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The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn

B. R. MIN[1,2], W. C. McNABB[2], T. N. BARRY[1]*, P. D. KEMP[3], G. C. WAGHORN[2] AND M. F. McDONALD[4]

[1] *Institute for Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand*
[2] *Nutrition Group, AgResearch, Grasslands Research Centre, Private Bag 11008 Palmerston North, New Zealand*
[3] *Institute of Natural Resources, Massey University*
[4] *Institute of Veterinary, Animal and Biomedical Science, Massey University, Palmerston North, New Zealand*

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SUMMARY

A grazing experiment, conducted for 55 days (from 4 March to 29 April) in the late summer/autumn of 1997, at Massey University, Palmerston North, New Zealand, compared the reproductive efficiency and wool growth of ewes grazing *Lotus corniculatus* (birdsfoot trefoil) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) dominant pasture (pasture). Half the ewes grazing each forage were given daily oral polyethylene glycol (PEG: molecular weight 3500) supplementation to inactivate the condensed tannins (CT) in lotus. A rotational grazing system with 200 mixed age ewes (54·2 ± 0·88 kg/ewe; 50 ewes/treatment) was used.

The effect of forage species and PEG supplementation upon voluntary feed intake (VFI), concentration of plasma metabolites, reproductive efficiency, wool production and wool characteristics was measured during two synchronized oestrous cycles. The ewes were restricted to maintenance feeding for the first 12 days of each oestrous cycle and then increased to *ad libitum* for the 6 days prior to and including ovulation. Lotus contained 17 g total CT/kg dry matter (DM) in the diet selected. There were only trace amounts of total CT in pasture. *In vitro* organic matter digestibility (OMD) was higher for lotus (0·82 *v.* 0·74) than for pasture, whilst lotus contained less nitrogen (N; 37·8 *v.* 44·5 g/kg OM).

Mean ovulation rates (OR) for CT-acting and PEG sheep grazing pasture and lotus were respectively 1·33 *v.* 1·35 and 1·78 *v.* 1·56, with corresponding lambing percentages being 1·36 *v.* 1·36 and 1·70 *v.* 1·42. Fecundity (number of corpora lutea/ewe ovulating) was greater for ewes grazing lotus than pasture ($P < 0·01$), and tended to be greater for CT-acting than for PEG sheep grazing lotus ($P = 0·06$). In unsupplemented sheep, ewes grazing lotus had increased plasma concentrations of branched chain amino acids (BCAA; 57%) and essential amino acids (EAA; 52%) compared to ewes grazing pasture.

In ewes grazing pasture, PEG administration had no effect on plasma concentrations of urea and free amino acids, VFI, reproductive efficiency and wool production. However, in sheep grazing lotus, plasma concentrations of urea were significantly lower and concentrations of most amino acids were significantly higher for CT-acting than for PEG supplemented ewes (CT not acting); there was no difference in VFI between these two groups. Compared to ewes grazing pasture, ewes grazing lotus had similar VFI but produced more wool with longer staples and thicker fibre diameter, with there being no effect of PEG supplementation.

It was concluded that feeding lotus increased the efficiency of both reproduction and wool production without an increase in VFI, and that a possible cause was the action of CT in increasing plasma EAA and especially BCAA concentration.

* To whom all correspondence should be addressed. Email: T. N. Barry@massey.ac.nz

INTRODUCTION

Most New Zealand (NZ) vegetative pastures contain high concentrations of nitrogen (N; 25–35 g/kg dry matter (DM)) and metabolizable energy (ME; 11–12 MJ/kg DM), and carbohydrate digestion is efficient on such diets (Ulyatt & MacRae 1974; Waghorn & Barry 1987). However, 25–30 % of the N eaten is lost across the rumen because the rapid degradation of soluble protein to ammonia-N exceeds the capacity for microbial protein synthesis (MacRae & Ulyatt 1974; Ulyatt *et al.* 1975; Beever 1993). Therefore the performance of ruminants grazing on fresh pasture could be limited by protein supply, because protein absorption from the small intestine is low in relation to ME intake (Barry *et al.* 1982; Waghorn & Barry 1987).

Condensed tannins (CT) are polyphenolic compounds known to precipitate dietary proteins, with the extent of this reaction being dependent on the concentration, molecular weight and structure of the CT and on protein structure (Jones & Mangan 1977; Martin & Martin 1983; Asquith & Butler 1986; Spencer *et al.* 1988). The CT:protein complex is stable and insoluble at pH 3·5–7·0 and medium concentrations of CT in *Lotus corniculatus* (30–35 g/kg DM) have reduced protein solubility and degradation in the rumen (Min *et al.* 1998*a*), increased the absorption of essential amino acids (EAA) from the small intestine by 62 % (Waghorn *et al.* 1987*a*) and increased the flow of cysteine to body synthetic reactions (Wang *et al.* 1994). In long term grazing experiments with sheep, the increase in EAA absorption caused by the action of CT in *L. corniculatus* increased wool growth by 12 % during summer (Wang *et al.* 1996*a*; Min *et al.* 1998*b*) and increased milk protein secretion by 14 % in mid and late lactation during spring (Wang *et al.* 1996*b*).

Increased protein absorption has been implicated in increasing the ovulation rate (OR) of ewes (Smith 1991), and this was illustrated by an increase in ewes showing multiple ovulations when given abomasal infusions of lactalbumin and soya protein isolate (73 *v.* 55 %; Cruickshank *et al.* 1988). Subsequent work correlated this response to an increase in plasma concentration of branched chain amino acids (BCAA; valine + leucine + *iso*-leucine; Waghorn *et al.* 1990). It therefore seems possible that the increased supply of protein and, especially of BCAA, caused by the action of CT could be used to increase reproductive efficiency in grazing ewes.

The objectives of the present investigation were to measure effects of CT in *L. corniculatus* upon reproductive efficiency and wool production in grazing ewes during autumn. Perennial ryegrass/white clover dominant pasture, containing only trace amounts of CT, was grazed by similar animals as a control diet.

MATERIALS AND METHODS

Experimental design

A grazing trial involving 200 mixed age Romney ewes, including 100 rising 2-year-olds mated for the first time, was conducted at Massey University, Palmerston North, NZ, from 4 February 1997 (late summer) to 29 April (autumn) 1997 (85 days). The experiment was a 2×2 factorial design, using two types of forage (*L. corniculatus v.* perennial ryegrass/white clover pasture), with half the ewes grazing each forage receiving a twice daily oral supplement of polyethylene glycol (PEG; MW 3500; PEG-supplemented group). The PEG binds with CT, preventing the CT from binding with protein (Jones & Mangan 1977; Barry & Manley 1986). Effects of CT can be quantified by comparing unsupplemented ewes (CT-acting) with ewes given PEG (CT-inactivated). The experiment was conducted over three oestrous cycles, with oestrus being synchronized in each cycle for all ewes. Reproductive efficiency was measured as OR in three synchronized oestrous cycles using laparoscopy and as lambs born/ewe; data are expressed in terms of fertility (ewes cycling/ewes mated) and fecundity (number ovulations/ewe ovulating). Wool production was determined by shearing the ewes at the end of the experiment (from 4 February to 29 April 1997); both fleece weight and wool processing characteristics were measured.

Forages

Pure vegetative *L. corniculatus* (birdsfoot trefoil; cv. Grasslands Goldie) and pasture were grazed in breaks by the ewes, with each break lasting 3 or 4 days. Measurement of herbage mass before and after grazing and collection of samples of feed on offer (cut to soil level) and diet selected (using sheep fistulated in the oesophagus; OF) were performed as described by Min *et al.* (1998*b*). Pre-and post-grazing herbage mass were determined weekly, immediately before and after grazing, by cutting eight random quadrats ($6 \times 0{\cdot}125$ m^2) per paddock to ground level and drying at 90 °C for 17 h. A further eight samples per paddock were cut to ground level, pooled, and stored at −20 °C for nutritive value analysis of feed on offer. The diet selected was determined using six OF Romney sheep, which allowed sampling for organic matter digestibility (OMD), total nitrogen (N) and CT. Samples were stored −20 °C, and then freeze dried and ground for chemical analysis.

Grazing management

Total grazing days (TGD; Min *et al.*, 1998*b*) were calculated using Equation 1.

$$TGD = \frac{HM \times PA}{n \times FA} \quad (1)$$

where HM is herbage mass (kg DM/ha), PA is paddock area (ha), *n* is number of animals, and FA is feed DM allowance/head per day (kg). This management was intended to provide vegetative high quality forage at all times. The relatively short period of grazing each break (3–4 days) was in order to provide reasonably constant levels of available feed at all times.

All 200 ewes were rotationally grazed on the pasture during the first 30 days, which included the first ovulation cycle, with feed allowance restricted to 1·5 kg DM/ewe per day, so that the ewes were fed at close to the maintenance level of energy intake. They were then randomly allocated to treatment groups, which were balanced for age of ewe, and grazed on either pasture (100 ewes) or lotus (100 ewes) during the second and third ovulation cycles (34 days), with and without PEG supplementation ($n = 50$ ewes/group). During ovulation cycles 2 and 3 the feed allowance was kept at maintenance for the first 12 days of each cycle, and then increased to *ad libitum* allowance (*c.* 2·2 kg DM/ewe) for the 6 days prior to and including ovulation. Day of ovulation was defined as day zero. After ovulation, the feed allowance was reduced to maintenance again. The rationale for this was that a minimum of 6 days of increased protein supply immediately prior to ovulation was required to increase ovulation rate in sheep (Smith *et al.* 1983; Stewart & Oldham 1986). *Ad libitum* feeding over this period was designed to give the action of CT in *L. corniculatus* maximum opportunity for increasing EAA supply over this critical period, whilst restricted feeding ensured that all ewes on both forages were kept close to the maintenance level of energy intake at all other times. At the end of oestrous cycle 3, the ewes continued grazing either lotus or pasture for a further 21 days, with and without PEG supplementation, taking the total period of lotus feeding to 55 days. The groups were then joined and grazed on pasture until lambing.

Animals

Mean initial liveweight (LW) was 52·1 kg (S.D. 0·38) for rising 2-year-old ewes and 56·3 kg (S.D. 0·49) for ewes aged 3 years or older. At the start of the experiment, all ewes were weighed, tagged and drenched with anthelmintic (Ivomec; Merck, Sharp & Dohme, NZ Ltd) to control internal parasites, and treated for external parasites (Wipeout; Coopers Animal Health NZ). Anthelmintic (Ivomec) was then given at monthly intervals to all ewes. The animals were weighed at fortnightly intervals, and feed supply adjusted if needed to keep the ewes at maintenance. All ewes were shorn before the experiment commenced and again at the conclusion of the grazing experiment (29 April). The dose of PEG was calculated on the basis of estimated daily VFI and the CT content in lotus, with the objective of administering 1·8 g PEG/g CT, this being the minimum amount to bind all the CT and prevent binding with soluble protein (Barry & Forss 1983). The PEG (71 g/d during maintenance and 110 g/d during *ad libitum* feeding) was administered as a 50% w/v solution, given daily as two equal doses at 08·00 and 16·00 h. The VFI was measured using slow release chromium capsules (Cr_2O_3 matrix; Nufarm, Auckland, NZ), according to the method described by Parker *et al.* (1989) and Min *et al.* (1998*b*). Sixteen ewes per treatment group were used to estimate VFI. Four rumen-fistulated Romney sheep were grazed on each forage for 27 days to measure the Cr release rate of capsules suspended in the rumen. Measurements started on day 5 after chromium capsules insertion, and proceeded at 3-day intervals until day 27.

Synchronization of oestrus and determination of ovulation rate

Ovulation was synchronized using controlled release intravaginal devices (CIDR; type G; Carter Holt Harvey; containing 0·3 g progesterone). They were inserted for 12 days of the first ovulatory cycle and were inserted for 8 days of the second and the third cycles. Ewes were mated with vasectomized teaser rams fitted with tupping crayons (four rams per group; during 8 days) at the first and the second ovulatory cycles and then entire rams fitted with tupping crayons (five rams per group; during 25 days) were mated with ewes at the third cycle. Ovulation rate was determined by counting corpora lutea (CL) using laparoscopy (Kelly & Allison 1976) *c.* 7 days after oestrus started. A total of three laparoscopy measurements were made (4 March, cycle 1; 20 March, cycle 2 and 7 April, cycle 3), following the initial synchronization. Subsequent lambing records were collected, including birth rank and birth weight.

Plasma samples

Blood samples (2×7 ml) were taken from the jugular vein of 80 sheep grazing each forage (40 PEG and 40 CT-acting) on day -8 (maintenance feed) and on day -1 (*ad libitum*) before ovulation during each oestrous cycle to measure plasma urea concentration. Samples for plasma amino acid analysis were taken from the same sheep on day -1 at the third cycle only. The blood samples were collected into vacutainers with

Na-EDTA (Becton Dickinson, USA) as an anti-coagulant and placed on ice. Sample preparation for cysteine and urea were performed using the methods described by Min *et al.* (1998*b*). One vacutainer was held on ice and centrifuged (3200 ***g*** for 20 min at 4 °C) to obtain blood plasma for cysteine and urea analysis. The samples for cysteine analysis was treated with 0·5 ml 0·75% sodium dodecyl sulphate (SDS), 9 mM Na-EDTA, 0·1 ml 200 mM phosphate buffer pH 8·0 containing 90 mM dithiothreitol (DTT) in order to release the protein-exchangeable cysteine. The concentration of cysteine was determined by Continuous Flow Auto Analyzer (CFAA; Technicon, Dublin, Ireland) using the method described by Gaitonde (1967) modified to include acid ninhydrin reagent, following incubation at 95 °C.

Whole blood from the second vacutainer tube was used for the analysis of all other amino acids. Whole blood (1 ml) was mixed with 1 ml 200 μM norleucine in milliQ water (added as internal standard) and 0·1 ml 200 mM phosphate pH 8·0 containing 80 mM DTT in a Centrisart tube (Sartorius; MW cut-off 10000; Gottingen, Germany), vortexed and then centrifuged at 1000 ***g*** for 15 min followed by 6000 ***g*** for 85 min. The clear deproteinized supernatant was filtered through a 0·2 μm syringe filter into an micro-centrifuge tube and stored at −85 °C for analysis. All amino acid concentrations except cysteine were determined using a HPLC (Waters Associates, USA) equipped with an ion-exchange column (Na^+-form AA analysis column; Waters Associates, USA). Amino acids were detected using post-column derivatization with ninhydrin. Absorbance was measured at 570 nm. Plasma urea concentration was determined with an enzymatic ultraviolet colourimetric assay that utilizes glutamate dehydrogenase and NADH, using an Auto-Analyzer (COBAS FARA, Basel, Switzerland).

Laboratory analyses

Forage and faeces

Samples of feed offered and diet selected were stored at −20 °C, freeze-dried and ground to pass through a 1 mm diameter sieve for laboratory analysis. Acetone/water-extractable, protein-bound and fibre-bound CT fractions in forages and OF extrusa were determined using a butanol-HCl colorimetric procedure (Terrill *et al.* 1992). All CT concentrations were determined using CT extracted from *L. pedunculatus* as a reference standard (Jackson *et al.* 1996). Total N was determined by the Kjeldahl method, OM by ashing samples for 16 h at 550 °C and *in vitro* OMD by the enzymatic method of Roughan & Holland (1977). Chromium in faeces was determined by the method of Costigan & Ellis (1987). As extractable and protein-bound CT would be dissolved in the initial *in vitro* extraction steps, but are known to be indigestible *in vivo* (Terrill *et al.* 1994), extractable and protein-bound CT (% OM) values were deducted from all *in vitro* OM digestibilities.

Wool samples

Fleeces were weighed at shearing to determine greasy fleece weight, with samples of 200–300 g being taken from both the left and right mid-side areas for laboratory analyses. A standard greasy wool washing procedure and measurements of staple length (cm), mean fibre diameter (MFD; μm) and wool colour were made using the methods described by Min *et al.* (1998*b*).

Calculation of data and statistical analyses

Ovulation data were analysed in terms of fertility (ewes ovulating/ewes mated) and fecundity (CL/ewe ovulating). The fecundity and lambing percentage of ewes giving birth are presented in the form of a percentage of ewes in the group having multiple (one, two or more ovulations). For statistical analysis the data were transformed using a Logistic Regression Model (SAS 1995) and treatment effects established using the chi-squared procedure, as described by Smith (1985). The other treatment means for plasma urea and plasma AA concentrations were analysed by General Linear Models procedures using the Statistical Analysis System package (SAS 1985), with the factors fitted being forage type, PEG supplementation and the interaction. Data are presented as mean values, together with the standard error of the mean (S.E.); the number of observations contributing to each mean is denoted in all tables by the letter *n*.

RESULTS

Forages

Both pasture and lotus were in a vegetative state throughout the experiment. Pre- and post-grazing herbage mass were generally similar for both forages and were higher during *ad libitum* than maintenance feeding (Table 1).

Chemical composition

Total CT concentration in the lotus was 23 g/kg DM for feed offered and 17 and 9 g/kg DM for OF extrusa representing the diet selected at the beginning and end of grazing each break (Table 2). Only trace amounts of total CT were detected in pasture. Most CT in the lotus on offer was readily extractable (65%), with much smaller amounts being protein-bound (32%) or fibre-bound (3%; Table 2). In the diet selected (OF extrusa), a much lower component was readily extractable (23–35%), with the largest component being protein-bound (55–62%).

Table 1. *Pre-grazing and post-grazing forage mass* (*dry matter*; *DM* (*t/ha*)) *of* Lotus corniculatus (*cv. Grasslands Goldie*) *and perennial ryegrass/white clover pasture*

	Forage mass (t DM/ha)				
	Pasture		Lotus		
	Pre-grazing	Post-grazing	Pre-grazing	Post-grazing	S.E.
Maintenance intake period*					
n	8	8	5	5	
DM (t/ha)	2.49	1.12	2.06	0.61	0.144
Ad libitum period†					
n	3	3	3	3	
DM (t/ha)	2.80	1.44	3.23	1.40	0.208

* Fed for the initial 12 days in each cycle (8 Feb–20 Feb, 27 Feb–8 Mar and 14–25 Mar) before *ad libitum* feeding started.
† Fed for the final 5 days in each cycle (21–26 Feb, 9–14 Mar and 25–30 Mar), with day of ovulation = day 0.

Table 2. *Organic matter* (*OM*), *total N* (*N*) *and condensed tannin* (*CT*) *contents and* in vitro *OM digestibility of feed offered and diet selected by sheep grazing* Lotus corniculatus (*cv. Grassland Goldie*) *and perennial ryegrass/white clover pasture*

	Feed on offer		Diet selected (OF extrusa)*				
			Early†		Late†		
	Pasture	Lotus	Pasture	Lotus	Pasture	Lotus	S.E. (D.F. 28)
n	7	7	6	6	4	4	
OM (g/kg DM)	893	900	851	844	857	848	6.88
In vitro OMD (% OM)							
Unadjusted	64.9	78.7	81.8	84.4	76.3	86.8	3.60
Adjusted†	64.8	76.4	81.6	82.9	76.0	86.0	3.59
Total N (g/kg OM)	41.5	29.0	49.6	41.1	42.3	43.3	0.43
Condensed tannin (g/kg DM)							
Extractable CT	0.3	14.9	1.1	5.7	1.6	2.1	0.49
Protein bound CT	0.5	7.4	1.2	9.0	1.5	5.6	0.43
Fibre bound CT	0.3	0.8	1.8	1.8	1.0	1.3	0.26
Total CT	1.1	23.1	4.1	16.5	4.1	9.0	0.62
(%) Bound CT	73.0	35.5	73.1	65.4	61.0	77.0	–

* OF: Oesophageal fistulae.
† Early: samples taken at the start of grazing each break; Late: samples taken at the end of grazing each break.
‡ After deducting extractable and protein-bound CT, as described in laboratory analysis.

For both forages, *in vitro* OMD and total N concentration were higher for the diet selected than for feed on offer. *In-vitro* OMD was slightly higher for lotus than for pasture in both feed on offer and OF extrusa. Lotus contained lower contents of N in both feed on offer and early OF extrusa than pasture, but was similar for late OF extrusa.

Plasma metabolites

There were no differences in plasma urea concentration between the four groups of animals during ovulatory cycle 1, when all animals grazed pasture without PEG administration. Plasma urea concentrations in sheep grazing lotus during ovulatory cycles 2 and 3 were significantly lower for CT-acting than for PEG-supplemented animals (Table 3) during both maintenance and *ad libitum* ($P < 0{\cdot}01$) feeding, but PEG did not affect plasma urea concentrations of sheep fed pasture.

For sheep grazing pasture, PEG supplementation generally had no effect on plasma amino acid concentration (Table 4). Comparing the unsupplemented groups, plasma concentration of most amino

Table 3. *Plasma concentration of urea* (*mM*) *in ewes grazing perennial ryegrass/white clover pasture and* Lotus corniculatus (*cv. Grasslands Goldie*), *with and without twice-daily oral administration of polyethylene glycol* (*PEG*; *MW* 3500)

	Pasture		Lotus		
Oestrous cycles	PEG-sheep	CT-acting	PEG-sheep	CT-acting	S.E. (D.F. 76)
n	20	20	20	20	
1st cycle (Both groups grazed on pasture)					
Maintenance intake period	6.9	6.5	7.1	6.9	0.22
Ad libitum intake	8.4	8.3	8.5	8.4	0.25
2nd cycle (Ewes grazed on either pasture or lotus)					
Maintenance intake period	4.6	4.5	5.4	4.5	0.19
Ad libitum intake	5.1	4.8	9.2	8.3	0.20
3rd cycle (Ewes grazed on either pasture or lotus)					
Maintenance intake period	6.1	6.2	8.6	7.7	0.22
Ad libitum intake	4.5	4.1	7.5	6.6	0.23

Table 4. *Plasma concentration of amino acids* (μM) *in ewes grazing perennial ryegrass/white clover pasture and* Lotus corniculatus (*cv. Grasslands Goldie*) ad libitum, *with and without twice-daily oral administration of polyethylene glycol* (*PEG*; *MW 3500*)

	Pasture		Lotus		
Amino acids	PEG-sheep	CT-acting sheep	PEG-sheep	CT-acting sheep	S.E. (D.F. 69)
n	18	18	19	18	
EAA*					
Valine	138	126	161	199	5.2
Leucine	77	69	93	108	3.8
Iso-leucine	52	45	58	72	3.3
Tyrosine	46	41	41	49	2.8
Phenylalanine	30	30	30	38	2.7
Histidine	54	56	59	67	3.8
Tryptophan	106	105	177	247	6.4
Lysine	103	89	100	129	5.2
Arginine	34	51	71	68	5.7
Threonine	114	99	77	106	5.5
Methionine	12	12	12	15	1.8
Cysteine ($n = 10$)	28	33	33	35	1.5
NEAA†					
Asparagine	17	27	21	25	3.2
Serine	85	85	62	86	4.7
Glutamate	188	186	192	254	6.4
Proline	61	68	69	96	5.3
Glycine	366	338	330	480	10.8
Alanine	139	116	120	149	5.5
BCAA‡	267	241	312	379	12.8
EAA*	786	742	894	1128	35.9
NEAA†	856	819	793	1091	35.8

* Essential amino acids (including BCAA and cysteine).
† Non-essential amino acids.
‡ Branched-chain amino acids (valine, leucine and iso-leucine).

Table 5. *The effect of grazing ewes on* Lotus corniculatus *or perennial ryegrass/white clover pasture, and of supplementation with polyethylene glycol (PEG; MW 3500), on ovulation rate (CL/ewe mated), lambing (lambs born/ewe mated) and lamb birth weight (kg/lamb)*

	Pasture		Lotus		
	PEG-sheep	CT-acting	PEG-sheep	CT-acting	S.E. (D.F. 196)
Ovulation rate (OR)					
n	50	50	50	50	
First cycle	1.29	1.42	1.34	1.45	0.090
Second cycle	1.23	1.35	1.32	1.43	0.081
Third cycle	1.35	1.33	1.56	1.78	0.080
Lambing (lambs born/ewe mated)	1.36	1.36	1.42	1.70	0.097
Birth weight					
Single					
*n**	29	32	27	21	
Mean value	5.21	5.28	5.12	5.00	0.153
Twin					
*n**	28	24	40	42	
Mean value	4.44	4.16	4.60	4.35	0.184
Triplet					
*n**	3	3	—	15	
Mean value	4.93	3.03	—	3.80	1.031

* Number of lambs.

Table 6. *The effect of grazing ewes on* Lotus corniculatus *or perennial ryegrass/white clover pasture, and of supplementation with polyethylene glycol (PEG; MW 3500), on fecundity at ovulation (number of CL/ewe cycling) and at lambing (lambs born/ewe lambing)*

	Pasture			Lotus			
	Fecundity			Fecundity			
	1	2	3–4	1	2	3–4	S.E. (D.F. 97)
Ovulation rate (% OR)							
n		50/group			50/group		
Cycle 1							
PEG-sheep	59.6	40.4	0.0	51.1	48.9	0.0	0.40
CT-acting	52.2	45.7	2.2	51.1	46.8	2.1	0.40
Cycle 2							
PEG-sheep	74.0	26.0	0.0	62.5	35.4	2.1	0.45
CT-acting	69.4	26.5	4.1	59.2	38.9	2.0	0.45
Cycle 3							
PEG-sheep	66.7	31.4	2.0	40.8	55.1	4.1	0.60
CT-acting	69.4	28.6	2.0	30.6	61.2	8.2	0.60
Lambing	(%)						
n		25/group			25/group		
2 years old							
PEG	81.8	18.2	0.0	68.2	31.8	0.0	0.77
CT-acting	82.6	17.4	0.0	63.6	31.8	4.6	0.77
3 years and older							
PEG	50.0	45.5	4.5	48.0	52.0	0.0	0.61
CT-acting	59.1	36.4	4.6	29.2	58.3	12.5	0.61

Table 7. *Organic matter intake* (*OMI*), *liveweight gain* (*LWG*), *wool production and wool processing characteristics of sheep grazing* Lotus corniculatus *and perennial ryegrass/white clover pasture, with or without polyethylene glycol* (*PEG*; *MW 3500*) *supplementation* (*85 days*)

	Pasture		Lotus		
	PEG-sheep	CT-acting	PEG-sheep	CT-acting	S.E. (D.F. 195)
OMI (kg/ewe/d)					
n	16	13	16	16	
Mean value	1.98	1.83	1.85	1.70	0.087 (D.F. 57)
LWG					
n	50	50	49	50	
Mean value (g/day)	4.5	18.6	33.8	40.3	6.88
Wool weight					
n	50	49	49	49	
Greasy fleece (kg)	1.32	1.38	1.64	1.56	0.029
Clean fleece	1.14	1.09	1.31	1.35	0.027
(kg) (g/day)	13.5	12.9	15.4	15.9	0.32
Clean belly (g)	86.5	84.6	86.1	95.9	4.49
Efficiency of wool production (g clean wool/kg OMI/day)	7.35	7.01	8.38	8.67	0.175
Wool characteristics					
Staple length (cm)	4.12	4.00	4.27	4.37	0.082
(mm/day)	4.85	4.70	5.02	5.14	0.092
Mean fibre diameter (μm)	38.7	39.7	40.1	40.8	0.50
Colour					
Brightness*	65.0	64.9	63.8	64.9	0.25
Yellowness†	0.18	0.17	0.49	0.15	0.078

* Measured as Y value.
† Calculated as Y-Z values.

acids were significantly higher ($P < 0{\cdot}01$) in sheep grazing lotus than those grazing pasture, with the increase being 52% for total EAA, 57% for BCAA and 25% for methionine. Most of the lotus effect can be explained by the action of CT, which significantly increased plasma concentration of all EAA except arginine ($P < 0{\cdot}01$).

Reproductive rate

Effects of the nutritional treatments upon mean ovulation rate, lambing percentage and lamb birth weight are shown in Table 5, whilst treatment effects upon ewe fecundity are given in Table 6. Mean birth weight of single, twin and triplet lambs was respectively 5·2, 4·4 and 3·9 kg per lamb, and these were not affected by the nutritional treatments (Table 5).

Ewe fertility increased from 92% in cycle 1 to 98% in cycle 2 and then 100% in cycle 3, and was not affected by forage type, PEG supplementation or age of ewe (2 years *v.* 3 years and older). Fecundity at ovulation was consistently less for rising 2-year-old than for older ewes ($P < 0{\cdot}01$), but there were no interactions between age of ewe and the nutritional treatments. PEG supplementation had no effect upon the fecundity of ewes grazing pasture (Table 6). Fecundity of ewes grazing lotus was greater than that of ewes grazing pasture during cycle 3 ($P < 0{\cdot}01$), with this trend becoming apparent during cycle 2 ($P < 0{\cdot}11$). During cycle 3, fecundity of CT-acting ewes grazing lotus was greater than that of PEG-supplemented ewes (CT not acting; $P = 0{\cdot}06$).

Fertility at lambing was 90·1% for rising 2-year-old ewes and 95·1% for older ewes ($P > 0{\cdot}05$), and was not affected by the nutritional treatments. Fecundity at lambing was less for rising 2-year-old than for older ewes ($P < 0{\cdot}001$), with no interactions between age and nutritional treatments, and was greater for ewes that grazed lotus than pasture during cycles 2 and 3 of ovulation ($P < 0{\cdot}05$), with a component of this due to action of CT in the ewes that grazed lotus ($P = 0{\cdot}12$; Table 6). The increased fecundity of ewes grazing lotus is indicated by fewer ewes having only one CL and giving birth to one lamb, and to more ewes having multiple ovulations and giving birth to two or more lambs. These effects were apparent in both nutritional treatment groups and both ages of ewe grazing lotus, but the effect was of greater magnitude in the CT-acting than the PEG supplemented ewes and the CT effect tended to be greater in older ewes.

Voluntary feed intake, liveweight gain, wool production and wool processing characteristics

VFI was similar for ewes grazing pasture and lotus, and was not affected by PEG supplementation (Table 7). Liveweight gain was low, but was higher in sheep grazing lotus than pasture ($P < 0{\cdot}001$). Clean fleece weight ($P < 0{\cdot}001$), efficiency of wool production (g clean wool/kg OMI per eaten; $P < 0{\cdot}001$), staple length (cm; $P < 0{\cdot}01$) and mean fibre diameter ($P < 0{\cdot}01$) were all significantly higher in ewes grazing lotus than those on pasture, with no effects due to PEG supplementation. In ewes grazing lotus, PEG supplementation significantly increased wool yellowness ($P < 0{\cdot}05$) and reduced brightness ($P < 0{\cdot}01$), but had no effect in ewes grazing pasture.

DISCUSSION

The most significant findings in this study were that ewes grazing lotus rather than pasture for two oestrus cycles during autumn increased both lambing percentage (25%) and wool production (14%) with no changes in VFI, thereby increasing the efficiency of both lamb and wool production. The increase in reproductive rate was due to increases in fecundity at both ovulation and birth, with no effect on fertility. Responses to PEG supplementation showed that a major reason for the effect of lotus on reproductive rate was its CT content. The CT in the diet probably increased EAA absorption (Waghorn *et al.* 1987*a*, *b*, 1990). The reduced plasma urea concentration and increased plasma concentration of most free amino acids in CT-acting compared to PEG supplemented sheep grazing lotus in the present experiment is consistent with these findings. This is the first report of forage CT increasing reproductive rate in ewes. The close similarity between cycle 3 OR and lambing data suggests that there was minimal embryonic loss in this experiment.

There is strong evidence showing that an improved rate of nutrition, especially protein content in the diet, can result in an increased OR (Smith 1991). The importance of protein nutrition was confirmed by Cruickshank *et al.* (1988), who found that abomasal infusions of lactalbumin and soya protein isolate increased the number of ewes having multiple ovulations from 55 to 73%. Subsequent work reported that the strongest correlation was found between OR and the plasma concentration of BCAA ($r = 0{\cdot}95$) and EAA ($r = 0{\cdot}61$) (Waghorn 1986; Waghorn *et al.* 1990). This has been confirmed by the findings that intravenous infusion of a BCAA mixture (33·1 g total BCAA/ewe per day) over a 5 day period in the late stages of the oestrous cycle (before luteolysis), produced an increase in OR (2·4 *v.* 1·5; Downing & Scaramuzzi 1991; Downing *et al.* 1995). One of the explanations for the increased fecundity of ewes grazing lotus in the present experiment may be their higher circulating plasma concentration of BCAA relative to pasture-fed ewes, especially in the CT-acting group. The critical period for protein supplementation to increase OR is the last 6 days before ovulation (Stewart & Oldham 1986) which is why the feeding level was increased to *ad libitum* over this critical period in this study. An additional possibility is that rumen ammonia production on the pasture and lotus + PEG diets may have elevated plasma ammonia concentration to the point where it reduced the survival of ova (Kaur & Arora 1995) and that action of CT in lotus reduced this. Plasma ammonia concentration needs to be measured in future experiments of this type.

Nutrition (protein and energy) influences ovarian function (including OR) through modulating the secretion of gonadotrophins (i.e. follicular stimulating hormone (FSH) (Davis *et al.* 1981; McNatty *et al.* 1985; Thompson & Smith 1988), but studies in sheep (Cruickshank *et al.* 1990; Downing & Scaramuzzi 1991; Downing *et al.* 1995) have not shown consistent effects of protein nutrition on peripheral concentrations of gonadotrophins in ovariectomized ewes. It has been suggested that changes in metabolic hormones such as growth hormone (GH), insulin and insulin-like growth factor (IGF), which consistently accompany the nutrition-induced alteration in body energy, protein balance and muscle protein synthesis (Garlick *et al.* 1983; Pell & Bates 1990; Downing *et al.* 1995), can affect ovarian function, either directly or by modulating gonadotrophin actions at the ovarian level (Smith 1991; Gong & Webb 1996). Furthermore, both insulin and EAA, especially intravenous infusion of BCAA, have been shown to increase the sensitivity of muscle protein synthesis *in vivo* to insulin (Garlick & Grant 1988; Biolo & Wolfe 1993). However, it has been suggested that the sensitivity of the muscle to insulin might be facilitated by EAA (including BCAA) and that the increase in protein synthesis after feeding might be associated with the simultaneous presence of both these factors. In addition, branched-chain amino transferase iso-enzyme (cytosolic) is found only in the ovaries, placenta and brain (Hutson *et al.* 1988, 1992), whereas mitochondrial iso-enzyme (branched-chain amino transferase) is expressed most in tissue. Therefore BCAA may have a direct stimulating effect on the ovaries, increasing OR, by an as yet unknown mechanism. Further studies are needed on the uptake of BCAA by the ovary and on the action of BCAA within the ovary, including any increase in the numbers of CL released.

Although wool growth was greater in sheep grazing lotus than pasture, there was no wool growth response on lotus due to the action of CT in this study. Wool growth is well known to respond slowly to changes in nutrition and it may well be that a longer period of

PEG supplementation is needed. Min *et al.* (1998*b*) reported that 18 weeks of PEG supplementation was required before the effect of CT on wool characters was apparent.

The availability of sulphur-containing AA (SAA) and post-ruminal supplementation with SAA has markedly increased wool growth (Black & Reis 1979; Reis 1979). More recently, studies with *L. pedunculatus* (McNabb *et al.* 1993) and with *L. corniculatus* (Wang *et al.* 1994) showed that the action of CT increased the irreversible loss rate (ILR) of cystine from blood plasma, mainly due to reducing the loss of SAA (30%) in the rumen. The increase in clean fleece weight (19%) from feeding lotus in the present experiment could be due to CT increasing the absorption of SAA and also that of all other EAA. This is similar to the result obtained by Min *et al.* (1998*b*), who found CT in lotus increased the efficiency of wool production, suggesting that CT from lotus may have altered the chemical composition of wool, especially changing the proportion of individual proteins in wool. Lotus feeding in the present experiment also increased length of wool growth and fibre diameter.

In ewes grazing lotus, PEG supplementation significantly increased wool yellowness and reduced brightness, whilst this effect was not apparent in ewes grazing pasture. Therefore, it appears that an interaction between PEG and lotus caused increased wool yellowness and reduced brightness. This effect was eliminated by the action of CT. However, the exact nature of the interaction between PEG and lotus which lead to changes in wool colour is unclear and further research is necessary to resolve this question. Min *et al.* (1998*b*) reviewed factors influencing yellowness in NZ crossbred wool and concluded that a major likely cause was degradation products of aromatic amino acids in the fleece and their subsequent adsorption onto wool fibres. Whilst, in part, this may have contributed to changes in wool colour in ewes fed lotus and supplemented with PEG, other factors must also be involved because similar changes in wool colour in ewes fed pasture with and without PEG supplementation did not occur, yet degradation of aromatic amino acids would also have occurred in those animals.

This study has shown that feeding the CT-containing legume *Lotus corniculatus* can be used to increase both the efficiency of reproduction and wool production in grazing ewes, with more lambs and wool being produced without any change in feed intake. Further research is required to define the length of feeding time on lotus to give maximum response in reproductive rate. The action of CT in lotus also improved wool quality and reduced yellowness.

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