The effect of whole body cysteine supplementation on cysteine utilization by the skin of a well-fed sheep

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ABSTRACT

Research, in both New Zealand and Australia, is being directed towards enhancing the sulphur amino acid supply of sheep by either plant or animal modification. This study investigated the effects of such improved cysteine supply in well-fed sheep on both whole body and skin metabolism of cysteine. The level of cysteine supplementation chosen (2g/d) represented about a 50% increase in absorbed cysteine. Cysteine was supplied by jugular infusion to avoid loss during gut absorption. At this level of cysteine supplementation a preliminary trial on sheep fed either 700 g or 1250 g lucerne chaff showed no significant effect on wool production but a significant increase in wool sulfur (S) concentration over a one month period of treatment.

In a second trial, two groups of five, 40kg, cryptorchid Romney sheep were offered 1200 g/d of lucerne chaff and one group was supplemented with 2g/d cysteine for five days prior to measurement, with the other group receiving saline only. Cysteine supplementation increased whole body cysteine utilization (irreversible loss of ^{35}S-cysteine) from 4.7 ± 0.4 to 6.4 ± 0.3 g/d, while appearance of ^{35}S-cysteine in the oxidation pool increased by 37%. Cysteine supplementation resulted in an increase in circulating cysteine concentration (33.8 ± 5.6 to 51.1 ± 12.0 μM) but blood flow to the skin patch (measured by dye dilution) was reduced from 0.29 ± 0.03 to 0.21 ± 0.02ml. $min^{-1}.g^{-1}$ skin. Hence the cysteine flux through the skin (blood flow times concentration) was unchanged. Nevertheless uptake of ^{35}S-cysteine by the skin increased with supplementation from 12.8 ± 2.7 to 22.8 ± 2.7%, resulting in a significant increase in skin utilization of cysteine for protein synthesis.

The results suggest that enhancing cysteine supply within the sheep does not proportionately improve cysteine availability for protein synthesis. Instead a large proportion of the extra supply is oxidised. Additionally, although enhanced whole body cysteine supply does not improve skin cysteine supply (or flux), increased arterial cysteine concentration appears to stimulate skin cysteine transport into cells so that increased cysteine utilization for protein synthesis is still achieved.

Keywords: Cysteine; skin utilization; protein synthesis; wool.

INTRODUCTION

It has been well established that the sulphur containing amino acids (SAAs), methionine and cysteine, are the first limiting amino acids for wool growth and that supplementation with either SAA at low planes of nutrition will enhance both wool production and the sulphur rich protein content of wool (Reis, 1979; Reis, 1989; Fratini *et al.*, 1994). Less well established are the effects of SAA supplementation on wool production and wool composition from sheep fed at above maintenance levels of intake. Black and Reis (1979), in a simulation model of the utilization of SAA, predicted that the amount of wool grown and the proportion of sulphur rich proteins in wool would increase in response to both increased SAA supply and increased skin blood supply. However the model did not attempt to study interactive responses between SAA supply and skin blood flow. It is clear however, from the Black and Reis (1979) model that as SAA supply increases, the rate of increase in wool growth rate should decline while the rate of increase in proportion of the sulphur rich protein should accelerate. This paper reports the results of a preliminary study examining the effects on wool production of improved cysteine supply given to a well-fed sheep, together with a more detailed study of the changes in cysteine metabolism in both the whole body and skin during such cysteine supplementation.

MATERIALS AND METHODS

Preliminary Trial

Four groups of five, 40kg, 12-15 month old cryptorchid Romney sheep were housed indoors in metabolism crates and accustomed to overhead feeders and the lucerne chaff diet. Two groups of sheep were offered 1400g/d lucerne chaff (crude protein 17%, 9.2 MJ ME/kg DM) at hourly intervals while the other 2 groups were offered 700 g/d lucerne chaff with both groups given fresh water available *ad libitum*. After an adjustment period all sheep had midside wool sites clipped to skin level and a jugular catheter inserted. One group of 5 sheep at 700 g/d (Lo+Cys) and one group at 1400g/d (Hi+Cys) were then continuously infused with 2g/d of cysteine in sterile physiological saline through the jugular catheter. The remaining two groups (LoCont and HiCont) were infused with physiological saline only. Infusion continued for a month, during which time feed refusals were measured, after which wool was harvested from the midside patches (10x10cm). The wool was scoured and conditioned for calculation of wool growth per unit area of the midside patch and then a subsample analysed for sulphur (S) concentration (Antram *et al.*, 1991).

Main Trial

Independent groups of 5, 40kg, 12-15 month old

cryptorchid Romney sheep were accustomed to lucerne chaff and metabolism crates as for the preliminary trial, except only 1200g/d lucerne chaff was offered to each animal. After a two week period of adjustment, sheep underwent surgery as described in Harris *et al.* (1989) to have fine bore polyvinyl chloride catheters implanted in descending lateral branches of the deep circumflex iliac artery (A1) and vein (V) supplying and draining an area of skin on the flank, the saphenous artery (A2) and into both jugular veins. Patency of all catheters, except those in the jugular, was maintained by infusion of 150ml/d heparinized (30i.u./ml) sterile saline. Starting immediately after surgery, one group of 5 sheep (+Cys) was continuously infused with 2g/d cysteine in sterile physiological saline via the jugular, while the other group (Cont) received an infusion of saline only.

Five days after surgery, each sheep was infused for 6 hours with ^{35}S-cysteine (NEN Research Products, Dupont, Wilmington, USA) at the rate of 10 μCi/g and 0.34 g/min into the remaining jugular catheter. Between the 5th and 6th hour of infusion skin blood flow was measured using dilution of an infusion of para-aminohippuric acid (PAH) into Al as described by Harris *et al.* (1989). Blood samples, for measurement of arterial (A2) and venous (V) cysteine concentration and specific radioactivity (SRA) for calculation of whole body irreversible loss rate (ILR) and utilization of cysteine for skin protein synthesised, were sampled and prepared prior to storage as described for 'total' thiol fraction in Lee *et al.* (1993). At the end of the infusion period sheep were killed with an overdose of barbiturate and the patch size visualised using coloured latex, then dissected and weighed as described in Harris *et al.* (1989).

Sample analysis and calculations

Separation and measurement of radioactivity of ^{35}S associated with reduced and oxidised cysteine and the combined cysteine oxidation products (including SO_4, cysteine sulphinic acid and taurine) in samples of each infusate and TCA precipitated blood were made as described in Lee *et al.* (1993) except that radioactivity in each fraction was monitored using a flow through scintillation counter (ß-Ram, INSUS Systems, Fairfield, NJ) in conjunction with the HPLC system. Determination of cysteine, calculations of ILR, skin blood flow and utilization of ^{35}S cysteine by the skin patch have been described elsewhere (Lee *et al.*, 1993). Treatments were compared by analysis of variance (SAS, 1985).

RESULTS

Preliminary Trial

Despite a 16% increase in wool production by the Lo+Cys and HiCont groups compared with that found in the LoCont group, small animal numbers in each group, and the relatively short period of treatment, resulted in large variances so that these differences were not significant. However, in response to cysteine supplementation wool S concentration increased significantly ($P<0.001$) resulting in a significant ($P<0.01$) increase in the overall daily production of wool S by 34% and 27% respectively in the cysteine supplemented low and high intake groups (Table 1).

TABLE 1: Preliminary Trial. Effect of cysteine supplementation (+Cys) on wool production, wool S concentration and wool S output at two levels of intake (Lo and Hi) (means ± s.e., n=5).

	LoCont	Lo+Cys	HiCont	Hi+Cys
Intake (g/d)	700	700	1320 ± 46	1230 ± 109
Wool Production ($mg/cm^2/d$)	1.37 ± 0.10	1.61 ± 0.09	1.59 ± 0.08	1.70 ± 0.15
S in wool (%)	2.76 ± 0.07	3.16 ± 0.06	2.77 ± 0.09	3.31 ± 0.07
S output in wool ($\mu g/cm^2/d$)	37.9 ± 3.4	50.8 ± 5.5	44.4 ± 3.7	56.6 ± 6.2

Main Trial

There was no significant difference between the +Cys and Cont sheep in intake after surgery including that on the day of measurement. Arterial cysteine concentration was elevated in the +Cys sheep, but not significantly. However, the measured ILR of cysteine (at steady state equal to the entry rate) was increased significantly by about 1.7g a day - less than the 2g of cysteine a day that was infused (Table 2). At plateau there was a significantly greater proportion of ^{35}S associated with the combined oxidation products in the protein-free blood (Table 2).

TABLE 2: Main Trial. Whole body parameters of cysteine utilization by sheep offered 1200g lucerne chaff with (+Cys) or without (Cont) supplementary cysteine at 2g/d (Means ± s.e., n=5).

	Cont	+Cys	Effect[1]
Body weight (kg)	44.1 ± 2.7	42.2 ± 1.5	NS
Intake on day of measurement (g/d)	977 ± 105	1020 ± 61	NS
Arterial cysteine concentration (μM)	36.4 ± 4.5	60.6 ± 11.7	P<0.09
Cysteine ILR (g/d)	4.67 ± 0.36	6.37 ± 0.29	P<0.01
% ^{35}S in protein free blood oxidation pool	43.6 ± 2.2	59.9 ± 1.5	P<0.001

[1]Significance of difference between treatment means.

The patch of skin for which blood flow and cysteine metabolism were measured varied greatly in size between animals, although there was no significant difference between the treatment groups in mean skin patch weight (Table 3). This variation is accounted for by expressing values in relation to weight (g) of tissue supplied and drained. Blood flow to the skin was significantly reduced in the +Cys group (Table 3) which, when combined with the elevation in circulating cysteine concentration (Table 2), means that the cysteine flux past the skin (amount of cysteine per g of tissue per minute) was unchanged by the cysteine supplementation. Although cysteine flux was the same in both treatment groups, the +Cys sheep took up a greater proportion of that flux for protein synthesis.

DISCUSSION

At the above maintenance intake of lucerne chaff consumed by the Hi+Cys sheep in the preliminary trial, there was only a small (7%) non-significant increase in wool produc-

TABLE 3: Main Trial. Blood flow and cysteine utilization by the skin of sheep offered 1200g lucerne chaff with (+Cys) or without (Cont) supplementary cysteine at 2g/d (Means i s.e., n=5).

	Cont	+Cys	Effect[2]
Skin patch weight (g)	74 ± 16	60 ± 6	NS
Blood flow to patch (g/min/g tissue)	0.29 ± 0.03	0.21 ± 0.02	P<0.05
Cysteine flux past skin (nmol/min/g tissue)	10.8 ± 1.2	11.3 ± 2.4	NS
% Uptake ^{35}S-cys for protein synthesis	12.8 ± 2.7	22.8 ± 2.7	P<0.03
Estimated skin protein synthesis as % whole body ILR[1]	20.7 ± 5.7	21.2 ± 5.4	NS

[1]Assuming skin weight to be 10% of body weight.
[2]Significance of difference between treatment means.

tion associated with a larger (20%) significant increase (P<0.001) in the proportion of wool S, compared with the HiCont sheep. From previous work, such increases in the proportion of wool S are almost entirely associated with an increase in the proportion of ultra-high S proteins of wool i.e. those proteins generally contained in the fibre matrix and containing a cysteine content of ~ 30% (Broad *et al.*, 1970). The preliminary trial only lasted one month and wool growth and S content effects would not have completely stabilized until at least two months of treatment (Lee and Williams, 1993). Nevertheless the main effects are already apparent after one month of treatment and, as predicted by the Black and Reis (1979) model of wool growth, across the low and high intakes the response to additional cysteine is curvilinear with respect to wool production (increasing only marginally), while the wool S content continues to increase with increasing cysteine supply. These responses appear to be obtained regardless of whether the additional cysteine is supplied directly, as in the present trial, or by improving the total dietary intake including cysteine (Lee and Williams, 1993).

The more detailed trial of cysteine metabolism in the skin and whole body was undertaken only a few days after cysteine supplementation commenced because of the short lifetime of the surgical preparation. Total intake was lower in this trial, perhaps because of the greater handling of the surgically modified animals, nevertheless this intake was still estimated to be at 1.2 x maintenance metabolisable energy (Geenty and Rattray, 1987). The measurements of cysteine ILR in the Cont group showed a relatively high cysteine utilization on this level of intake (4.67g/d Cys ILR, compared with 1.75g/d in 36 kg sheep fed 1100g/d lucerne chaff by Williams *et al.* (1972), or 1.05g/d in 30kg sheep fed 800g/d of concentrate diet by Pisulewski and Buttery (1985)). The increase in cysteine ILR measured in the +Cys sheep (1.7g/d) compares favourably with the 2g/d additional cysteine infused, the difference accounted for by measurement error.

There was a significant increase in whole body oxidation of cysteine in the +Cys sheep as measured by appearance of ^{35}S in the combined oxidation pool (SO_4, cysteine sulphinic acid and taurine) in the blood. In the study of Sun *et al.* (1994) of sulphur metabolism in Romney sheep fed lucerne chaff at similar intakes the total S oxidation pool was shown to have a concentration of approximately 1 mM (Lee, Pers. comm), while combined urinary and faecal excretion of S was 2g/d. Using these figures together with results from the current trial it can be estimated that the increase in plateau ^{35}S in the protein-free blood pool from 44 to 60% (Table 2) is associated with an increase from 0.4g/d cysteine oxidation in the Cont sheep to 1.3g/d in the +Cys sheep, ie. about half of the supplementary cysteine is oxidised.

Even though half of the supplementary cysteine had been oxidised, arterial cysteine concentration was increased in the +Cys sheep. At the same time, blood flow to the skin of the +Cys sheep significantly dropped, resulting in the same flux of cysteine passing the skin as in the Cont sheep. Given this constant cysteine flux there was still a greater proportional uptake of the ^{35}S by the skin of the +Cys sheep by 78%. This is higher than the 30% increase in wool S output found in the preliminary trial, but comparisons between a 6 hour and 30 day measurement are obviously vulnerable to error. Nevertheless from both the results it is clear that there is a significant increase in the rate of movement of cysteine into the skin of the +Cys sheep and that, as suggested by Lee and Williams (1993), it is the concentration of cysteine in the blood (not the cysteine flux) which must act as the regulator of the synthesis of matrix proteins during the keratinization of the fibre. It is noteworthy that homeostasis is maintained in cysteine flux past the skin by changing blood flow, and at the same time the proportion of whole body cysteine utilization (as measured by ILR) directed to skin protein synthesis is also constant (20.7% in Cont vs 21.2% in the +Cys sheep).

CONCLUSIONS

Supplementary cysteine supply to the sheep given a low intake results in an increase in both wool production and the S content of the wool produced. At above maintenance intakes the increase in wool production is reduced but the S content of the wool continues to increase, despite significant loss of the supplementary cysteine to oxidation. The increase in wool S must be directly linked to increased circulating cysteine in the blood, however, because fibre growth is not proportionately affected, bulb cell production changes are probably not related to blood cysteine concentration.

What the effects of increased proportions of ultra-high S proteins have on the resultant wool characteristics such as strength are unclear (Reis, 1992). Unless improved characteristics can be clearly demonstrated cysteine, supplementation would seem inappropriate in most New Zealand farming situations with adequate feed supply.

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