The effect of condensed tannin containing diets on whole body amino acid utilisation in Romney sheep: consequences for wool growth

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ABSTRACT

Three groups of sheep housed in metabolism crates were fed *Lotus corniculatus* (low tannin), *L. pedunculatus* (medium tannin) and ryegrass pasture (control) respectively. After a period of adaptation to each of the diets, labelled ^{35}S-cysteine and ^{3}H-phenylalanine were infused (separately) for 6 hours via a jugular catheter. Blood samples were taken at 5 and 6 hours and irreversible loss rates were determined from radioactivity and pool size measurements for cysteine and phenylalanine for sheep on each diet. Blood concentrations of cysteine, glutathione and phenylalanine, wool production and dry matter intake were also determined. Significant treatment differences were found for circulating cysteine, (36.2 ± 4, 36.3 ± 2.8, 60 ± 3.9 μM), glutathione (1.76 ± 0.14, 1.08 ± 0.1, 1.63 ± 0.14 mM) and cysteine ILR (16.5 ± 3.2, 27.3 ± 2.2, 35.6 ± 3.1 m moles d^{-1}) for the *L. corniculatus,* pasture and *L. pedunculatus* treatments respectively. Phenylalanine blood concentrations and phenylalanine ILR were not significantly different among treatments. Although *L. pedunculatus,* which contains most tannin, significantly improved cysteine supply and utilization at whole body level, this was not reflected in gains in wool production.

Keywords Condensed tannin, cysteine, glutathione, phenylalanine, irreversible loss rate, wool, sheep.

INTRODUCTION

Cysteine is the first limiting amino acid for both skin growth and wool production (Black and Reis, 1979). Therefore dietary conditions which affect transport processes across the gut wall, and hence the supply of cysteine to the circulating blood pool, are of importance. Because skin and wool impose a heavy demand on cysteine supply, the aim of much research, now and in the past, has focused on ways to enhance cysteine blood levels to effect gains in wool growth (Reis *et al.*, 1990 and others). Tannin containing diets are known to effect changes in amino acid supply, especially essential amino acids (Waghorn *et al.*, 1987).

In this work we present new data on the effect of diets containing different types and levels of tannin on blood thiol (cysteine and glutathione) and essential amino acid (phenylalanine) concentrations and irreversible loss rates, and the consequences of these effects in relation to wool growth.

METHODS

As part of a larger trial, three groups of sheep (n=5) aged 6-8 months were housed in metabolism crates and fed freshly cut *Lotus corniculatus* (low tannin), *L. pedunculatus* (medium tannin) and ryegrass (no tannin; control) from overhead feeders at hourly intervals. Fresh water was available *ad libitum.* After a period of adaptation to each of the diets, labelled ^{35}S-cysteine and ^{3}H-phenylalanine in sterile physiological saline were infused (separately) at about 1 μCi min^{-1} for 6 hours into one side of a bilateral jugular catheter. At 5 and 6 hours blood samples were withdrawn by syringe from the other catheter into chilled polypropylene tubes on ice, containing 0.1 ml of 15% (w/v) sodium-EDTA or 0.1 ml of 100 I.U. ml^{-1} heparin for the cysteine and phenylalanine samples respectively.

For the determination of thiols (cysteine, glutathione) an aliquot of whole blood (2g) labelled with ^{35}S-cysteine was mixed with an equal volume of 0.5% (w/v) sodium dodecyl sulphate A.R., vortexed and held at room temperature for 15 minutes to release protein exchangable cysteine. Chilled 20% trichloroacetic acid (TCA) (2g) was added to precipitate protein, vortexed and centrifuged at 2000 g for 20 minutes at 4°C. The supernatant was filtered through 0.45 μm cellulose acetate and stored at -85°C prior to analysis. The ^{3}H-phenylalanine labelled blood samples (2 g) were mixed with an equal volume of 10% 5-sulphosalicylic acid (SSA), containing 200 μM norleucine as an internal standard, vortexed, centrifuged and filtered as described for the cysteine samples, before storage at -20°C prior to analysis.

Radioactivities of ^{35}S associated with whole blood cyst(e)ine and glutathione, and with oxidation products (SO_4^{2-}, cysteinesulphinic acid and taurine), were determined after chromatographic separation of the labelled compounds in TCA extracts on an HPLC cation exchange column (Bio Rad aminex-9, Na^+).

Concentration of thiols in whole blood TCA extracts were determined by reverse phase HPLC after simultaneous derivatisation of reduced thiols with a fluorigenic benzofurazan reagent (Lee *et al.*, 1992). Phenylalanine concentration measurements were made using a LKB amino acid ion-exchange analyser, after the SSA extract had been passed through Dowex 50W-X8 ion exchange resin and the amino acid fraction eluted with 4M NH_4OH, rotary evaporated to remove ammonia, and taken up in 0.2M lithium citrate buffer. Determination of ^{3}H radioactivity was also made on this sample.

From subsequent radioactivity and pool size measurements for each of the amino acids, irreversible loss rates (ILR; equivalent to entry rate at steady state) were obtained for each diet. ILR was calculated by dividing the infusion rate (dpm min^{-1}) by the specific radioactivity (dpm nmole^{-1}) in the TCA supernatant. Wool production (midside patch method) and dry matter intakes (DMI) were also determined.

Analysis of variance to test for difference among treatment means was made using the SAS statistical package.

RESULTS

Wool production, DMI and body weight at the end of the treatment periods for the three groups of sheep are given in Table 1. There were no significant differences among treatments for body weight or wool output, although there was a trend for sheep on the *Lotus* diets to grow more wool. In the larger trial with 9 animals on each treatment, from which the present work was a subset, the wool productions were 1.76 ± 0.34, 1.08 ± 0.31 and 1.30 ± 0.40 (mean ± s.d.) for the *L. corniculatus*, pasture and *L. pedunculatus* respectively, with *L. corniculatus* showing significantly greater wool production than either of the other diets (P<0.01). Individual variation however was large in both the main trial and the subset in the present study. Significant differences in DMI among the treatment groups were found, with the highest tannin containing diet, *L. pedunculatus*, markedly depressing intake.

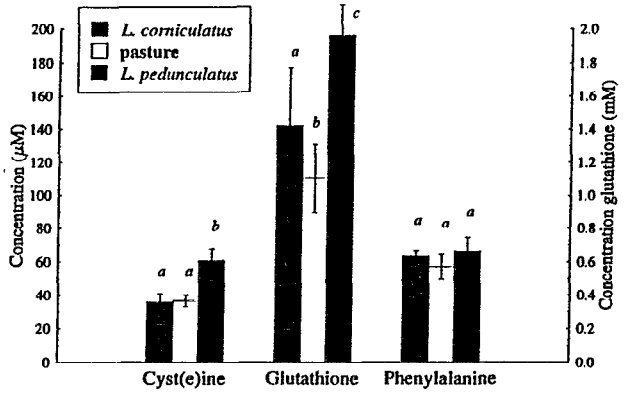
TABLE 1 Wool growth, dry matter intake (DMI) and terminal body weight for treatment groups (mean ± sd, n=5).

Group (diet)	Wool Growth (mg cm^{-2} d^{-1})	DMI (g)	Body Weight (kg)
L. corniculatus	1.73 ± 0.4 a[1]	900 ± 100 a	29.9 ± 1.5 a
Pasture	1.23 ± 0.3 a	745 ± 29 b	27.6 ± 0.8 a
L. pedunculatus	1.45 ± 0.3 a	614 ± 76 c	27.0 ± 2.2 a

[1]Means with different letters denote significant differences at the 5% probability level

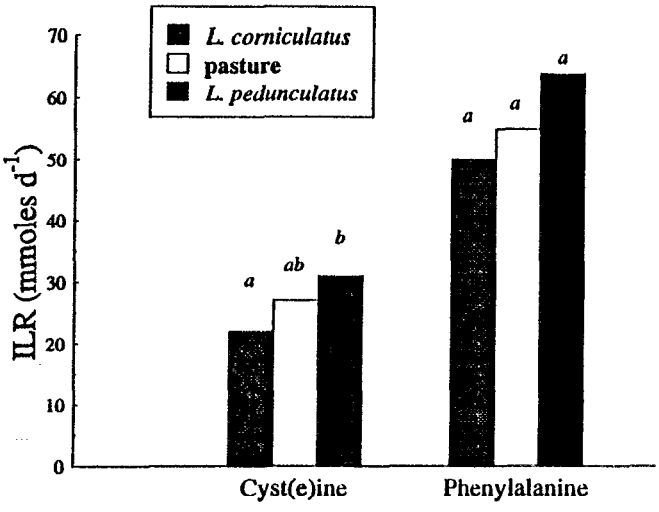
Mean concentration of cysteine, glutathione and phenylalanine in circulating blood for the three diet groups are shown in Figure 1. Compared with pasture and *L. corniculatus*, the *L. pedunculatus*, which had a medium concentration of tannin, significantly increased both cysteine and glutathione concentration in whole blood. Diet however had no effect on phenylalanine concentrations.

FIGURE 1 Mean concentration of cysteine, glutathione and phenylalanine in blood of sheep fed *L. corniculatus*, pasture and *L. pedunculatus* (LSD's for cysteine, glutathione and phenylalanine are: 8.2, 295 and 10 µM respectively. Bars indicate standard deviation with intreatments; different letters denotes significant differences at the P<0.05 level 1).



Similarly, significant treatment differences were found for cysteine ILR, with means for sheep fed *L. corniculatus* diet markedly lower than those for *L. pedunculatus* (21.6 ± 5.7 and 30.7 ± 5.7 m moles d^{-1} respectively). Cysteine ILR for pasture fed sheep (27.0 ± 2.6 mmoles d^{-1}) were intermediate between the two *Lotus* diets but differences were not significant (figure 2). No treatment effects on phenylalanine ILR were found, although treatment trends were similar to those for cysteine.

FIGURE 2 Mean irreversible loss rate (ILR) for cysteine and phenylalanine in sheep fed *L. corniculatus*, pasture and *L. pedunculatus* (LSD's for cysteine ILR and phenylalanine ILR are 6.5 and 15 mmoles d^{-1} respectively. Different letters denote significant differences at the P<0.05 level.



Across the diets, circulating concentrations of cysteine were significantly positively correlated with circulating glutathione (r=0.70; P<0.01; n=14) and cysteine ILR (r=0.58; P<0.002; n=14). Circulating phenylalanine was significant positively correlated with phenylalanine ILR (r=0.49; P<0.05; n=14). Phenylalanine ILR and cysteine ILR were significantly positively correlated (r=0.55; P<0.025; n=14).

TABLE 2 Effect of diet on circulating blood amino acid concentrations, irreversible loss rate (ILR), and wool output after adjustment of treatment means for dry matter intake by covariate analysis.

Variable	Diet (adjusted treatment means)			mean SD	significance level	
	L. cornic.	pasture	L. ped.		(diet)	(DMI)
cysteine (µM)	36	36	60	5.6	***	NS
glutathione (µM)	1757	1085	1633	201	**	*
phenylalanine (µM)	65	57	65	6.8	NS	NS
cysteine ILR (mmol day^{-1})	16	27	35	4.5	*	NS
phenylalanine ILR (mmol day^{-1})	42	55	70	10.7	NS	NS
wool growth (mg cm^{-2} d^{-1})	1.9	1.22	1.29	0.35	NS	NS

Because of the differences in DMI among the diets (Table 1), blood amino acid concentrations, ILR measurements and wool growth data were re-analysed for covariance with DMI. The adjusted treatment means and significance of the covariate are given in Table 2. DMI had no effect on circulating cysteine concentrations, therefore the high cysteine concentration in whole blood of the *L. pedunculatus* sheep can be attributed to a diet effect *per se* (not intake). However blood glutathione concentration was negatively (P<0.01) correlated with intake (r=-0.64; n=14). The significant covariate effect (P<0.05) is reflected in the adjusted treatment means for whole blood glutathione with

higher and lower levels (1757 and 1633 μM) for the *L. corniculatus* and *L. pedunculatus* diets respectively, compared with the measured concentration shown in Figure 1. DMI had no effect on any of the other variables measured, although there was a trend ($P<0.06$) for a negative effect of DMI on cysteine ILR.

Although sheep fed the *Lotus* tended towards a higher wool growth, differences were not significant, nor was there any relationship between blood cysteine, glutathione, cysteine ILR or phenylalanine ILR and wool growth. However blood glutathione was positively correlated with wool growth across treatments when it was corrected for DMI.

DISCUSSION AND CONCLUSIONS

Measurement of the ILR (flux) of an amino acid through the blood pool provides a simple, non-destructive method from which the rate of whole body protein synthesis can be repeatedly estimated on the same animal. As detailed elsewhere (e.g. Waterlow *et al.*; 1978) there are limitations and uncertainties associated with the procedure: ILR is the sum of whole body protein synthesis and oxidation and thus is an overestimate of protein synthesis alone. However because ILR is based on the blood free amino acid specific radioactivity (SRA) rather than the SRA of the true precursor of peptide bond synthesis, ILR is an underestimate of true whole body protein synthesis. To some extent these uncertainties cancel out. In addition, ILR gives no estimation of the contribution of individual tissues and assumes these contributions remain unaltered between treatments. Whole body ILR measurements for most amino acids are dominated by the demands of the viscera and carcass with skin and wool protein metabolism usually accounting for only 10% of the ILR (Harris and Lobley, 1991). However, because of the high proportions of cysteine in skin and wool protein relative to other tissues, cysteine ILR should show a greater sensitivity than other amino acids (such as phenylalanine) to changes in wool production. In sheep with submaintenance intakes, wool production (and hence cysteine utilization) may even become the dominant protein synthesis sink in the body (Harris *et al.*, 1990).

There is some evidence that low circulating levels of glutathione (Hopkins *et al.*, 1975) and cysteine (Williams *et al.*, 1972; Williams, 1986) are associated with higher rates of wool production. The relationship between amino acid ILR and wool production is less clear. In one study (Williams *et al.*, 1972) no relationship was found between cysteine ILR and wool production in genetically selected high fleece-weight merinos, while a later study (Williams, 1976) showed a lower ILR of cysteine in high wool producing sheep. Similarly, Sun *et al.*, (1992) showed a lower ILR of phenylalanine in high wool producing sheep, but our own studies show a *positive* correlation between both leucine ILR and cysteine ILR and wool production (Lee, unpublished data). Some of these apparent contradictions may be a consequence of genetic capability for amino acid utilisation and skin metabolism predisposition being overridden by effects of diet as well as the relative insensitivity of ILR to peripheral metabolism.

In the present trial, feeding of a medium tannin diet *(L. pedunculatus)* reduced feed intake and this intake effect confounded the results when compared with other diets. This effect of tannin on intake has also been observed by Waghorn *et al.*, 1990. Nevertheless, *L. pedunculatus* was shown to give higher circulating blood cysteine and a higher cysteine ILR. Although it was not significant, there was a similar trend in phenylalanine ILR, but there was no commensurate increase in wool production. Overall, because the ILR of both phenylalanine and cysteine were positively correlated across all the diets, it is probable that the effects on ILR were primarily on visceral and carcass metabolism rather than skin and wool.

The higher circulating levels of both cysteine and glutathione with the *L. pedunculatus* treatment are less easy to explain. With glutathione there is a confounding effect of intake, nevertheless, both the tannin containing diets have higher circulating levels than the pasture diet. According to the model of Black and Reis (1979) increases in wool production will result in lower circulating levels of cysteine but in the current trial none of the differences in circulating metabolites are associated with changes in wool production. The low, and highly variable levels of intake with *L. pedunculatus* may mean that these sheep were just above or just below maintenance, resulting in some cases net protein catabolism. However as wool production or body weight was not impaired these cannot have been extreme catabolic effects. This suggests that, not only the type and level of tannin but perhaps other unknown dietary effects within *L. pedunculatus* are confounding the results. Further work is necessary to resolve this.

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