Nutritive value of *Lotus corniculatus* L. containing low and medium concentrations of condensed tannins for sheep

G.C. WAGHORN, A. JOHN, W.T. JONES AND I.D. SHELTON

Biotechnology Division
DSIR, Palmerston North

ABSTRACT

A reduction in nitrogen degradation in the rumen of sheep fed fresh *Lotus corniculatus* containing 2.3-3.5% condensed tannin (CT) in dry matter has been demonstrated in two experiments. Protection from degradation appeared to favour amino acids essential (EAA) to sheep so that in the presence of 2.2% CT, 50% more EAA and 14% more non-essential amino acids (NEAA) reached the abomasum. Apparent absorption of EAA from the small intestine was 62% higher, and for NEAA 9% lower than in sheep fed the same diet where CT was made ineffective by ruminal infusion of polyethelene glycol. An increased absorption of EAA could explain the high nitrogen retention reported in this and previous experiments where sheep have been fed forages containing low to medium concentrations of CT.

CT did not affect digestion of carbohydrate fractions in the rumen, or over the whole digestive tract. Aspects of microbial growth, rumen pool size, fractional outflow rates and particle size of rumen digesta were determined and are discussed.

Keywords *Lotus corniculatus;* condensed tannins; sheep; amino acids; digestion.

INTRODUCTION

Lotus corniculatus, also known as birdsfoot trefoil, is a perennial forage legume. It is widespread throughout the world, will grow on poorly drained infertile soils and is drought tolerant (Seaney, 1973). Although *Lotus spp.* are currently of minor importance in New Zealand pastures they are noted for their high nutritive value when fed to sheep (John and Lancashire, 1981; Ulyatt, 1981) and do not induce bloat in cattle (Jones and Mangan, 1977).

The high nutritive value and absence of bloat with *Lotus spp.* is often attributed to the presence of condensed tannins (CT), which range from very low to quite high concentrations (0.25-10.6%) in dry matter (DM). Chewing releases CT from intracellular vacuoles enabling the CT to complex with plant proteins under the near neutral pH conditions of the rumen. The stable protein-CT complex prevents micorbial degradation of protein in the rumen, but the complex dissociates at pH < 3.5 allowing protein hydrolysis and absorption to occur in the abomasum and small intestine (Jones and Mangan, 1977). Studies with sheep fed *Lotus pedunculatus* substantiate the protein binding capabilities of CT, but the high levels (6-11% of DM) reduced DM intakes and apparent digestibility of both DM and nitrogen (N) (Barry and Duncan, 1984; Barry and Manley, 1984).

The experiments reported here were designed to investigate processes of digestion in sheep fed *Lotus corniculatus* containing low (up to 0.6%) and medium (0.6-6%) concentrations of CT, and to explain the high nutritive value and N retention in relation to a low apparent N digestibility typical of forages containing CT.

METHODS

Animals and Diet

Two experiments are reported. Both used young Romney wether sheep (45 kg live weight), fitted with rumen and duodenal (Experiment 1) or abomasal (Experiment 2) 'T' piece cannulae. Sheep were held indoors and individually housed in metabolism crates. Water was not provided, but salt lick was available. In both experiments lotus was harvested from pure swards at 0800 h daily and cut into short (50 mm) lengths to facilitate feeding by constant feeder, at hourly intervals.

Experiment 1

This experiment compared 2 cultivars of Lotus: Empire, containing low concentration of CT, and Maitland which contained medium concentrations of CT (Table 1). Six sheep were used, 3 fed Empire and 3 fed Maitland. First cut lotus was fed during February-March after which the treatments were reversed and regrowth was fed during April-May. Feeding levels were judged to be maintenance (800 g DM/d), and 2 weeks were allowed for sheep to become used to the dietary règimen after which digesta flow through the duodenum was determined

with Cr-EDTA and ruthenium-phenanthroline markers administered as a continuous infusion (Faichney, 1975). Rumen volumes were estimated by decline in rumen Cr-EDTA concentration post-infusion. This was followed by a 10 d nitrogen balance (Ulyatt and Egan, 1979), after which the rumen contents were removed, weighed, sampled for particle size determination by wet sieving (Waghorn *et al.*, 1986) and returned to the animal.

The principal measurements included digesta and microbial flux at the duodenum (John and Ulyatt, 1984), rumen pool sizes, fractional outflow rates (K_0) and sites of digestion, total digestion of feed components, and N retention.

Experiment 2

First cut Maitland lotus fed *ad libitum* for 7 d and 90% of *ad libitum* thereafter to 8 wether sheep, 4 of which received an intra-ruminal infusion of 50 g/d of polethylene glycol (PEG), (PEG group). PEG selectively binds to CT and prevents CT from binding to proteins (Jones and Mangan, 1977). The remaining 4 sheep (Control group) received an equivalant infusion of water.

PEG infusions commenced on day 14 of lotus feeding and continued until slaughter on day 24. Rumen ammonia levels were determined prior to and after 3 d of PEG infusion. Faecal collection bags were fitted on day 17 for digestibility determination. Liquid and solid phase markers (^{51}Cr-EDTA and ^{103}Ruthenium-phenanthroline) were infused from day 17 until slaughter to determine digesta flow and site of digestion (Faichney, 1975). Sheep were killed by an overdose of phenobarbitone and a midline incision made, the terminal ileum located, sectioned and diegesta gently 'milked' from about 4 m of terminal ileum for determination of marker concentration.

Analytical and Statistical Methods

In Experiment 1 the fibre components were determined by the method of Van Soest and Wine (1967) and CT by the method of Broadhurst and Jones (1978). Microbial aspects are described by John and Ulyatt (1984) and other chemical analyses are those used by Waghorn *et al.*, 1987. In Experiment 2 amino acid concentrations were determined in abomasal and ileal digesta and in lotus after hydrolysis (Association of Official Analytical Chemists, 1982). A full description of methods used in Experiment 2 is presented in Waghorn *et al.*, (1987).

Statistical comparisons are by analyses of variance. Data are presented as means with pooled standard errors.

RESULTS

Experiment 1 (Low and medium CT lotus cultivars)

Empire contained 0.5% CT in DM compared with about 3.5% in DM with Maitland (Table 1). Differences between cultivars in other components were minor, although the cellulose and neutral detergent fibre (NDF) contents of lotus were higher in first cut (19.0, 39.4% of DM) than regrowth (14.4 and 33.5% of DM respectively). Mean concentrations of other components (% of DM) were: total-N, 3.52; amino acid-N, 2.37; soluble sugars, 9.9; pectin, 5.3; hemicellulose, 6.1; lignin, 10.4. *In vitro* solubility of Fraction 1 protein (W.T. Jones, *pers. comm.*) from macerated plant tissue in phosphate buffer (pH 7.2) was 95% for Empire and 5% for Maitland, suggesting the CT content of Maitland was sufficient to precipitate virtually all of the soluble protein, whereas that of Empire had a minimal effect on protein solubility.

TABLE 1 Digestion of nitrogenous constituents in sheep fed either first cut or regrowth Empire and Maitland cultivars of *Lotus corniculatus* (Experiment 1). Data are means and pooled standard errors.

	First cut		Regrowth		SE
	Empire	Maitland	Empire	Maitland	
Condensed tannin (% feed DM)	0.46	3.29	0.59	3.48	—
Nitrogen intake (g/d)	29.0	25.1	28.9	29.1	0.17
Nitrogen digestion (%)	78.9	69.4	77.0	75.4	0.43
Rumen NAN pool (g)	8.6	10.5	11.0	12.6	0.29
Rumen ammonia-N (mg/*l*)	334	225	284	229	14
NAN digested in rumen (%)	−5.9	−2.4	38.8	25.6	5.3
Feed N digested in rumen (%)	38.8	24.8	66.4	50.5	1.99
Rumen NAN K_0 (per d)[1]	3.35	2.46	1	1.74	0.115
Duodenal NAN flux (g/d)	30.7	25.7	17.7	21.9	1.12
Duodenal microbial N flux (g/d)	12.9	6.8	8.0	7.5	0.237
Duodenal feed N flux (g/d)	17.8	18.9	9.7	14.4	0.703
Apparent N absorption from intestine (g/d)	24.6	18.0	11.0	14.7	1.20
N retention (g/d)	4.5	5.8	1.1	4.4	0.67

Abbreviations: DM dry matter; NAN non-ammonia-nitrogen; N nitrogen
[1] Fractional outflow rate of NAN (duodenal flux/rumen pool)

The larger rumen non-ammonia-N (NAN) pool size in sheep fed Maitland ($P<0.05$) and lower ammonia-N concentration ($P<0.05$, Table 1) are consistent with a lower solubility and reduced deamination of plant proteins when CT were present. The percentage of feed-N digested in the stomach was always lower ($P<0.01$) with Maitland than Empire. Microbial-N flux to the duodenum was lower with Maitland than Empire when first cut lotus was fed (Table 1) but the overall difference between cultivars was not significant. Apparent N absorption from the intestines were similar for the 2 cultivars, so that the higher N retention with Maitland ($P<0.05$, Table 1) appeared to be due to improved utilisation of absorbed-N rather than an increase in N absorption.

Sheep fed Maitland had a larger rumen organic matter (OM) pool than with Empire ($P<0.05$) and a lower fractional outflow rate ($P<0.05$, Table 2), but there were no differences between cultivars, or cuts, in the distribution of DM between rumen particle size fractions. There were no differences between Empire and Maitland in the proportions of OM or NDF intake that were digested in the rumen, although proportions were higher ($P<0.01$) with regrowth than first cut lotus.

Fractional water outflow from the rumen was similar for both cultivars. Water flux from the rumen (7.1 ± 0.49 *l*/d) was considerably higher than feed water intake (4.2 ± 0.33 *l*/d)but only half that of duodenal water flux (14.0 ± 0.33 *l*/d). The importance of endogenous secretions as saliva and from the abomasum are apparent.

Large differences in digestion of first cut and regrowth lotus were evident. Regrowth lotus had a higher proportion of feed-N digested in the rumen ($P<0.01$), a lower fractional outflow rate ($P<0.01$) and a lower duodenal NAN flux ($P<0.01$) with a consequent lower intestinal apparent N absorption ($P<0.01$) and N retention ($P<0.05$, Table 1). Regrowth lotus was also associated with larger rumen DM ($P<0.01$) and water ($P<0.05$) pools, and the amount of organic matter digested in the rumen was higher ($P<0.01$) with regrowth than first cut so the fractional outflow of organic matter and water were both lower ($P<0.05$) with regrowth lotus (Table 2). Organic matter digestibility and the proportion of digestible OM digested in the rumen was higher ($P<0.01$) and the duodenal flux lower ($P<0.01$) with regrowth than first cut lotus (Table 2).

Experiment 2 (Medium CT lotus with and without PEG infusion).

The Maitland lotus fed in this experiment was first cut. On a DM basis it contained a lower proportion of CT (2.2%) and N (2.7%) but more NDF (48.5%) than first cut Maitland in Experiment 1. Other components were similar (soluble sugars, 8.8%; pectin, 4.5%; starch, 4.4%; lignin, 10.3%; ether extract, 4.7% and ash 8.8% of DM).

Despite the high DM intakes, 1400 and 1461 g/d (Table 3), data from Control and PEG sheep in experiment 2 substantiated many of the observations with Maitland and Empire treatments in Experiment 1. Control sheep had similar N intakes but lower ($P<0.01$) rumen ammonia-N concentrations than PEG sheep. Total digestibility of DM and NDF were similar but N digestion was lower in Control sheep ($P<0.001$, Table 3). Significantly less DM and N was

TABLE 2 Pools, fluxes and digestion of dry matter, organic matter and water in sheep fed either first cut or regrowth Empire and Maitland cultivars of *Lotus corniculatus* (Experiment 1). Data are means and pooled standard errors.

	First cut		Regrowth		SE
	Empire	Maitland	Empire	Maitland	
Organic matter intake (g/d)	682	712	715	690	11.5
Organic matter digestion (%)	68.8	67.5	73.4	73.8	0.2
Rumen organic matter pool (g)	240	298	315	325	7.7
DOM digested in rumen (%)	44.3	51.6	71.3	72.6	2.7
Feed OM digested in rumen (%)	30.6	34.8	52.9	53.6	1.20
Feed NDF digested in rumen (%)	27.1	33.2	57.1	56.1	0.73
Rumen organic matter K_0 (per d)[1]	2.07	1.56	1.19	1.00	0.078
Duodenal OM flux (g/d)	473	464	337	319	11.5
Rumen DM distribution between partical size fractions (%)					
>2 mm	10.9	12.2	11.9	11.1	1.09
0.5+1.0 mm	14.3	16.6	17.9	15.0	0.41
<0.25 mm	23.5	24.0	26.6	24.0	0.54
Residues	23.6	24.2	24.3	28.5	0.81
Solubles	25.9	22.0	18.2	20.1	0.38
Rumen water pool (*l*)	2.21	2.85	2.79	2.96	0.08
Rumen water K_0 (per d)[2]	3.15	2.95	1.86	2.65	0.15

Abbreviations: DOM digestible organic matter; OM organic matter; NDF neutral detergent fibre; DM dry matter

[1] Fractional outflow rate of OM (duodenal flux/rumen pool)

[2] Fractional outflow rate of water (rumen outflow/rumen pool)

TABLE 3 Digestion of first cut Maitland *Lotus corniculatus* by sheep with or without an intra-ruminal infusion of polyethylene glycol (Experiment 2). Data are means and pooled standard errors.

	Control	PEG	SE	Treatment effect
Dry matter intake (g/d)	1400	1461	2.7	NS
Nitrogen intake (g/d)	37.8	39.5	0.7	NS
DM digestion (%)	69	71	0.5	NS
NDF digestion (%)	60	62	0.7	NS
N digestion (%)	70	78	0.5	***
Rumen NH_3-N concentration (mg/*l*)	367	504	15.5	**
DM digested in rumen (% of intake)	42	47	0.8	*
NDF digested in rumen (% of intake)	48	49	1.1	NS
N digested in rumen (% of intake)	12	21	1.7	*
Abomasal DM flux (g/d)	874	825	20	NS

Abbreviations: PEG polyethylene glycol; DM dry matter; NDF neutral detergent fibre; N nitrogen; NH_3 ammonia

digested in the rumen of Control sheep ($P<0.05$), without any change in NDF digestion (Table 3).

The flux of N was higher at the abomasum ($P<0.05$), ileum ($P<0.01$) and faeces ($P<0.001$) of Control sheep than PEG sheep (Table 4). In Control sheep an abomasal amino acid (AA) flux of 164.1 g/d represented 78% of AA intake, compared with 124.1 g/d (60% of intake) in PEG sheep ($P<0.01$). In addition to the high AA flux, abomasal digesta of Control sheep comprised a higher proportion of most amino acids essential to sheep (EAA) and some amino acids not essential to sheep (NEAA) than PEG sheep, so that Control sheep had a significantly higher flux of threonine, valine, isoleucine ($P<0.001$), leucine, histidine and lysine ($P<0.01$). There were smaller increases in the flux of phenylalanine and arginine and of glutamate, proline and glycine ($P<0.05$) in Control sheep.

Total-AA flux through the ileum was higher ($P<0.01$) in Control (67.9 g/d) than PEG (46.5 g/d) sheep, suggesting CT may not have released all plant protein for enzymic digestion and absorption in the small intestine. However more total-AA were absorbed from the intestine of Control sheep than PEG sheep ($P<0.01$). Control sheep absorbed 62% more EAA and 9% less NEAA than sheep infused with PEG to remove the effects of CT. Apparent digestibility of EAA in the small intestine were similar for Control and PEG sheep (Table 4) but were lower in Control sheep for aspartate ($P<0.001$), serine, glutamate, alanine ($P<0.01$) and proline ($P<0.05$).

DISCUSSION

Both experiments have demonstrated a reduction in rumen degradation of plant proteins without affecting rumen NDF digestion, when sheep were fed lotus containing 2.2-3.6% CT. The differences in OM digestion and rumen OM pool sizes between Empire and Maitland in Experiment 1 are unlikely to be due to CT, as results from Experiment 2 showed that binding of CT with PEG did not alter digestion, other than N digestion. Larger differences were evident between regrowth and first cut lotus. Larger rumen OM pools with regrowth are unexpected in view of a lower NDF content, yet a more extensive ruminal digestion of both OM and plant N occurred. The lower nutritive value of regrowth lotus is consistent with reports of autumn *v* spring grass (e.g. Macrae *et al.*, 1985).

Sainfoin (*Onobrychis viciifolia* Scop.) also contains medium concentrations of CT and when fed to sheep resulted in a higher N retention but lower apparent N digestibility than grasses and clovers fed at similar levels of intake (Thomson *et al.*, 1971; Ulyatt *et al.*, 1977), which could not be accounted for in terms of urea-N recycling (Egan and Ulyatt, 1980). The relationship between duodenal (and abomasal) NAN fluxes and N intake in sheep fed fresh sainfoin and lotus species is summarised in Fig. 1 and shows a superior NAN conservation in the rumen compared to non-CT containing grasses and legumes. High concentrations of CT in fresh forage DM are associated with significantly ($P<0.05$) higher duodenal NAN/N intake ratios, than low CT concentrations (Fig. 2), although high concentrations are nutritionally disadvantageous to sheep (Barry and Duncan, 1984; Barry and Manley, 1984).

TABLE 4 Net fluxes and apparent absorption of nitrogen and essential amino acids (EAA) and non-essential amino acids (NEAA) by control and polyethylene glycol (PEG) treated sheep fed *Lotus corniculatus* (Experiment 2). Data for PEG group are adjusted to the same nitrogen intake (37.8 g/d) as the Control group. Data are means and pooled standard errors.

	Control	PEG	SE	Treatment effect
Nitrogen flux at the abomasum (g/d)	33.4	29.8	0.63	*
Nitrogen flux at the ileum (g/d)	13.5	8.8	0.51	**
Nitrogen flux to the faeces (g/d)	11.3	8.3	1.18	***
Flux of AA through the abomasum (g/d)				
EAA	95.6	63.9	3.20	**
NEAA	68.5	60.0	2.10	†
Apparent digestibility of AA in the small intestine (%)[1]				
EAA	0.69	0.65	0.015	NS
NEAA	0.55	0.69	0.016	**
Apparent absorption of AA from the small intenstine (g/d)				
EAA	58.8	36.1	2.60	**
NEAA	37.4	41.3	1.88	†

[1] Proportion of AA flowing at the abomasum apparently absorbed from the small intestine.

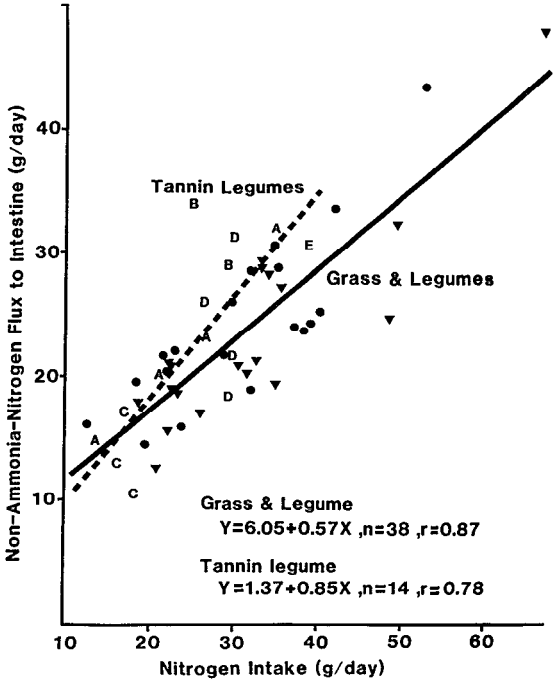

FIG. 1 Nitrogen intake (X) and non-ammonia-nitrogen flux to the intestine (Y) in sheep fed fresh tannin containing legumes (A, B, C, D, E) and fresh grasses (•) and legumes (▼) without tannin. Source: Tannin data A. Egan and Ulyatt (1980); B. Barry and Manley (1984); C. Barry *et al.* (1986); D. Experiment 1; E. Experiment 2; Grass legume data Egan (1974); Macrae and Ulyatt (1974); Egan and Ulyatt (1980); Corbett *et al.* (1982); D.A. Dellow unpublished data; G.C. Waghorn unpublished data.

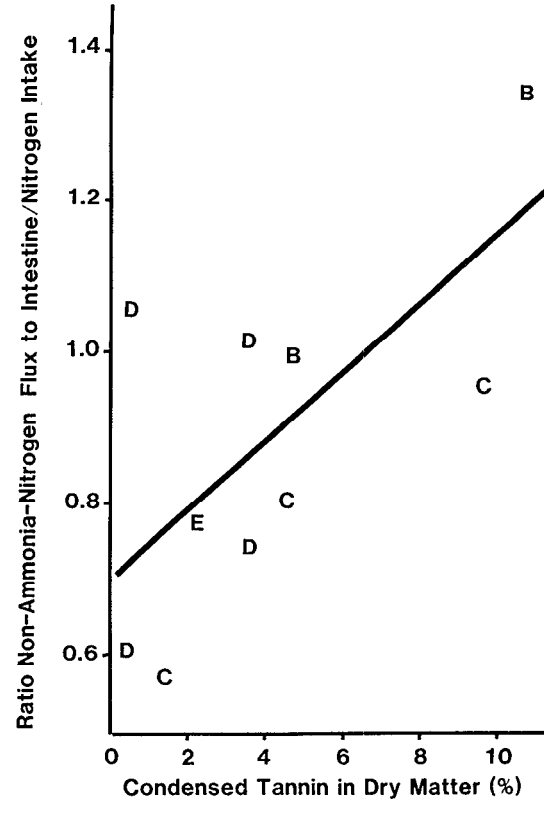

FIG. 2 The relationship between condensed tannin concentration in lotus dry matter (X), and the ratio of non-ammonia-nitrogen flux to the intestine:nitrogen intake (Y) ($Y = 0.71 + 0.045\ X$, $r = 0.66$, $P < 0.05$). Data are those indicated for Fig. 1, with Sainfoin data omitted because the CT were of a different form.

Nutritive value is dependent on both the intake and the quality of nutrients absorbed. Both lotus and sainfoin have a slightly lower nutritive value than white clover but a markedly superior nutritive value to Ruanui ryegrass (Ulyatt, 1981). Part of the high nutritive value may be due to the high ratio of EAA:NEAA in abomasal digesta of Control sheep (1.40) compared to PEG sheep (1.07) and values from duodenal digesta of sheep fed Ruanui ryegrass (1.09) and white clover (1.10) (Macrae and Ulyatt, 1974).

Variation in EAA:NEAA ratios is important because an optimal ratio of 10.4 g AA absorbed per MJ metabolizable energy (ME) intake has been indicated for both sheep (Agricultural Research Council, 1980; Hogan, 1982) and dairy cows (Tamminga, 1981). In Experiment 2, lotus contained about 10.6 MJ ME/kg DM intake so that the ratio of g AA absorbed (excluding arginine and sulphur containing AA; Waghorn *et al.*, 1987) per MJ ME intake was 6.50 (Control group) and 5.24 (PEG group). However the high proportion of EAA absorbed by Control sheep suggests a fixed optimum of 10.4 g AA/MJ ME intake is not always appropriate.

CONCLUSIONS

Medium concentrations of CT are nutritionally advantageous to ruminants fed fresh forages. They prevent bloat in cattle (Jones and Mangan, 1977) and promote higher rates of N retention than can be achieved from comparable CT free forages fed to sheep. We suggest the high rates of N retention are attributable to an elevated absorption of EAA, but at present cannot explain the mechanism by which EAA are protected from degradation in the rumen.

ACKNOWLEDGEMENTS

We thank J.C. Gurnsey, B.R. Sinclair, A. Allen and G. Filby for technical assistance and chemical

analyses and Dr V.J. Thomas for assistance with statistical analyses.

REFERENCES

Agricultural Research Council 1980. *The nutrient requirements of ruminant livestock.* Commonwealth Agricultural Bureaux, Slough.

Association of Official Analytical Chemists 1982. Calculated protein efficiency ratio. *Journal of the Association of Official Analytical Chemists* **65:** 496-497.

Barry T.N.; Duncan S.J. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep 1. Voluntary intake. *British journal of nutrition* **51:** 485-491.

Barry T.N.; Manley T.R. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep 2. Quantitative digestion of carbohydrates and proteins. *British journal of nutrition* **51:** 492-504.

Broadhurst R.B.; Jones W.T. 1978. Analysis of condensed tannins using acidified vanillin. *Journal of the science of food and agriculture* **29:** 788-794.

Corbett J.L.; Furnival E.P.; Inskip M.W.; Pickering F.S. 1982. Protein digestion in grazing sheep. In *Forage protein in ruminant animal production.* British Society of Animal Production. Occasional Publication No. 6. p. 141-143.

Egan A.R. 1974. Protein-energy relationships in digestion products of sheep fed on herbage diets differing in nitrogen concentration and digestibility. *Australian journal of agricultural research* **25:** 613-630.

Egan A.R.; Ulyatt M.J. 1980. Quantitative digestion of fresh herbage by sheep. VI. Utilisation of nitrogen in five herbages. *Journal of agricultural science, Cambridge* **94:** 47-56.

Faichney G.J. 1975. The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In *Digestion and metabolism in the ruminant.* Eds. I.W. McDonald and A.C.I. Warner. University of New England Publishing Unit, Armidale. p. 277-291.

Hogan J.P. 1982. Digestion and utilization of proteins. In *Nutritional limits to animal production from pasture.* Ed. J.B. Hacker. Commonwealth Agricultural Bureaux, Slough. p. 245.

John A.; Lancashire J.A. 1981. Aspects of feeding and nutritive value of *Lotus* species. *Proceedings of the New Zealand Grasslands Association* **42:** 152-159.

John A.; Ulyatt M.J. 1984. Measurement of protozoa, using phosphatidyl choline, and bacteria using nucleic acids or diaminopimelic acid, in the duodenal digesta of sheep fed chaffed lucerne hay (*Medicago sativa* L.) diets. *Journal of agricultural science, Cambridge* **102:** 33-44.

Jones W.T.; Mangan J.L. 1977. Complexes of the condensed tannins of sainfoin (*Onobiychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by polyethelene glycol and pH. *Journal of the science of food and agriculture* **28:** 126-136.

Macrae J.C.; Ulyatt M.J. 1974. Quantitative digestion of fresh herbage by sheep. II. Sites of digestion of some nitrogenous constituents. *Journal of agricultural science, Cambridge* **82:** 309-319.

Macrae J.C.; Smith J.S.; Dewey P.J.S.; Brewer A.C.; Brown D.S.; Walker A. 1985. The efficiency of utilization of metabolizable energy and apparent absorption of amino acids in sheep given spring- and autumn-harvested dried grass. *British journal of nutrition* **54:** 197-209.

Seaney R.R. 1973. Birdsfoot trefoil. In *Forages. The science of grassland and agriculture* 3rd Edition. Eds. M.E. Heath, D.S. Metcalfe and R.F. Barnes. Iowa State University Press, Ames. p. 177-188.

Tamminga S. 1981. Nitrogen and amino acid metabolism in dairy cows. Ph.D. Thesis, Wageningen, p. 122.

Thomson D.J.; Beever D.E.; Harrison D.G.; Hill I.W.; Osbourne D.F. 1971. The digestion of dried lucerne (*Medicago sativa* L.) and dried sainfoin (*Onobrychis viciifolia* Scop.) by sheep. *Proceedings of the Nutrition Society* **30:** 14A.

Ulyatt M.J. 1981. The feeding value of herbage: can it be improved? *New Zealand agricultural science* **15:** 200-205.

Ulyatt M.J.; Lancashire J.A.; Jones W.T. 1977. The nutritional value of legumes. *Proceedings of the New Zealand Grasslands Association* **39:** 107-118.

Van Soest P.J.; Wine R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Agricultural Chemists* **50:** 50-55.

Waghorn G.C.; Reid C.S.W.; Ulyatt M.J.; John A. 1986. Feed comminution, particle composition and distribution between the four compartments of the stomach in sheep fed chaffed lucerne hay at two feeding frequencies and intake levels. *Journal of agricultural science, Cambridge* **106:** 287-296.

Waghorn G.C.; Ulyatt M.J.; John A.; Fisher M.T. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British journal of nutrition* **57:** 11-25.