·74 | 0·016 |
| Rumen digestion | | | | |
| Proportion of intake | 0·42 | 0·41 | 0·59 | 0·026 |
| Proportion of total digested | 0·63 | 0·57 | 0·81 | 0·039 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·25 | 0·31 | 0·14 | 0·030 |

Table 3. *Quantities of energy eaten, flowing past the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Energy flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Energy (MJ/d) | | | | |
| Eaten | 10·7 | 9·8 | 10·8 | 0·54 |
| Duodenum | 6·9 | 6·4 | 4·7 | 0·29 |
| Faeces | 3·8 | 3·0 | 3·1 | 0·30 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·64 | 0·69 | 0·71 | 0·019 |
| Rumen digestion | | | | |
| Proportion of intake | 0·35 | 0·35 | 0·56 | 0·029 |
| Proportion of total digested | 0·54 | 0·50 | 0·79 | 0·045 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·29 | 0·35 | 0·15 | 0·035 |

Table 4. *Quantities of nitrogen eaten, flowing at the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| N flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| N (g/d) | | | | |
| Eaten | 16·7 | 15·5 | 17·5 | 0·86 |
| Duodenum | 18·1 | 14·7 | 11·8 | 0·41 |
| | (16·1)† | (12·6)† | (10·0)† | (0·41)† |
| Faeces | 7·0 | 3·5 | 3·7 | 0·27 |
| Duodenum: eaten | 1·09 | 0·95 | 0·68 | 0·031 |
| | (0·97)† | (0·81)† | (0·58)† | (0·045)† |
| Apparent digestibility | | | | |
| Proportion of intake | 0·58 | 0·77 | 0·79 | 0·014 |
| NAN digested in post-rumen region | | | | |
| g/d | 9·1 | 9·1 | 6·3 | 0·32 |
| Proportion NAN arriving at duodenum | 0·57 | 0·72 | 0·63 | 0·021 |
| g/g total N intake | 0·55 | 0·59 | 0·36 | 0·037 |
| g/MJ ME intake | 1·65 | 1·64 | 1·01 | 0·122 |
| Amino acid absorption (proportion ME)* | 0·19 | 0·18 | 0·11 | 0·014 |

NAN, non-ammonia-N; ME, metabolizable energy.

* Calculated from NAN absorbed post-ruminally using conversion factor derived from MacRae & Ullyatt (1974).

† NAN.

For sheep fed on control lotus, mean values of FDPR, FDR and FOR (with SE) for OM were respectively 0·084 (SE 0·009), 0·040 (SE 0·005) and 0·044 (SE 0·005). It was not possible to calculate values for sheep fed on PEG-treated lotus, as the quantity of PEG present in the rumen at slaughter was unknown and hence rumen pool size of non-PEG OM could not be calculated.

*N digestion*

Duodenal flows of both total N and NAN increased with increasing dietary CT concentration (Table 4). The ratios, duodenal total N flow:total N intake (DN) and duodenal NAN flow: total N intake (DNAN) were related to dietary reactive CT concentration (g/kg DM) by the equations:

$$DN = 0{\cdot}64\ (\text{SE}\ 0{\cdot}047) + 0{\cdot}0049\ (\text{SE}\ 0{\cdot}00075)\ CT \quad r\ 0{\cdot}937;\ P < 0{\cdot}001;\ \text{RSD}\ 0{\cdot}0750, \qquad (6)$$

$$DNAN = 0{\cdot}54\ (\text{SE}\ 0{\cdot}053) + 0{\cdot}0047\ (\text{SE}\ 0{\cdot}00084)\ CT \quad r\ 0{\cdot}916;\ P < 0{\cdot}01;\ \text{RSD}\ 0{\cdot}0848. \qquad (7)$$

Eqn (7) is similar in slope to but different in intercept from the relations (eqn (8)) reported by Barry & Manley (1984) for primary growth *Lotus pedunculatus*:

$$DNAN = 0{\cdot}79\ (\text{SE}\ 0{\cdot}016) + 0{\cdot}0052\ (\text{SE}\ 0{\cdot}00027)\ CT \quad r\ 0{\cdot}997;\ P < 0{\cdot}05. \qquad (8)$$

Post-rumen digestibility of NAN in the intestines was greater for sheep fed on low-PEG lotus than for sheep fed on control or high-PEG diets ($P < 0{\cdot}05$). Consequently, the total amount of NAN apparently absorbed post-ruminally, together with calculated amino acid absorption, were similar for the control and low-PEG diets, but much lower for the high-PEG diet ($P < 0{\cdot}05$).

Although both the concentration and total amount of free ammonia in the rumen tended to increase as dietary reactive CT concentration was reduced by PEG treatment (Table 5), the trend failed to attain significance ($P < 0{\cdot}05$), and in fact rumen ammonia concentration was quite high in sheep fed on control lotus. Rumen water pool size and CrEDTA FOR (Table 5) were unaffected by nutritional treatment, but FOR for Ru-P tended to decrease with increasing PEG application rate, although the trend did not attain significance ($P > 0{\cdot}05$).

*N retention*

Faecal N excretion differed only slightly between the two groups of sheep fed on PEG-treated lotus, and urinary N excretion was similar; hence values for the two groups have been combined in the calculation of N retention (Table 6). Although N consumed was similar for sheep fed on control and PEG-treated lotus, the higher total CT concentration of control lotus was associated with a 3·7 g/d increase in faecal N excretion and a 5·2 g/d reduction in urinary N excretion. Consequently, the higher dietary CT concentration was associated with an increase in N retention of 1·5 g/d and large increases in N retained as a proportion of N consumed and apparently absorbed, but due to experimental variation these effects did not attain significance ($P > 0{\cdot}05$).

*Digestion of water-soluble CHO and of pectin*

Water-soluble CHO was completely digested on all diets, with flows in faeces being zero for every animal. A slightly larger proportion of the soluble CHO ingested was digested in the rumen of sheep fed on control diet than in those fed on PEG-treated lotus ($P < 0{\cdot}05$; Table 7).

Apparent digestibility of pectin was high (Table 8) and in the range 0·92–0·94 for all three diets. A mean of 0·88 of this was digested in the rumen for sheep fed on control and low-PEG lotus, but this increased to 0·95 for sheep fed on high-PEG lotus ($P < 0{\cdot}05$). Rumen pool size, FDPR and FDR for pectin showed no difference between diets, but FOR increased linearly ($P < 0{\cdot}05$) with increases in dietary reactive CT concentration.

Table 5. *Rumen pool sizes of ammonia and water together with fractional outflow rates of CrEDTA and ruthenium phenanthroline (Ru-P) indigestible markers, in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350)*

(Mean values with their standard errors for three animals per diet)

| | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Rumen ammonia N | | | | |
| Concentration (mg/l) | 275 | 287 | 389 | 53·2 |
| Pool size (mg) | 777 | 1020 | 939 | 197·3 |
| Water | | | | |
| Pool size (l) | 2·61 | 3·66 | 2·48 | 0·400 |
| Marker fractional outflow rate | | | | |
| CrEDTA (/h) | 0·058 | 0·048 | 0·050 | 0·0138 |
| Ru-P (/h) | 0·050 | 0·041 | 0·035 | 0·0069 |

Table 6. *Quantities of nitrogen (g/d) eaten, excreted in faeces and urine and retained in sheep fed on control and polyethylene glycol-treated (molecular weight 3350)* Lotus pedunculatus

(Mean values with their standard errors for three and six sheep fed on control and PEG-treated lotus respectively)

| | Control lotus | | PEG-treated lotus | |
|---|---|---|---|---|
| N intake and excretion | Mean | SEM | Mean | SEM |
| Intake | 18·4 | 1·69 | 18·4 | 1·31 |
| Faeces | 7·7 | 0·61 | 4·0 | 0·47 |
| Urine | 8·1 | 1·33 | 13·3 | 1·03 |
| Retained | | | | |
| g/d | 2·6 | 1·22 | 1·1 | 0·94 |
| proportion intake | 0·13 | 0·063 | 0·06 | 0·049 |
| proportion absorbed | 0·21 | 0·098 | 0·08 | 0·076 |

*Hemicellulose and cellulose digestion*

Apparent digestibility of hemicellulose (Table 9) and of cellulose (Table 10) were generally over 0·70 and not affected by PEG addition. Rumen hemicellulose digestion as a proportion of intake (HCI) and as a proportion of total digested (HCD) decreased with increasing dietary reactive CT concentration (g/kg DM) according to the equations:

$$\text{HCI} = 0{\cdot}61\ (\text{SE}\ 0{\cdot}030) - 0{\cdot}0014\ (\text{SE}\ 0{\cdot}000\,51)\ \text{CT} \quad r\ 0{\cdot}729;\ P < 0{\cdot}05;\ \text{RSD}\ 0{\cdot}0506, \qquad (9)$$

$$\text{HCD} = 0{\cdot}88\ (\text{SE}\ 0{\cdot}039) - 0{\cdot}0021\ (\text{SE}\ 0{\cdot}000\,64)\ \text{CT} \quad r\ 0{\cdot}770;\ P < 0{\cdot}05;\ \text{RSD}\ 0{\cdot}0650. \qquad (10)$$

Some 0·92 of the total cellulose digested was digested in the rumen of sheep fed on control lotus, and although this progressively increased to 0·98 as reactive CT concentration was lowered, the trend did not attain significance ($P > 0{\cdot}05$). For both hemicellulose and cellulose, there was some tendency for rumen pool size to increase, and hence rumen FDPR to decrease, when PEG was added to the diet, but in all cases these effects failed to attain

Table 7. *Quantities of soluble carbohydrate (CHO) eaten and flowing past the duodenum (g/d) in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Soluble CHO flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Soluble CHO (g/d) | | | | |
| Eaten | 44·0 | 41·2 | 46·8 | 2·31 |
| Duodenum | 3·2 | 10·8 | 10·0 | 2·11 |
| Rumen digestion | | | | |
| Proportion of intake | 0·93 | 0·74 | 0·78 | 0·049 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·07 | 0·26 | 0·22 | 0·050 |

Table 8. *Quantities of pectin eaten, flowing past the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Pectin flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Pectin (g/d) | | | | |
| Eaten | 28·0 | 25·3 | 29·5 | 1·44 |
| Duodenum | 5·0 | 4·7 | 3·2 | 0·54 |
| Faeces | 2·3 | 1·6 | 1·8 | 0·25 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·92 | 0·94 | 0·94 | 0·007 |
| Ruman digestion | | | | |
| Proportion of intake | 0·82 | 0·82 | 0·89 | 0·016 |
| Proportion of total digested | 0·89 | 0·87 | 0·95 | 0·018 |
| Rumen pool (g) | 6·4 | 6·8 | 5·9 | 0·89 |
| Fractional disappearance rate (/h) | 0·218 | 0·217 | 0·255 | 0·0318 |
| Fractional degradation rate (/h) | 0·184 | 0·189 | 0·233 | 0·0289 |
| Fractional outflow rate (/h) | 0·047 | 0·029 | 0·022 | 0·0056 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·10 | 0·12 | 0·05 | 0·017 |

significance ($P > 0·05$). For both these structural carbohydrate constituents, PEG addition had no effect on rumen FDR, but rumen FOR of sheep fed on lotus treated with either rate of PEG were similar and lower than that of sheep fed on control lotus ($P < 0·05$). Post-rumen digestion of hemicellulose and cellulose, expressed as a proportion of each flowing past the duodenum daily, increased as dietary concentration of reactive CT increased with the trend attaining significance for hemicellulose ($P < 0·05$). At all levels of PEG addition, post-rumen digestion of hemicellulose was notably higher than that of cellulose and lignin, and was especially high for control lotus.

Table 9. *Quantities of hemicellulose eaten flowing past the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Hemicellulose flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Hemicellulose (g/d) | | | | |
| Eaten | 51·6 | 45·9 | 52·1 | 2·38 |
| Duodenum | 26·5 | 21·6 | 20·2 | 1·54 |
| Faeces | 14·5 | 15·6 | 14·7 | 1·15 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·72 | 0·66 | 0·72 | 0·018 |
| Rumen digestion | | | | |
| Proportion of intake | 0·49 | 0·52 | 0·61 | 0·029 |
| Proportion of total digested | 0·68 | 0·79 | 0·85 | 0·040 |
| Rumen pool (g) | 34·6 | 40·0 | 37·1 | 3·39 |
| Fractional disappearance rate (/h) | 0·068 | 0·060 | 0·061 | 0·0059 |
| Fractional degradation rate (/h) | 0·036 | 0·036 | 0·038 | 0·0045 |
| Fractional outflow rate (/h) | 0·032 | 0·024 | 0·023 | 0·0023 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·23 | 0·14 | 0·11 | 0·030 |
| Proportion flowing at duodenum | 0·45 | 0·29 | 0·29 | 0·050 |

Table 10. *Quantities of cellulose eaten, flowing past the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Cellulose flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Cellulose (g/d) | | | | |
| Eaten | 84·8 | 78·3 | 80·8 | 3·81 |
| Duodenum | 26·7 | 22·0 | 21·9 | 1·68 |
| Faeces | 21·5 | 19·3 | 21·3 | 1·47 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·75 | 0·75 | 0·74 | 0·013 |
| Rumen digestion | | | | |
| Proportion of intake | 0·69 | 0·72 | 0·73 | 0·021 |
| Proportion of total digested | 0·92 | 0·95 | 0·98 | 0·034 |
| Rumen pool (g) | 51·0 | 56·9 | 54·6 | 5·33 |
| Fractional disappearance rate (/h) | 0·080 | 0·071 | 0·064 | 0·0079 |
| Fractional degradation rate (/h) | 0·058 | 0·055 | 0·047 | 0·0016 |
| Fractional outflow rate (/h) | 0·022 | 0·017 | 0·017 | 0·0071 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·06 | 0·03 | 0·01 | 0·026 |
| Proportion flowing at duodenum | 0·18 | 0·12 | 0·02 | 0·085 |

Table 11. *Quantities of lignin eaten, flowing past the duodenum and excreted in the faeces in sheep fed on* Lotus pedunculatus *treated with zero, low and high rates of polyethylene glycol (PEG; molecular weight 3350), together with the partition of digestion into that digested in the reticulo-rumen and post-rumen regions of the digestive tract*

(Mean values with their standard errors for three animals per diet)

| Lignin flow and digestion | Control lotus | Low-PEG lotus | High-PEG lotus | SEM |
|---|---|---|---|---|
| Lignin (g/d) | | | | |
| Eaten | 97·3 | 98·6 | 112·0 | 5·38 |
| Duodenum | 83·5 | 84·7 | 79·9 | 3·95 |
| Faeces | 75·9 | 73·4 | 78·7 | 4·13 |
| Apparent digestibility | | | | |
| Proportion of intake | 0·22 | 0·26 | 0·30 | 0·020 |
| Rumen digestion | | | | |
| Proportion of intake | 0·14 | 0·14 | 0·28 | 0·013 |
| Proportion of total digested | 0·67 | 0·55 | 0·96 | 0·081 |
| Post-rumen digestion | | | | |
| Proportion of intake | 0·08 | 0·11 | 0·02 | 0·026 |
| Proportion flowing at duodenum | 0·09 | 0·13 | 0·02 | 0·029 |

### *Lignin digestion*

Apparent digestibility of lignin (ADL; proportion eaten) was low (Table 11) and decreased with increasing dietary reactive CT concentration:

$$\text{ADL} = 0{\cdot}31\ (\text{SE}\ 0{\cdot}021) - 0{\cdot}0010\ (\text{SE}\ 0{\cdot}00034)\ \text{CT} \quad r\ 0{\cdot}761;\ P < 0{\cdot}05;\ \text{RSD}\ 0{\cdot}0346. \qquad (11)$$

Lignin digested in the rumen as a proportion of both that eaten and totally digested was similar for sheep fed on control and low-PEG lotus, but was increased in sheep fed on high-PEG lotus ($P < 0{\cdot}01$).

### *Interrelations between the digestion of lignin and structural CHO*

When expressed as a proportion of each nutrient ingested, each unit of lignin digested in the rumen was associated with 0·75 (SE 0·248; $P < 0{\cdot}05$) units of hemicellulose digested in the rumen but only 0·20 (SE 0·185; $P > 0{\cdot}05$) units of cellulose digested in the rumen. When expressed as a proportion of each nutrient flowing at the duodenum, post-rumen digestion of hemicellulose and cellulose were not related to post-rumen lignin digestion ($P > 0{\cdot}05$).

## DISCUSSION

### *Effects of PEG addition on chemical composition*

The objective of spraying lotus with PEG was to reduce the concentration of CT that was available to react with plant proteins when the plant was disintegrated, and this objective was clearly attained. Two side-effects were also produced in addition, in that PEG treatment increased the apparent concentration of lignin, and increased the concentration of water-soluble CHO in the plant and reduced its rate of degradation in the rumen. However, both effects were smaller in magnitude than the primary effect produced on CT concentration. CT and lignin are both polyphenolic compounds, and their monomers are synthesized in plants by the same shikimic acid biochemical pathway (Harkin, 1973; Wong, 1973). It is

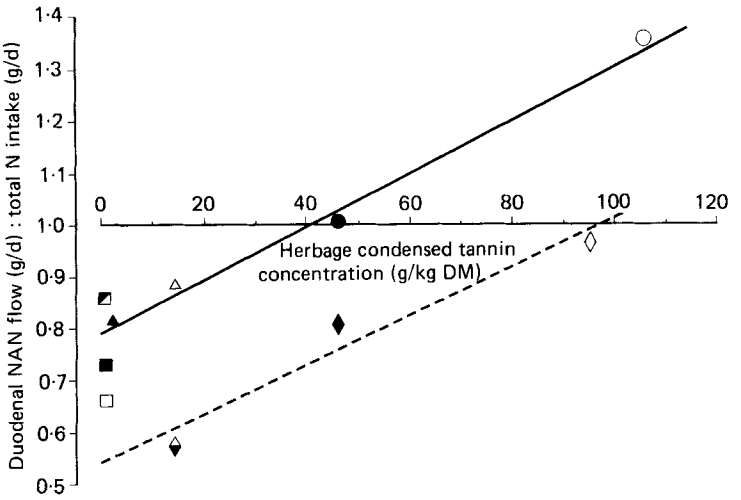


Fig. 1. Duodenal non-ammonia nitrogen (NAN) flow per unit total N intake as a function of dietary condensed tannin concentration in sheep fed on *Lotus* sp. (◊), Control; (♦), low-polyethylene glycol (PEG; molecular weight 3350); (◈), high-PEG-treated secondary-growth *Lotus pedunculatus* fed in the present investigation; (○), high-, (●), low-tannin primary-growth *Lotus pedunculatus* (Barry & Manley, 1984); (△), high-, (▲), low-tannin primary-growth *Lotus corniculatus* (John & Lancashire, 1981). Non-tannin-containing species: (□), perennial ryegrass (*Lolium perenne*); (■), white clover (*Trifolium repens*); (◩), short-rotation ryegrass (MacRae & Ulyatt, 1974). (—), Lotus primary growth (eqn (8), p. 129)); (– – –), lotus secondary growth (eqn (7), p. 129). DM, dry matter.

therefore probable that PEG binds to lignin by the same mechanism as it binds to CT, thus accounting for the increased concentration of 'artefact' lignin following PEG treatment. The effects of PEG treatment on water-soluble CHO are more difficult to explain. The most logical explanation is that PEG in some way inhibits enzymes involved in the degradation of this component, thus accounting for its increased concentration in the plant and reduced rumen degradation when the forage was sprayed with PEG. Apart from these effects on lignin and water-soluble CHO, there is no evidence that PEG affected any dietary component other than CT, and the principal effects found in the present study are therefore due to changes in CT concentration induced by PEG treatment.

### *N and CHO digestion*

Barry & Manley (1984) concluded that the main effects of CT in primary growth *Lotus pedunculatus* were to increase duodenal flow of NAN, and to cause reductions in rumen digestion of hemicellulose and pectin but not of cellulose. These effects are confirmed in the present study, but duodenal flow of NAN was less for secondary than for primary growth, although the rate of increase per unit CT was similar for both primary and secondary growth (Fig. 1). Duodenal NAN comprises undegraded dietary protein, microbial protein, endogenous protein from abomasal secretions and nucleic acids. For sheep grazing a range of fresh forages, Corbett & Pickering (1983) found microbial protein flowing at the duodenum to be 35–40% more for primary than for secondary growth. It may well be that the difference between the two intercepts in Fig. 1 is due to greater microbial protein synthesis for sheep fed on primary growth lotus, whilst the relative constancy of the slopes is due to effects of CT in increasing duodenal flow of undegraded dietary protein. The low value of the intercept in the present study (eqn (7)) illustrates inefficient use of N in the

Table 12. *Comparison of rates of rumen carbohydrate (CHO) degradation and outflow in sheep fed on* Lotus pedunculatus *and forage kale* (Brassica oleracea) *differing markedly in the ratio, readily fermentable CHO: structural CHO and in lignin content*

| | Control high-tannin lotus | High-PEG, low-tannin lotus | Forage kale* |
|---|---|---|---|
| Dietary concentration (g/kg organic matter) | | | |
| Water-soluble CHO | 88 | 98 | 334 |
| Pectin | 58 | 58 | 141 |
| Hemicellulose | 104 | 104 | 61 |
| Cellulose | 168 | 168 | 85 |
| Readily-fermentable CHO: structural CHO | 0·54 | 0·54 | 3·25 |
| Lignin | 196 | 232 | 61 |
| Fractional degradation rate (/h) | | | |
| Pectin | 0·18 | 0·23 | 0·62 |
| Hemicellulose | 0·036 | 0·038 | 0·039 |
| Cellulose | 0·058 | 0·047 | 0·30 |
| Fractional outflow rate (/h) | | | |
| Pectin | 0·047 | 0·022 | 0·017 |
| Hemicellulose | 0·032 | 0·023 | 0·021 |
| Cellulose | 0·022 | 0·017 | 0·027 |
| Water | 0·058 | 0·050 | 0·155† |

* From Barry *et al.* (1984).
† From Barry & Manley (1985).

rumen, and a large net absorption of N from the rumen as ammonia, in sheep fed on secondary growth lotus containing negligible concentrations of CT.

Predicted concentrations of CT to increase duodenal NAN flow to the same level as N intake (i.e. no loss or gain of N across the rumen) were 41 g/kg DM for primary lotus (eqn (8)) and 98 g/kg DM for secondary-growth lotus (eqn (7)). Choice of an optimum concentration of CT in lotus depends on a balance between its positive effect in increasing duodenal NAN flow (eqns (6) and (7)) and its negative effect in depressing apparent digestibility of energy and OM (eqns (4) and (5)) and rumen digestion of hemicellulose (eqns (9) and (10)) and of pectin (Table 8). Based on these criteria, a suitable compromise for both secondary and primary growth would appear to be about 30–40 g/kg DM.

### *N retention and recycling*

Presence of CT in sainfoin (Egan & Ulyatt, 1980) and in *Lotus corniculatus* (John & Lancashire, 1981) has been associated with increased N retention in sheep, and this was confirmed in the present study for *Lotus pedunculatus*, with a major reason being increased efficiency of use of apparently absorbed N. Rumen ammonia concentration was still relatively high in sheep fed on control lotus, when it might have been expected that the reduction in dietary protein degradation caused by the high CT concentration (95 g/kg DM) would result in a low rumen ammonia concentration. Egan & Ulyatt (1980) associated higher N retention of sheep fed on sainfoin (*Onobrychis vicifolia* Scop.) than on white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) with increased rate of recycling N into the rumen, presumably as salivary urea; this effect may have operated in sheep fed on control lotus in the present study, and be induced by dietary CT.

*Degradation and outflow from the rumen*

For the carbohydrate components, sheep appeared to respond to high dietary concentrations of CT by increasing FOR, with no change in rumen FDR. Indeed, increased rate of rumen outflow seems the principal reason for reduced digestion of hemicellulose and pectin in the rumen of sheep fed on diets high in CT concentration. Decreased rumen FOR for carbohydrates is matched by decreased rumen FOR for Ru-P but not CrEDTA; PEG is osmotically active, and it may be that increased PEG intake increased water flow into the rumen through the epithelium and so prevented any decline in FOR for CrEDTA as dietary CT concentration was reduced.

An indication of factors governing rates of breakdown and outflow from the rumen can be gained by comparing FOR and FDR of sheep fed on lotus in the present investigation with those of sheep fed on forage kale, the two diets representing extremes in the ratio, readily fermentable:structural CHO (Table 12). Increasing the ratio as a result of kale feeding produced marked increases in rumen FDR of pectin and cellulose, accompanied by a large increase in water FOR. Hemicellulose had the lowest FDR of all the carbohydrate constituents, and this remained constant irrespective of diet. It is possible that hydrolysis of lignin–hemicellulose bonds may be rate-limiting for rumen hemicellulose digestion, and hence may be the rate-limiting step in rumen cell wall digestion. This is supported by the much closer association of rumen lignin digestion with that of hemicellulose rather than cellulose (p. 131). The lack of a relation between post-rumen digestion of lignin and structural CHO indicates that, following the digestion of some lignin in the rumen, the large post-rumen digestion of hemicellulose occurs independent of any association with lignin.

Apart from effects attributable to CT, FOR was broadly similar for the two diet types, notably for hemicellulose and cellulose, supporting the concept that FOR may be partly a function of the animal, and probably regulated by rumen contractions.

In conclusion, increasing concentration of CT confers beneficial effects on nutritive value in increasing duodenal NAN flow and N retention, and detrimental effects in restricting apparent digestibility of OM, energy and lignin and rumen digestion of hemicellulose and pectin. A compromise concentration, which will also confer bloat resistance, is suggested as 30–40 g CT/kg DM for both primary and secondary growth. This will serve both as a guide in the utilization of 'Grasslands Maku' lotus in NZ grazing systems, and as a value to aim for in the transfer of genes coding for leaf CT production from species such as lotus into white clover, as discussed by Barry & Reid (1986).

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