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The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L.

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1. Sheep were used to evaluate the nutritional consequences of a low condensed-tannin concentration (22 g/kg dry matter (DM)) in lotus (*Lotus corniculatus* L.) (control group) compared with lotus given to sheep receiving intraruminal polyethylene glycol (PEG) infusion (PEG group). PEG selectively binds to tannins and prevents tannins from binding proteins.

2. DM intakes (1430 (SE 28) g/d) and digestibility of energy (663 (SE 4·5) kJ/MJ intake) were similar for both groups but the apparent digestion of nitrogen was lower in the control sheep (0·70) than in the PEG sheep (0·78; $P < 0·001$).

3. The proportion of N apparently digested before the abomasum (i.e. in the rumen) was lower ($P < 0·05$) in control sheep (0·12) than in PEG sheep (0·21; $P < 0·05$). Rumen ammonia concentrations were lower ($P < 0·001$) in control sheep than in PEG sheep. The proportion of neutral-detergent fibre (NDF) digested in the rumen was similar for both groups (0·48 (SE 0·012)) but less energy was digested in the rumen of the control (0·42) than of the PEG sheep (0·47; $P < 0·05$).

4. The flux of essential amino acids (EAA) through the abomasum of control sheep was 50% greater than that in PEG sheep; flux of non-essential amino acids (NEAA) was 14% higher in control than in PEG sheep. Apparent digestibility of EAA in the small intestine was similar for both treatments (0·67), but NEAA were less well digested in the control (0·55) than in the PEG sheep (0·69).

5. The presence of tannins in the control group increased net apparent absorption of threonine (57%), valine (89%), isoleucine (94%), leucine (30%), tyrosine (41%), phenylalanine (93%), histidine (90%) and lysine (59%), and reduced NEAA absorption by 10%, compared with PEG sheep.

Tannins are widespread in the plant kingdom and appear to be beneficial to ruminants in some instances. Condensed tannins bind to proteins to form stable complexes in the pH range 3·5–7·0 but dissociate at pH $< 3·5$ and $> 8·5$ (Jones & Mangan, 1977). Plant proteins should therefore be bound and protected from microbial degradation in the rumen (pH 5·5–7·0) and released in the abomasum, enabling hydrolysis and absorption of amino acids (AA) to occur in the small intestine.

Conservation of dietary protein in the rumen of sheep fed on tannin-containing legumes has been demonstrated with both sainfoin (*Onobrychis viciifolia* Scop.) (Thomson *et al.* 1971; Ulyatt *et al.* 1977; Egan & Ulyatt, 1980) and lotus (*Lotus corniculatus*) (John & Lancashire, 1981; Barry & Manley, 1984). Sainfoin diets have a lower nitrogen digestibility and a higher N retention than grasses and clovers fed at similar levels of intake (Ulyatt *et al.* 1977; Egan & Ulyatt, 1980). The higher N retention of sainfoin could not be explained in terms of urea-N recycling (Egan & Ulyatt, 1980). Sheep fed on a lotus cultivar containing 14·5 g condensed tannin/kg dry matter (DM) retained more N than those fed on a cultivar containing 2·5 g condensed tannin/kg DM (John & Lancashire, 1981). Lotus species containing high concentrations of condensed tannins (60–110 g/kg DM) reduce voluntary intake and result in a low N digestibility (0·48–0·71) when fed to sheep (Barry & Duncan, 1984; Barry & Manley, 1984).

The objective of the present experiment was to feed sheep high intakes of a lotus cultivar containing low levels of condensed tannins and to remove the effects of the tannins from half the animals by an intraruminal infusion of polyethylene glycol (PEG) (Jones & Mangan, 1977). High levels of intake were intended to maximize the potential for N loss from the rumen (Ulyatt & Egan, 1979) and therefore maximize the potential for tannins

to bind, and later release, plant proteins. The effects of tannins were evaluated on the basis of nutrient digestion before the abomasum, in the small intestine and in the whole digestive tract. Although carbohydrate and mineral components have been measured, most emphasis has been placed on the digestion of nitrogenous constituents and the availability of individual AA for absorption.

EXPERIMENTAL

Feed

The lotus (cv. Maitland) was harvested from a vigorously growing stand 300–400 mm high in a pre-bloom vegetative state. The lotus was harvested daily at 08·00 hours and was cut to 30–50 mm lengths with a chaff cutter immediately after harvest. One-third of the daily requirement was placed on belt feeders (which delivered feed in hourly increments) by 09·00 hours, and the remaining two-thirds stored at 4° until 16.00 hours and then placed on belt feeders.

Lotus was given for a total of 24 d, and was offered *ad lib.* for the first 7 d and thereafter at 90% of *ad lib.* intake. A rapid DM determination by microwave oven enabled a consistent daily allocation of DM. Feed DM was confirmed by drying at 100 ° for 24 h. Fresh feed was subsampled daily and pooled samples stored at −20° for analysis.

Animals

Eight 18-month-old Romney Marsh wethers, mean weight 44 (SE 1·0) kg, had been fitted with rumen and abomasal 'T' piece cannulas 6 months previously, and were accustomed to handling. The sheep had been used for a previous experiment (Waghorn *et al.* 1987) and had been allowed 5–8 weeks grazing ryegrass (*Lolium multiflorum* Lam.)–clover (*Trifolium repens* L.) pasture before returning to metabolism crates for the present experiment.

Measurement sequence

The sheep were randomly divided into two groups (four sheep per group). One group received a continuous infusion of 50 g PEG/d (molecular weight 3500; in 380 ml water) into the rumen from day 14 until slaughter (PEG group) and the others received 430 ml water/d (control group). A 20 ml sample of strained rumen fluid was collected from each sheep immediately before and 3 d after infusions commenced, to determine ammonia levels. Rumen NH_3 concentration indicates the effectiveness of PEG in binding tannins, enabling microbial degradation of unbound plant proteins (Jones & Mangan, 1977; John & Lancashire, 1981).

Sheep were fitted with harnesses and faecal collection bags on day 17 for digestibility measurements. Faeces were collected each day and frozen until analysed. Liquid- and solid-phase markers were added to the intrarumen infusate from day 18 until slaughter: CrEDTA was infused at 280 mg chromium/d as the liquid-phase marker (Binnerts *et al.* 1968) and ^{103}Ru phenanthroline complex at 9 μCi/d as the solid-phase marker (Tan *et al.* 1971). Abomasal digesta were sampled (50–100 ml) at 6–8 h intervals from days 21 to 24 to represent a two-hourly sampling over 24 h (Ulyatt & Egan, 1979). Samples were refrigerated (4 °) immediately, and when the final samples were obtained the twelve samples from each sheep were combined on an equal volume basis and frozen until analysed.

Feeding and marker infusion continued until 5 min before slaughter on day 24 of the experiment. Sheep were killed by intravenous administration of sodium pentabarbital, a midline incision made in the abdomen and the terminal ileum located and sectioned. Ileal digesta were gently 'milked' from about 4 m of the terminal ileum, yielding 250–350 g wet material which was stored at −20 ° for analyses. Ileal digesta were collected within 2 min of death.

Analyses

Flow of digesta through the abomasum and ileum was determined by the double-marker method of Faichney (1975). This requires both Cr concentrations and ^{103}Ru activity to be determined in whole digesta and supernatant (29000 ***g***) fractions. Whole digesta were prepared for Cr analyses by 'wet ashing' 0·3 g DM in 10 ml concentrated nitric acid for 3 d and resuspension in 2 M-hydrochloric acid (Grace, 1983). The supernatant fraction was diluted with an equal volume of 4 M-HCl. Cr concentrations were determined by inductively coupled argon plasma spectrometry (ICAPS) (Lee, 1981). ^{103}Ru activity in whole digesta and supernatant fraction was determined using a gamma counter (Packard).

On the basis of these determinations, abomasal digesta flows were calculated and digesta from each sheep reconstituted (Faichney, 1975) for DM determination and chemical analysis. Very low values for Cr in the ileal supernatant fraction suggested matrix interference in the analyses, so that ileal flow rates were calculated from ^{103}Ru activity/unit ileal DM, and were not reconstituted for analysis.

Feed, digesta and faecal samples were freeze dried and ground for determination of ash, neutral-detergent fibre (NDF), lignin, energy, N and total (hydrolysed) AA concentrations. Structural carbohydrates were determined by sequential neutral-detergent, then acid extractions (Robertson & Van Soest, 1980); energy by bomb calorimetry; and N by auto-analysis of NH_3 following Kjeldahl digestion (Williams & Twine, 1967). AA concentrations were determined in feed (duplicate) and in abomasal and ileal digesta after hydrolysis (Association of Official Analytical Chemists, 1982) and separation on an LKB analyser (Fisher, 1983). Sulphur-containing AA could not be determined accurately by this technique, and are not reported. Methods for determining monosaccharides, starch, pectin and lipids in feed have been summarized by Ulyatt & Macrae (1974).

Rumen NH_3 was determined by autoanalysis (Technicon Industrial Systems, 1973) and condensed tannins by acidified vanillin (Broadhurst & Jones, 1978). Mineral elements in plant, digesta and faecal samples were determined by the same 'wet ashing' procedures as described for Cr, and analyses by ICAPS.

All concentrations of nutrients in digesta and faeces, and fluxes of digesta and faeces, are expressed on a 'PEG free' basis. Fluxes of nutrients are determined from DM flux and concentration of nutrients in DM. Apparent absorption is the disappearance between two sites. All presentations of AA results as ratios of EAA:NEAA exclude S-containing AA, and include tyrosine as an EAA because the only source is phenylalanine. This also applies to the discussion of results from other experiments.

Statistical analyses

Means are presented with the standard errors of the means, or with pooled standard errors, as appropriate. Comparison between control and PEG treatments were made by analysis of variance.

RESULTS

Lotus DM was 165 (SE 6·3) g/kg over the duration of the experiment. Lotus DM composition was (g/kg): 88 ash, 84 monosaccharides, 44 starch, 45 pectin, 485 NDF, 103 lignin, 27 N, 47 diethyl-ether-extractable lipid, and contained 18·72 kJ gross energy (GE) per g. The concentration of condensed tannins was 21·7 g/kg lotus DM. Feed intakes by the control and PEG groups during the digestion period were 1400 and 1461 g DM/d respectively (not significant, Table 1).

Rumen NH_3 concentrations (μg/ml rumen fluid) before PEG infusion were similar for the control (348 (SE 42·6)) and PEG (320 (SE 5·8)) treatments but, after 3 d of PEG infusion,

Table 1. *Intake, digestibility and flow of polyethylene glycol (PEG)-free digesta through the abomasum and terminal ileum of sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland))* *with and without administration of PEG*

(Mean values for four animals per treatment with their pooled standard errors)

	Control sheep (mean)	PEG sheep (mean)	Pooled SEM
DM intake (g/d)	1400	1461	27·3
Digestibility (proportion of intake):			
DM	0·69	0·71	0·005
Energy	0·66	0·67	0·005
NDF	0·60	0·62	0·007
Flow through the abomasum (g/d):			
DM	874	825	20·3
Abomasal digesta	23743	25270	1380
Flow through the ileum (g/d):			
DM	544	514	14·9
Ileal digesta	6860	6397	258
Faecal DM output (g/d)	442	427	13·7

DM, dry matter; NDF, neutral-detergent fibre.

Table 2. *Concentration (g/kg dry matter (DM)) of metabolites and energy in polyethylene glycol (PEG)-free abomasal and ileal contents and faeces of sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland))* *with and without infusion of PEG*

(Mean values for four animals per treatment with their pooled standard errors)

	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference
Abomasal digesta:				
Ash	182	201	3·9	$P < 0·10$
Nitrogen	38	38	0·4	NS
AA	192	162	6·0	$P < 0·05$
NDF	405	441	7·8	NS
Lignin	124	133	4·1	NS
Energy (kJ/g DM)	17·4	17·8	0·20	NS
Abomasal supernatant fraction:				
Ash	182	182	3·0	NS
Ammonia (μg/ml)	205	211	5·2	NS
Ileal digesta:				
Ash	158	153	5·0	NS
N	25	18	0·5	$P < 0·001$
AA	106	84	2·5	$P < 0·01$
NDF	553	615	26·0	$P < 0·05$
Lignin	172	192	4·7	$P < 0·05$
Energy (kJ/g DM)	18·1	18·4	0·25	NS
Faeces:				
Ash	107	104	2·0	NS
N	26	20	0·5	$P < 0·01$
NDF	620	640	5·3	$P < 0·10$
Lignin	236	236	3·3	NS
Energy (kJ/g DM)	20·3	21·3	0·11	$P < 0·01$

AA, amino acids; NDF, neutral-detergent fibre; NS, not significant.

Table 3. *Nitrogen and non-ammonia-N (NAN) flow through the alimentary tract, and sites of digestion in sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland)) with and without infusion of polyethylene glycol (PEG)*

(Mean values for four animals per treatment with their pooled standard errors)

	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference
Intake of N* (g/d)	37·8	37·8	—	—
Abomasal N:				
Total N flow (g/d)	33·4	29·8	0·63	$P < 0·05$
NH_3 flow (g/d)	3·9	4·0	0·12	NS
NAN flow (g/d)	29·5	25·8	0·58	$P < 0·05$
NAN (mg/g N intake)	781	683	15·2	$P < 0·05$
Ileal N flow (g/d)	13·5	8·8	0·51	$P < 0·01$
Faecal N (g/d)	11·3	8·3	0·118	$P < 0·001$
Apparent digestion of N (g/d):				
Total	26·5	29·5	0·18	$P < 0·001$
Rumen (pre-abomasal)	4·4	7·9	0·63	$P < 0·05$
Small intestine	19·9	21·0	0·79	NS
Large intestine	2·2	0·50	0·54	NS
Apparent digestion of N (proportion of intake):				
Total	0·70	0·78	0·005	$P < 0·001$
Rumen (pre-abomasal)	0·12	0·21	0·017	$P < 0·05$
Small intestine	0·52	0·56	0·021	NS
Large intestine	0·06	0·01	0·014	NS

* Values for PEG sheep have been adjusted to a dry matter intake of 1400 g/d. NS, not significant.

rumen NH_3 increased ($P < 0·001$) to 504 (SE 28·7), while the control group remained unchanged at 367 (SE 12·1) μg/ml rumen fluid.

Infusion of PEG did not affect the apparent digestibility of DM, energy or NDF or the flux of DM or whole digesta through the abomasum or ileum (Table 1).

N digestion

The principal effect of PEG infusion was on the digestion of N (Tables 2 and 3). Although the concentrations of N and NH_3 in abomasal digesta were the same for the two treatments (Table 2), the non-NH_3-N (NAN) flux through the abomasum (as a percentage of N intake) was greater in the control sheep ($P < 0·05$). The control sheep apparently digested less dietary N in the rumen (11·7%) compared with the PEG sheep (21·0%; $P < 0·05$) and had a higher ($P < 0·05$) concentration of AA in abomasal digesta than the PEG group (Table 2).

Ileal digesta of control sheep had higher N ($P < 0·001$) and AA ($P < 0·01$) concentrations than those of the PEG sheep (Table 2), so that the apparent digestion of N in the small intestine was similar for both treatments (Table 3).

Faecal N concentration was higher in the control sheep than in the PEG sheep ($P < 0·01$) and total apparent digestion of N (as a percentage of intake) was lower ($P < 0·001$) in the control sheep (70·1%) than in the PEG sheep (78·1%; Table 3).

AA digestion

Lotus contained 153 mg AA/g DM. Feed intakes by the control and PEG sheep were 214 (SE 6·5) and 224 (SE 5·3) g AA/d. Abomasal AA fluxes were 167 (SE 5·8) and 133 (SE

Table 4. *Amino acid composition (g/kg) in lotus* (Lotus corniculatus *L. (cv. Maitland)*), *abomasal and ileal digesta* of sheep fed on lotus with and without infusion of polyethylene glycol (PEG)*

(Mean values for four animals per treatment with their pooled standard errors)

			Abomasal digesta				Ileal digesta			
Amino acid	Essentiality	Lotus	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference
Asparagine		13·6	9·0	11·3	0·27	$P < 0·01$	13·3	11·0	0·24	$P < 0·01$
Threonine	E	5·3	7·3	6·3	0·13	$P < 0·05$	8·0	8·0	0·20	NS
Serine		5·7	5·0	6·5	0·25	$P < 0·05$	7·0	6·0	0·19	NS
Glutamate		12·2	11·8	13·3	0·31	NS	13·8	11·8	0·27	$P < 0·05$
Proline		5·5	4·8	4·8	0·18	NS	8·0	6·3	0·24	$P < 0·05$
Glycine		5·7	4·0	4·3	0·13	NS	4·0	4·5	0·14	NS
Alanine		4·0	6·5	7·8	0·19	$P < 0·05$	7·5	7·3	0·19	NS
Valine	E	5·9	6·5	4·8	0·19	$P < 0·01$	7·0	6·8	0·25	NS
Isoleucine	E	4·8	6·0	4·5	0·14	$P < 0·01$	5·5	5·8	0·19	NS
Leucine	E	9·0	9·0	8·8	0·13	NS	8·0	8·0	0·20	NS
Tyrosine	E[t]	3·6	5·0	5·0	0·20	NS	2·8	4·8	0·27	$P < 0·05$
Phenylalanine	E	5·5	6·7	5·3	0·24	$P < 0·05$	4·3	5·8	0·18	$P < 0·01$
Histidine	E	4·7	3·3	2·3	0·18	$P < 0·05$	2·0	2·3	0·28	NS
Lysine	E	8·4	8·5	7·3	0·19	$P < 0·05$	6·0	6·8	0·31	NS
Arginine	E	6·1	6·5	6·8	0·19	NS	ND	3·8	—	—

NS, not significant; ND, co-elution at arginine peak prevented arginine determination in ileal digesta of control sheep.

[t] Essential in that only source is phenylalanine.

* Composition of PEG-free digesta.

Table 5. *Amino acid (AA) intake, flux through abomasum and apparent loss in the rumen of sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland)) with and without infusion of polyethylene glycol (PEG)*

(Mean values for four animals per treatment with their pooled standard errors)

Amino acid	Essentiality	Intake* (g/d)	Flux of AA through abomasum (g/d)		Loss of AA in rumen (g/d)		Pooled SEM	Statistical significance of difference†
			Control sheep (mean)	PEG sheep (mean)	Control sheep (mean)	PEG sheep (mean)		
Asparagine		28·6	14·9	14·0	13·8	14·6	0·51	NS
Threonine	E	11·1	11·8	7·8	−0·7	3·3	0·33	$P < 0·001$
Serine		11·9	8·2	8·1	3·7	3·8	0·36	NS
Glutamate		25·6	19·7	16·5	5·9	9·1	0·58	$P < 0·05$
Proline		11·6	8·3	6·4	3·3	5·2	0·32	$P < 0·05$
Glycine		11·8	6·7	5·3	5·1	6·5	0·22	$P < 0·05$
Alanine		8·4	10·8	9·7	−2·4	−1·3	0·34	NS
Valine	E	12·3	10·8	6·2	1·5	6·1	0·36	$P < 0·001$
Isoleucine	E	10·1	9·6	5·6	0·5	4·5	0·33	$P < 0·001$
Leucine	E	18·9	14·7	10·8	4·2	8·1	0·47	$P < 0·01$
Tyrosine	E[t]	7·6	8·0	6·3	−0·4	1·4	0·43	NS
Phenylalanine	E	11·6	10·7	6·6	0·9	5·0	0·62	$P < 0·05$
Histidine	E	9·8	5·3	3·0	4·5	6·8	0·28	$P < 0·01$
Lysine	E	17·5	13·8	9·2	3·7	8·3	0·60	$P < 0·01$
Arginine	E	12·9	11·0	8·4	2·1	4·5	0·50	$P < 0·05$
EAA		111·8	95·6	63·9	16·2	47·9	3·20	$P < 0·01$
NEAA		97·9	68·5	60·0	29·4	37·9	2·10	$P < 0·10$

NS, not significant; EAA, essential, NEAA, non-essential AA.

[t] Essential in that only source is phenylalanine.

* Values for PEG sheep have been adjusted to an intake of 1400 g dry matter/d.

† Apparent loss in the rumen is the intake minus the abomasal flux, so that the test of significance applies to both sets of values.

8·2) g/d, suggesting a pre-abomasal apparent loss of 22 and 40% of dietary AA in the control and PEG sheep respectively.

Abomasal digesta from control sheep had significantly higher concentrations of valine and isoleucine ($P < 0·01$) and threonine, phenylalanine, lysine and histidine ($P < 0·05$), but lower concentrations of aspartate ($P < 0·01$) and of serine and alanine ($P < 0·05$) than those of PEG sheep (Table 4). The flux of AA through the abomasum (corrected to equal DM intakes) shows that the control sheep had a significantly greater flux of threonine, valine, isoleucine ($P < 0·001$), leucine, histidine, lysine ($P < 0·01$), glutamate proline, glycine, phenylalanine and arginine ($P < 0·05$) than the PEG sheep (Table 5).

The AA flux at the ileum was 58 (SE 3·5) and 43 (SE 2·0) g/d for the control and PEG sheep respectively. The apparent AA absorptions in the small intestine were 109 (SE 5·5) (control) and 90 (SE 7·3) (PEG) g/d, or 51 and 40% of the respective AA intakes.

Ileal digesta from control sheep contained higher concentrations of aspartate ($P < 0·01$), glutamate and proline ($P < 0·05$), and lower concentrations of phenylalanine ($P < 0·01$) and tyrosine ($P < 0·05$) than PEG sheep (Table 4). The control sheep had a higher flux at the terminal ileum of aspartate, serine, glutamate, proline, glycine, alanine, valine, isoleucine ($P < 0·01$), threonine and leucine ($P < 0·05$) than the PEG sheep.

The effects of PEG treatment on apparent absorption of individual AA in the small intestine, and their apparent digestibility, are summarized in Table 6. Control sheep

Table 6. *Amino acids (AA) apparently absorbed in the small intestine (SI)*, the absorption ratio control: polyethylene glycol (PEG) sheep and proportions of AA entering the abomasum that are absorbed, in sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland)) with and without infusion of PEG*

(Mean values for four animals per treatment with their pooled standard errors)

		Apparent absorption from SI (g/d)					Proportion apparently absorbed in the SI			
Amino acid	Essentiality	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference	Apparent absorption Control:PEG	Control sheep (mean)	PEG sheep (mean)	Pooled SEM	Statistical significance of difference
Asparagine		7·2	9·6	0·50	$P < 0·05$	0·75	0·48	0·63	0·020	$P < 0·001$
Threonine	E	7·2	4·6	0·24	$P < 0·01$	1·57	0·62	0·60	0·019	NS
Serine		4·1	5·7	0·37	NS	0·72	0·50	0·70	0·023	$P < 0·01$
Glutamate		11·8	11·7	0·39	NS	1·01	0·60	0·71	0·013	$P < 0·01$
Proline		3·6	4·0	0·39	NS	0·90	0·43	0·62	0·035	$P < 0·05$
Glycine		4·2	3·5	0·19	NS	1·20	0·63	0·65	0·014	NS
Alanine		6·5	6·8	0·29	NS	0·96	0·60	0·70	0·013	$P < 0·01$
Valine	E	6·6	3·5	0·24	$P < 0·001$	1·89	0·62	0·56	0·013	NS
Isoleucine	E	6·6	3·4	0·28	$P < 0·01$	1·94	0·68	0·60	0·017	NS
Leucine	E	9·9	7·6	0·35	$P < 0·05$	1·30	0·68	0·70	0·010	NS
Tyrosine	E[t]	6·2	4·4	0·36	$P < 0·05$	1·41	0·78	0·69	0·020	NS
Phenylalanine	E	8·3	4·3	0·55	$P < 0·05$	1·93	0·77	0·64	0·022	$P < 0·05$
Histidine	E	3·8	2·0	0·32	$P < 0·05$	1·90	0·71	0·66	0·041	NS
Lysine	E	10·2	6·4	0·55	$P < 0·05$	1·59	0·74	0·69	0·022	NS
Arginine	E	ND	6·8	—	ND	—	ND	0·81	—	—
EAA†		58·8	36·1	2·60	$P < 0·01$	1·62	0·69	0·65	0·015	NS
NEAA		37·4	41·3	1·88	NS	0·90	0·55	0·69	0·016	$P < 0·01$

NS, not significant; ND, co-elution at arginine peak prevented arginine determination in ileal digesta of control sheep; EAA, essential AA; NEAA, non-essential AA.
[t] Essential in that only source is phenylalanine.
* Values for PEG sheep have been adjusted to an intake of 1400 g dry matter/d.
† Arginine values are excluded from this comparison.

Table 7. *Intake and apparent absorption of macroelements before the abomasum (pre-abo), in the small intestine (SI) and in the large intestine (LI) of sheep fed on lotus* (Lotus corniculatus *L. (cv. Maitland)*)

(Values for diets with and without infusion of polyethylene glycol are combined mean values with their standard errors for eight animals)

	Intake (g/d)	Absorption (proportion of intake)	Absorption (g/d)					
			Pre-abo		SI		LI	
			Mean	SE	Mean	SE	Mean	SE
Calcium	12·47	0·05	−0·74	0·40	1·08	0·76	0·25	0·75
Potassium	56·81	0·89	3·52	2·24	41·09	2·73	5·88	1·64
Magnesium	2·71	0·29	0·72	0·05	0·03	0·12	0·04	0·13
Sodium	1·66*	0·67*	−11·32*	1·38	−4·69	1·66	17·11	1·40
Phosphorus	4·56	0·23	−6·63	0·42	7·54	0·55	0·15	0·32
Sulphur	3·67	0·47	0·62	0·13	0·65	0·15	0·48	0·19

* Actual intakes may have been increased from sodium chloride supplement (available in block form, free choice).

absorbed more valine ($P < 0·001$), threonine, isoleucine ($P < 0·01$), leucine, tyrosine, phenylalanine, histidine and lysine ($P < 0·05$) and less aspartate ($P < 0·05$) than PEG sheep. This indicates that the apparent absorption in the small intestine of AA essential to the sheep (EAA), including tyrosine, was 62% greater with 22 g condensed tannins/kg dietary DM than when the tannins were inactivated by PEG. About 10% less non-EAA (NEAA) were apparently absorbed from the small intestine in the control sheep, compared with the PEG sheep.

The apparent digestibility of AA in the small intestine of control sheep was lower for aspartate ($P < 0·001$), serine, glutamate, alanine ($P < 0·01$) and proline ($P < 0·05$) but, with the exception of phenylalanine, EAA digestibility was unaffected by tannins (Table 6).

Carbohydrate digestion

Effects of PEG on non-nitrogenous constituents were minor. Although the digestion of energy was similar in both treatments (0·66 (SE 0·004)) the proportion of energy intake digested in the rumen was lower ($P < 0·05$) in control sheep (0·42 (SE 0·011)) than in PEG sheep (0·47 (SE 0·012)). This was not due to differences in NDF digestion in the rumen. Control sheep digested 0·48 (SE 0·017) and PEG sheep 0·49 (SE 0·016) of NDF intake in the rumen.

There were no treatment effects on digestion of energy in the small (0·19 (SE 0·015)) and large (0·03 (SE 0·012)) intestines, or on digestion of NDF in the small (0·07 (SE 0·026)) and large (0·05 (SE 0·014)) intestines. The higher concentrations of NDF and lignin in ileal digesta of PEG sheep (Table 2) are probably a reflection of protein absorption from digesta of this group.

Mineral digestion

Intakes and apparent absorption of macroelements are summarized in Table 7. The only effect of PEG was to increase apparent absorption of sulphur, potassium and magnesium.

Apparent absorption of S was 1·67 (SE 0·051) g/d in control sheep compared with 1·88 (SE 0·021) g/d in the PEG group ($P < 0·01$). This effect was primarily due to a low apparent absorption pre-abomasum (0·29 (SE 0·092) g/d) in the control sheep compared with 0·94 (SE 0·081) g/d in the PEG sheep ($P < 0·01$).

Apparent absorption of K was also lower ($P < 0.05$) in control sheep (47·7 (SE 1·22) g/d) compared with PEG sheep (53·4 (SE 1·16) g/d). Apparent absorption of Mg was slightly lower when tannins were present, 0·73 (SE 0·020) g/d compared with the PEG sheep, 0·85 (SE 0·045) g/d ($P < 0.10$). The effect of PEG on K and Mg absorption could not be attributed to specific sites of digestion.

DISCUSSION

Possible effects of excessive amounts of PEG on digestion were avoided by infusing only 50 g/d (1·7 g PEG/g tannins) which would displace (Jones & Mangan, 1977) and completely bind all available tannins (Barry & Forss, 1983). The similarity of intakes, digestibility of energy and NDF suggests minimal effects of PEG on non-nitrogenous components.

The lower apparent digestion of N in the control group, compared with those receiving PEG infusion, is consistent with other reports (e.g. Egan & Ulyatt, 1980) of low N digestion in tannin-containing legumes. The increase in rumen NH_3 concentration with PEG infusion provided indirect evidence that PEG was able to remove the protein protection derived through the presence of tannins. The effect of tannins in reducing rumen proteolysis was further evidenced by the higher abomasal NAN and AA fluxes, and lower digestion of energy, but not NDF, and of S in the rumen of the control sheep. Although the apparent digestion of NAN in the small intestine was similar in both groups, the higher ileal AA and N concentrations in the control sheep suggested that the theoretical release of tannin-bound plant proteins for digestion in the small intestine was not complete.

The nutritional advantage conferred by tannins was primarily pre-abomasal and apparently a consequence of reduced microbial degradation of plant proteins in the rumen. In addition to increasing the AA flux to the abomasum, tannins appear to have affected a change in AA composition. The EAA:NEAA value in abomasal digesta was 1·40 for control sheep, compared with 1·08 for PEG sheep, so that at equal intakes the flux of abomasal digesta in control sheep contained 50% more EAA and 14% more NEAA than PEG sheep.

The EAA:NEAA value in lotus was 1·14 and in bacteria it is 1·08 (John, 1984) so that the value of 1·40 in abomasal digesta of control sheep could only arise from reduced microbial growth or selective protection of specific plant proteins, or both. Calculations of the effects of reduced microbial growth, or specific protection of Fraction 1 plant protein (ribulose-1,5-biphosphate carboxylase-oxygenase (*EC* 4.1.1.39); Mangan, 1982) or other plant proteins (65% of lotus protein; J. L. Mangan, personal communication) show that an alteration of the AA content of digesta protein leaving the rumen is theoretically possible. However, none of these options is able to effect an increase in the EAA:NEAA value recorded in the control sheep in this experiment.

John & Lancashire (1981) reported a 15% decrease in microbial N and a 36% increase in plant N leaving the stomach of sheep fed on *Lotus corniculatus* cv. Maitland, which contained 14·5 g condensed tannins/kg DM, compared with cv. Empire (2·5 g condensed tannins/kg DM); however, the AA fluxes were not determined. Studies of AA digestion in sheep have often showed small increases in the EAA:NEAA value between feed and duodenal digesta. Five dried diets (including dried sainfoin) had EAA:NEAA values of 0·97–1·05, and, when given to sheep, the EAA:NEAA values in duodenal digesta were 1·01–1·19 (Harrison *et al.* 1973). These authors also gave fresh red clover, where feed and digesta values were 1·00 and 1·10 respectively. In a study with sheep fed on fresh forages, MacRae & Ulyatt (1974) showed respective feed and duodenal EAA:NEAA values to be 0·91 and 1·10 with white clover (*Trifolium repens* L.), 1·01 and 1·09 with perennial ryegrass

(*Lolium perenne* L.) and 0·93 and 1·06 with short rotation ryegrass (L. *multiflorum* × *perenne*).

The possibility of analytical error being responsible for the very high EAA:NEAA value in abomasal digesta of control sheep (1·40) was considered. However, samples from both PEG and control sheep were treated in an identical manner from collection to AA determination. Also there was no reduction in the quality of AA separation or recovery of AA from hydrolysates of control digesta. Quality assurance material was routinely run with all batches of AA assayed, and gave AA concentrations well within expected analytical variation (Fisher *et al.* 1986).

The apparent digestibilities of EAA in the small intestine in the present experiment were slightly lower than comparable values from other green forages (MacRae & Ulyatt, 1974). The presence of tannins was also associated with a significant depression in apparent digestibility of NEAA in control sheep. It is possible that digestion of all plant AA were depressed by tannins in the control sheep, and that 'essential' endogenous AA secretions were reabsorbed more effectively than 'non-essential' endogenous AA. The net effect of the lower apparent digestibility of NEAA in the control sheep was largely overcome by the higher AA flux to the abomasum, compared with the PEG group.

An increased absorption of EAA could account for the nutritional superiority and higher N retention reported in sheep fed on forages with low levels of condensed tannins (Ulyatt *et al.* 1977; Egan & Ulyatt, 1980; John & Lancashire, 1981). This could be further enhanced by an increased arginine absorption, and stimulation of growth hormone release with a consequent promotion of protein synthesis (Grodsky, 1979). Although apparent absorption of arginine could not be measured in this experiment, the abomasal flux was significantly higher in the presence of tannins.

It is evident that low levels of condensed tannins have dual advantages to ruminants fed on fresh forages. They promote higher rates of N retention than can be achieved from comparable tannin-free forages, and also prevent ruminants bloating (Jones & Mangan, 1977). Further research is required to determine the mechanism by which the concentrations of EAA are selectively increased in abomasal digesta.

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