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

*5. Effects on the endocrine system and on adipose tissue metabolism

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1. Three experiments were conducted using *Lotus pedunculatus* containing high concentrations of condensed tannins (CT), and utilizing the principle that polyethylene glycol (PEG) application (molecular weight 3350) will irreversibly bind a portion of the CT and thus reduce the dietary reactive (i.e. non-PEG bound) CT concentration. Lotus diets containing 95, 45 and 14 g total reactive CT/kg dry matter (DM), induced by spraying with three PEG rates, were given to sheep at hourly intervals (600 g DM/d) for 21 d (Expt 1). In Expts 2 and 3, lambs grazed areas oversown with either lotus (89 g CT/kg DM) or clovers (*Trifolium repens* and *Trifolium pratense*; < 1 g CT/kg DM) for 42 and 92 d respectively. In Expt 2 half the animals grazing each forage received oral PEG (75 g/d), whilst in Expt 3 half the lambs were sired by rams selected respectively for low or high levels of subcutaneous fat deposition.

2. Hormone concentrations in plasma (Expt 1 only) were determined by radioimmunoassay. Rates of [U-^{14}C]-acetate and D-[U-^{14}C]glucose incorporation and oxidation by subcutaneous and abdominal adipose tissue removed at slaughter, together with rate of glycerol release, were determined during in vitro incubation in all three experiments.

3. Plasma concentration of growth hormone was positively and linearly related to dietary reactive CT concentration, whilst 3,5,3′-triiodothyronine (T_3) concentration tended to be negatively and linearly related to dietary reactive CT concentration. Diet CT concentration had no effect on plasma concentrations of the other hormones measured.

4. Feeding of lotus high in CT was associated with a consistent but non-significant increase in the rate of glycerol release from adipose tissue, which was reduced as dietary reactive CT concentration was lowered through PEG application, and a reduction in the lipogenesis:lipolysis value. Selection for leanness decreased acetate incorporation and increased glycerol release from adipose tissue, with the effect not interacting with the diet.

When tissues of *Lotus* sp. or sainfoin (*Onobrychis viciifolia* Scop) plants are disintegrated, such as during chewing, condensed tannins (CT) present in certain specialized cells are known to precipitate the soluble plant proteins by pH-reversible hydrogen bonding (Jones & Mangan, 1977). This action of CT, present in the leaves and stems of these two species, but not in those of white clover (*Trifolium repens*), red clover (*Trifolium pratense*) or lucerne (*Medicago sativa*), is known to increase non-ammonia-nitrogen (NAN) absorption from the intestines (Barry & Manley, 1984; Barry *et al.* 1986). The presence of CT has also been accompanied by increases in N retention in sheep, both for diets of sainfoin (Egan & Ulyatt, 1980), *Lotus corniculatus* (John & Lancashire, 1981) and *Lotus pedunculatus* (Barry *et al.* 1986). The presence of CT in *Lotus pedunculatus* has also been associated with reduced carcass fat content in grazing lambs, relative to lambs grazing white clover (Purchas & Keogh, 1984). The objectives of the present work were to evaluate the effects of dietary CT concentration on plasma hormone concentration and the metabolic properties of adipose tissue in sheep fed on *Lotus pedunculatus*.

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EXPERIMENTAL

Experimental design

Three experiments were conducted using *Lotus pedunculatus* (cv Grasslands Maku) oversown into acid, low-fertility soils. Under these conditions the CT concentration in the plant tissue is very high (approximately 90 g/kg dry matter (DM)). In Expts 2 and 3 the metabolic properties of adipose tissue removed from animals grazing areas oversown with high-tannin lotus were compared with those in tissue removed from lambs grazing areas oversown with a mixture of white clover and red clover, which contains only traces of CT.

Polyethylene glycol (PEG) of molecular weight (MW) 3350 forms hydrogen-bonded complexes with CT and thus reduces the amount of bonding between CT and plant proteins (Jones & Mangan, 1977; Barry & Manley, 1986). As PEG is inert in the digestive tract of ruminants, this treatment was used to reduce the nutritional effects attributable to CT by spraying lotus with PEG in Expt 1 and by oral administration of PEG in Expt 2.

Animals and diets

Concentrations of CT and of N in the legume diets used are shown in Table 1.

Expt. 1. The animals, diets and other experimental procedures used were as described by Barry *et al.* (1986). Briefly, nine wether sheep (58 kg), aged 18 months, were fed on vegetative, secondary-growth, fresh *Lotus pedunculatus* containing 95 g CT/kg DM for a pre-experimental period of 7 d. The diets given to two of the groups of three sheep were then sprayed with PEG (MW 3350) such that the concentration of total reactive CT (i.e. that not bound to PEG) was reduced to 45 and 14 g/kg DM, whilst the control lotus (95 g CT/kg DM) was sprayed with an equivalent volume of water. The three diets were then given (600 g DM/d) at hourly intervals from overhead belt feeders for an experimental period of 21 d. The experiment was conducted during March, a time of declining day length, and natural light in the animal house was supplemented by continuous fluorescent lighting.

An indwelling catheter was placed in the left jugular vein of all sheep on the first day of feeding the three experimental diets. Blood samples were withdrawn for measurements of hormone and metabolite concentrations at 12.30 hours (0·5 h after the hourly feed delivery) on days 11 and 12 and on days 18 and 19 of the experimental period, these times being before and after the measurement of digestion indices reported by Barry *et al.* (1986). At the end of the experiment, animals were anaesthetized and slaughtered (Barry *et al.* 1986), and samples of subcutaneous (shoulder) and abdominal adipose tissue removed and maintained in saline (9 g sodium chloride/l) at 39°.

Expt 2. Twenty-four Romney wether lambs (26 kg), aged 7 months, grazed areas of low fertility native tussock, oversown with either lotus or a mixture of white and red clovers, for 42 d. Twelve lambs grazing each forage received an oral drench once daily supplying 75 g PEG, with the remainder receiving an equivalent volume of water. During this experiment the clover was in short supply and the lotus was mature secondary regrowth, showing some flower development. All animals were weighed after a 24 h fast at the beginning and end of the experiment. They were then returned to their experimental plots and slaughtered (without anaesthetic) in groups of four (one per treatment groups) 3–5 d later, after an overnight fast (14 h) in all cases; adipose tissue was removed from the shoulder and abdominal areas as described for Expt 1. Further details of experimental procedures are described by Barry (1985).

Expt 3. Groups of sixteen male Coopworth x Romney lambs aged 5 months and weighing 21·3 kg grazed areas of acid, low fertility native tussock, oversown with either *Lotus pedunculatus* or a mixture of white clover and red clover, for 92 d. Herbage allowance was 4 kg/lamb per d so that, based on our experience, animal growth would not be limited

Table 1. *Composition and digestibility of the diets fed*

Experimental no.	Diet	Condensed tannins (g/kg DM)	Total nitrogen (g/kg DM)	Apparent energy digestibility
1	Control	95	31	0·64
	Low PEG	45	30	0·69
	High PEG	14	27	0·71
2	Lotus*	89	28	ND
	Clover†	<1	33	ND
3	Lotus	88	—	ND
	Clover	<1	—	ND

DM, dry matter; PEG, polyethylene glycol (molecular weight 3350); ND, not determined.
* *Lotus pedunculatus*.
† *Trifolium repens* and *Trifolium pratense*.

by feed availability. Half the lambs grazing each forage were sired by Coopworth rams selected respectively for high or low depths of carcass backfat using ultrasonic scanning techniques, hence referred to as selection for fatness or leanness; the dams were unselected Romney ewes. All the lambs were weighed after a 24 h fast at the beginning and end of the experiment. They were then returned to their experimental plots and four animals per diet (two fat and two lean) slaughtered (without anaesthetic) in groups of four (one fat and one lean grazing lotus and clover) 3–5 d later, after an overnight fast (14 h) in all cases, with adipose tissues being removed from the shoulder and abdominal areas. The animals formed part of a larger experiment described by Lowther & Barry (1985), where further experimental details are described.

Laboratory procedures

Blood samples were collected on ice, centrifuged at 4°, and portions of plasma for each hormone or metabolite determination (generally 0·5 ml) taken and stored at −20°. For each animal, the portions from each sample day were pooled and stored at −20°, to give composite samples for analysis. Before plasma was added, the proteinase inhibitor Trasylol (Bayer; Leverkusen, W. Germany) was added (1000 KIU/tube) to the tubes used for somatostatin and glucagon determination and the tubes lyophilized.

Hormone and metabolite concentrations were determined as described by Barry *et al.* (1985). Indices of the free (i.e. non-protein bound) concentrations of thyroid hormones in plasma were computed through multiplying total concentration by the thyroxine binding: globulin binding ratio (TBGbr), a measure of the degree of saturation of plasma proteins that transport thyroid hormones.

To measure the metabolism of adipose tissue the samples were sliced at approximately 0·3 mm intervals and 50-mg quantities were incubated in quadruplicate with shaking for 2 h at 37°. The tissue slices were placed in 2·5 ml of a medium consisting of Krebs-Ringer bicarbonate buffer with half the normal calcium concentration and containing (/l), in addition, 40 g bovine serum albumin, 0·3 g casein hydrolysate, 4·5 mmol sodium acetate, 2·5 mmol glucose, 67 μmol methionine, 49 μmol tryptophan, 20 mg adrenalin bitartrate and 10 mg ovine insulin (Pike & Roberts, 1980). Duplicate incubation flasks contained 9·2 kBq of either [U-^{14}C]sodium acetate or D-[U-^{14}C]glucose. The medium was gassed with oxygen:carbon dioxide (95:5 v/v). Incubations were terminated by the addition of 0·5 ml 1 M-sulphuric acid.

Acetate and glucose uptake were measured from the amount of ^{14}C incorporated into

chloroform–methanol-extractable material. Glycerol released into the incubation medium was determined by the enzymic method of Wieland (1974), taking the mean value from the four incubation flasks per sample. Rates of oxidation of acetate and glucose were measured by trapping liberated $^{14}CO_2$ in hyamine hydroxide (Pike & Roberts, 1981).

Statistical analyses

Plasma hormone and metabolite values in Expt 1 were analysed using one-way analysis of variance, with trends being established by examining linear and quadratic contrasts as functions of dietary total reactive CT concentration. Adipose tissue values in Expt 1 and 2 were analysed by a split-plot method, with nutritional treatments forming main plots and sampling sites forming subplots. Expts 2 and 3 were analysed as 2×2 factorials.

RESULTS

Expt 1

Plasma hormone concentrations. Plasma concentrations of total 3,5,3′-triiodothyronine (T_3) and the free T_3 index tended to be negatively and linearly related to dietary concentration of total reactive CT ($P < 0{\cdot}1$; Table 2). Plasma growth hormone (GH) concentration (μg/l) was positively and linearly related to dietary total reactive CT concentration (g/kg DM) by the relation:

$$\text{GH} = 1{\cdot}71\ (\text{SE}\ 0{\cdot}712) + 0{\cdot}032\ (\text{SE}\ 0{\cdot}0113)\ \text{CT} \qquad (1)$$

r 0·756, residual SD (RSD) 1·12, $P < 0{\cdot}05$.

Plasma concentration of the other hormones measured was not affected by dietary CT concentration ($P > 0{\cdot}05$).

Adipose tissue metabolism. Both the incorporation and oxidation of acetate and glucose were more rapid in subcutaneous than in abdominal adipose tissue, with the difference tending towards significance for acetate incorporation ($P < 0{\cdot}1$), and being significant for glucose oxidation ($P < 0{\cdot}01$) and glucose incorporation ($P < 0{\cdot}05$). There were no differences between sheep fed on the low- and high-PEG-treated diets in the incorporation and oxidation of acetate and glucose, and the mean overall values for sheep fed on PEG-treated diets (Table 3) did not differ from those of sheep fed on control lotus ($P > 0{\cdot}05$). The rate of glycerol release was not affected by site of adipose tissue sampling; however, the rate of glycerol release was consistently lower and the ratio, acetate incorporation: glycerol release, was consistently higher in sheep fed on PEG-treated lotus compared with control lotus, although the differences did not attain significance ($P > 0{\cdot}05$).

Expt 2

Live-weight gain (LWG) of control sheep grazing high-tannin lotus, and of control and PEG-drenched sheep grazing areas oversown with clover, were low at 26–28 g/d. Oral PEG supplementation increased LWG of sheep grazing high-tannin lotus to 70 g/d (SE of difference (SED) 9·2; $P < 0{\cdot}001$).

Subcutaneous adipose tissue was more metabolically active than abdominal adipose tissue, with the values (nmol/g wet tissue per h) being respectively 253 *v.* 128 for acetate oxidation (SED 19·2; $P < 0{\cdot}001$), 270 *v.* 127 for acetate incorporation (SED 51·7; $P < 0{\cdot}05$), 116 *v.* 95 for glucose oxidation (SED 18·6; $P > 0{\cdot}05$), 79 *v.* 57 for glucose incorporation (SED 6·6; $P < 0{\cdot}01$) and 420 *v.* 312 for glycerol released (SED 22·2; $P < 0{\cdot}001$). None of the nutritional treatments had any effect on the incorporation and oxidation of either acetate or glucose; however, glycerol release tended to be greater in adipose tissue from control than from PEG-treated sheep grazing high-tannin lotus (460 *v.* 356 nmol/g wet tissue per h), although the difference did not attain significance (SED 148·9; $P > 0{\cdot}05$). Corresponding

Table 2. *Expt 1. Plasma hormone concentrations in sheep fed on control and PEG-treated high-tannin* Lotus pedunculatus

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

Total reactive condensed tannin (g/kg dry matter)	Control lotus 95	Low PEG-treated lotus 45	High PEG-treated lotus 14	SEM (6 df)
Total T_4 (nmol/l)	66·0	55·7	67·3	5·48
Total T_3 (nmol/l)	1·16	1·29	1·57	0·131
TBGbr (relative units)	0·95	1·11	0·97	0·059
Free T_4 (relative units)	62·2	61·3	65·4	5·15
Free T_3 (relative units)	1·12	1·43	1·52	0·147
Growth hormone (μg/l)	4·8	2·8	2·3	0·64
IGF I (μg/l)	47·5	38·7	45·5	7·94
IGF II (μg/l)	987·0	1286·7	1056·3	118·9
Prolactin (μg/l)	14·4	17·7	17·7	4·03
Somatostatin (ng/l)	38·1	41·9	35·6	5·19
Insulin (mU/l)	20·0	22·4	22·4	4·15
Glucagon (ng/l)	152·4	94·5	239·1	73·62

T_4, thyroxine; T_3, 3,5,3′-triiodothyronine; TBGbr, thyroxine binding:globulin binding ratio; IGF, insulin-like growth factor.

Table 3. *Expt 1. Incorporation and oxidation of acetate and glucose, together with glycerol release (nmol/g wet tissue per h), in adipose tissue from sheep fed on control and PEG-treated high tannin* Lotus pedunculatus

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

Diet	Control lotus		PEG-treated lotus		SEM* (7 df)	
Source of adipose tissue	Abdominal	Subcutaneous	Abdominal	Subcutaneous	*n*3	*n*6
Acetate oxidation†	78·4	139·8	95·3	106·5	20·24	14·37
Acetate incorporation†	504·8	880·8	506·6	546·4	252·61	178·66
Glucose oxidation†	159·9	201·0	134·1	184·1	27·62	19·53
Glucose incorporation†	19·8	36·7	29·2	38·1	5·99	4·25
Glycerol release	328·3	313·3	193·0	161·0	124·80	88·25
Acetate incorporation/ glycerol release	1·56	2·63	4·65	6·46	1·91	1·35

PEG, polyethylene glycol (molecular weight 3350).

* Calculated from pooled main plot + subplot error mean square.

† Amount of [U-^{14}C]acetate and D-[U-^{14}C]glucose either incorporated into adipose tissue or oxidized to carbon dioxide.

values for sheep grazing areas oversown with clover were respectively 322 and 328 nmol/g wet tissue per h.

Expt 3

LWG of lambs sired by fat rams grazing areas oversown with lotus or clover were 123 and 121 g/d respectively; corresponding values were higher ($P < 0·05$), 131 and 138 g/d, for lambs sired by lean rams (SED 5·92 g/d).

Rates of acetate incorporation tended to be lower and rates of glycerol release tended to be higher in adipose tissue from lambs grazing lotus compared with those grazing clover (Table 4), although the differences did not attain significance ($P > 0·05$). There were no

Table 4. *Expt 3. Effects of oversowing with either lotus* (Lotus pedunculatus) *or clover* (Trifolium repens *and* Trifolium pratense), *and of sire selection for fatness or leanness, on incorporation and oxidation of acetate and glucose, together with glycerol release (nmol/g wet tissue per h) in subcutaneous adipose tissue from growing lambs*

(Adipose tissue was removed from two animals, selected for fatness or leanness that had grazed either lotus or clover diets; main effects are given for the means of four animals per treatment comparison)

	Diet*		Sire selection*		SEM (5 df)
	Lotus	Clover	Fat	Lean	
Acetate oxidation†	114	172	198	87	28·9
Acetate incorporation†	1556	1906	2394	1068	338·8
Glucose oxidation†	94	118	149	63	29·4
Glucose incorporation†	56	75	74	57	7·6
Glycerol release	1238	732	745	1226	314·2
Acetate incorporation/ glycerol release	1·7	3·5	3·4	1·7	0·88

* Main effects from analysis of variance; interaction not significant ($P > 0·05$).

† Amounts of [U-^{14}C]acetate and D-[U-^{14}C]glucose incorporated into adipose tissue or oxidized to carbon dioxide.

interactions between diet type and sire selection ($P > 0·05$). Sire selection for leanness lowered rates of acetate incorporation and oxidation ($P < 0·05$), tended to lower the rate of glucose oxidation ($P < 0·10$) in adipose tissue, and tended to increase glycerol release although this effect did not attain significance ($P > 0·05$).

Acetate incorporation (ACI) was related to glucose incorporation (GLI) and to acetate oxidation (ACO) by the relation (nmol/g wet tissue per h):

$$\text{ACI} = 39·8\ \text{GLI}\ (\text{SE}\ 12·59) - 867·9\ (\text{SE}\ 850·3) \quad r\ 0·791,\ \text{RSD}\ 615·3,\ P < 0·05. \qquad (2)$$

$$\text{ACI} = 8·4\ \text{ACO}\ (\text{SE}\ 3·01) + 533·6\ (\text{SE}\ 489·1) \quad r\ 0·751\ \text{RSD}\ 662·9\ P < 0·05. \qquad (3)$$

DISCUSSION

Endocrine adaptations to dietary CT concentration

Plasma concentrations of most of the hormones measured in the present study showed substantial changes following twice-daily feeding (Barry *et al.* 1982); the system of offering the daily ration as twenty-four equal feeds each 1 h apart (Expt 1) was designed to eliminate such variation. The system of taking samples at a fixed time point (12.30 hours; 0·5 h after a feeding cycle) could have limitations in the case of GH, which shows a pulsatile pattern of release. However, the pooling of samples taken at this time on four separate days will go some way to providing a mean value for GH concentration, and a comparison of effects produced by the nutritional treatments is considered valid as the same sampling procedure was used for animals fed on each diet.

The increase in plasma GH concentration with increasing dietary reactive CT concentration may be related to inactivation (precipitation) of gut-wall proteins by CT. When *Lotus pedunculatus* is disintegrated, approximately 90% of the CT is precipitated as a complex with plant protein (i.e. bound tannin) and 10% is released as free CT (Barry & Manley, 1986). On ingestion by the animal, free CT will react with and inactivate other proteins, including those of the rumen epithelium. A second possibility concerns the fate of protein-bound CT, which will be released with plant protein at the low pH (< 3·0) in the abomasum and the high pH (7·5–8·5) in the small intestine (Jones & Mangan, 1977).

Although the fate of this CT in the small and large intestines is unknown, a portion of it may likewise react with and inactivate the gut-wall proteins by H bonding. Reviews of recent experiments with growing ruminants suggest that GH stimulates N retention and reduces carcass fat deposition, with an increase in fat turnover (i.e. mobilization) being a suggested mechanism for explaining the second effect (Trenkle, 1980; Muir *et al.* 1983; Bauman & McCutcheon, 1985). Sheep fed on fresh lotus and sainfoin may thus respond to CT inactivating gut-wall proteins by increasing GH secretion, as a means of stimulating replacement protein synthesis. Increased GH secretion in response to this stimulus may also be a contributing factor in the increased N retention associated with feeding lotus and sainfoin containing CT (Egan & Ulyatt, 1980; John & Lancashire, 1981; Barry *et al.* 1986). A similar increase in plasma GH concentration has been observed in sheep fed on forage kale, where dimethyl disulphide is produced from rumen fermentation of S-methyl-L-cysteine sulphoxide, with this compound then inactivating thiol (–SH) groups of proteins (Barry *et al.* 1985). These authors suggested that the release of gut peptides was involved in the GH response, and a similar mechanism may be involved in the case of diets containing CT.

Adipose tissue metabolism

The measurements of adipose tissue metabolic activity recorded in the present investigation may have been affected by intravenous anaesthetic (Expt 1 only) and other factors. Overnight fasting (14 h) may have increased lipolysis, and adrenalin addition to the glucose incubations may have depressed glucose oxidation and stimulated its incorporation into lipid (Yang & Baldwin, 1973). However, any such effects would apply equally well to adipose tissue incubations from both control and treatment animals, and may not be of large magnitude, as the measured molar increment in acetate uptake per unit increase in glucose uptake (40 (SE 13); eqn (2)) was close to the theoretical value for tripalmitate synthesis (48).

Purchas & Keogh (1984) reported lower carcass fat content (approximately 25 g/kg) in lambs grazing *Lotus pedunculatus* than in lambs growing at a similar rate grazing vegetative white clover, which does not contain CT. This effect could be produced by two mechanisms. Firstly, the increased N retention associated with feeding fresh forages containing CT could lead to a dilution in fat concentration (g/kg). Secondly, lotus feeding appears to reduce the lipogenesis:lipolysis value, with the major factor in the present study being a consistent though non-significant increase in lipolysis, as measured by the rate of glycerol release. It is possible that the increased lipolysis in sheep fed on lotus high in CT was mediated by increased GH secretion; certainly, both were reduced when nutritional effects attributable to CT were reduced through oral PEG application.

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