

## THE IMPORTANCE OF METHIONINE FOR WOOL GROWTH IN SHEEP

P.J. REIS\*

## SUMMARY

Mixtures of essential amino acids were infused into the abomasum of sheep, and effects on wool growth were measured. Various mixtures containing methionine stimulated wool growth, whereas mixtures lacking methionine were not effective. In two experiments, the provision of cysteine in place of methionine failed to stimulate wool growth. These results indicate a specific role of methionine in controlling wool growth, other than to provide cysteine via transsulphuration.

## INTRODUCTION

The amounts and proportions of amino acids available to wool follicles have marked effects on the rate of wool growth (Reis 1979). The sulphur-containing amino acids, cyst(e)ine or methionine, are particularly effective for stimulating wool growth. The abomasal infusion of mixtures of amino acids simulating their proportions in casein enhanced the rate of wool growth, but wool growth was inhibited when methionine was omitted from these infusions (Reis and Tunks 1978). The present experiments were designed to investigate the importance of methionine for wool growth in various mixtures of essential amino acids, and to see whether cysteine could effectively replace methionine in these mixtures.

## MATERIALS AND METHODS

Mature Merino wethers, each fitted with an abomasal cannula near the pylorus, were kept indoors in metabolism cages. The ration, 600 g of a ground and pelleted mixture of lucerne hay (three parts) and oat grain (two parts), was offered once daily; drinking water was available *ad libitum*.

Effects on wool growth, of infusion of amino acids into the abomasum, were measured in five experiments. Seven different mixtures of amino acids (Table 1) were given for periods of 12 days, and wool growth was compared with that in a control period prior to infusion. In some experiments the effects of two mixtures of amino acids were also compared in two consecutive 12-day periods, in each of which half the sheep received one mixture and half the other. Amino acids were obtained from Tanabe Seiyaku Co. Ltd., Osaka, and Ajinomoto Co. Inc., Toyko. A peristaltic pump was used to maintain a steady rate of infusion of aqueous solutions into the abomasum over about 23 hours per day in a volume of 1-2 l.

The patterns of infusion of amino acid mixtures in the various experiments are described below:

Experiment 1: Mixture 1 (ten essential amino acids) and Mixture 2 (cysteine replacing methionine in Mixture 1) were compared in four sheep.

Experiment 2: Mixture 3 (five essential amino acids) and Mixture 4 (Mixture 3 with methionine omitted) were compared in four sheep.

Experiment 3: Mixture 3 (five essential amino acids) and Mixture 5 (cysteine replacing methionine in Mixture 3) were compared in two sheep.

Experiment 4: Mixture 6 (four essential amino acids) was given to five sheep.

Experiment 5: Mixture 7 (six essential amino acids) was given to four sheep.

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\* CSIRO, Division of Animal Production, P.O. Box 239, Blacktown, N.S.W. 2148.

TABLE 1 Composition of amino acid mixtures infused into the abomasum (g/day)

Amino acid	Mixture						
	1	2	3	4	5	6	7
L-Arginine	3	3	-	-	-	-	3
L-Histidine	2	2	-	-	-	-	2
L-Isoleucine	6	6	6	6	6	6	-
L-Leucine	7	7	7	7	7	7	-
L-Lysine	7	7	7	7	7	7	-
L-Methionine	3		3	-	-	3	-
L-Phenylalanine		7	-	-	-	-	7
L-Threonine	4	4	-	-	-	-	4
L-Tryptophan	1	1	-	-	-	-	1
L-Valine	5	5	5	5	5	-	5
L-Cysteine		2.4	-	-	2.4	-	-
Glycine	-		-	1.5	-	-	-

Responses in wool growth were assessed in two ways. The mass of clean, dry wool grown per day was assessed as described by Reis and Tunks (1978), using a clipping schedule which allowed for the estimated emergence time of wool fibres. Wool was removed from a defined area of skin (100-200 cm<sup>2</sup>) on the mid-side of each sheep, prior to infusion and for 8-day periods which corresponded to each infusion period. The autoradiographic technique of Downes *et al.* (1967) was used to measure changes in diameter, length growth and volume of fibres. Intravenous injections of tracer doses of L-[<sup>35</sup>S]cystine (c. 60 µCi/dose) were given at intervals of 4 or 6 days prior to infusion, and 4 days apart at the end of each 12-day infusion period (Reis and Tunks 1978), to measure wool growth prior to treatment and during the period days 9 to 12 of treatment inclusive. Fibre measurements and calculations were made as described by Reis and Tunks (1978). Fibres were sampled from four sites along one side of each sheep; a total of 40-80 fibres were measured per sheep with approximately equal numbers from each site. The statistical significance of differences was tested using a t-test for paired variates.

## RESULTS

Abomasal infusion of the ten essential amino acids (Mixture 1) approximately doubled wool growth in Experiment 1, by increasing both fibre diameter and length growth rate (Table 2). However, when methionine was replaced by an equimolar amount of cysteine (Mixture 2), wool growth was not significantly different from control values. In Experiment 2, a mixture of five essential amino acids including methionine (Mixture 3) also appreciably increased wool growth, but when methionine was omitted (Mixture 4) the infusion failed to alter wool growth. The results of Experiment 3 also indicated that the replacement of methionine with cysteine (Mixture 5) in this mixture of five essential amino acids was not effective for stimulating wool growth; these results with only two sheep were not analysed statistically. The infusion of four essential amino acids including methionine (Experiment 4) stimulated wool growth similarly to Mixture 3, but the infusion of the remaining six essential amino acids (Experiment 5) did not stimulate wool growth. In fact, fibre diameter and the amount of wool grown were slightly, but significantly, reduced by the latter treatment (Table 2).

TABLE 2 Effect of abomasal infusion of mixtures of amino acids on wool growth. Values are means for two sheep (Experiment 3), four sheep (Experiments 1, 2 and 5) or five sheep (Experiment 4). Values with different superscripts differ significantly at the 5% level of probability

Experiment	Treatment	Fibre diameter (μm)	Fibre length growth rate (μm/day)	Fibre volume x 10 <sup>-3</sup> (μm <sup>3</sup> /day)	Clean wool/midside area (mg/day)
1	Control	18.2 <sup>a</sup>	288 <sup>a</sup>	86 <sup>a</sup>	48 <sup>a</sup>
	Mixture 1	22.2 <sup>b</sup>	365 <sup>ab</sup>	160 <sup>b</sup>	102 <sup>b</sup>
	Mixture 2	18.3 <sup>a</sup>	323 <sup>ab</sup>	97 <sup>a</sup>	60 <sup>a</sup>
2	Control	19.1 <sup>a</sup>	262 <sup>a</sup>	86 <sup>a</sup>	127 <sup>a</sup>
	Mixture 3	21.2 <sup>b</sup>	333 <sup>a</sup>	132 <sup>a</sup>	174 <sup>ab</sup>
	Mixture 4	18.5 <sup>a</sup>	275	84 <sup>a</sup>	123
3	Control	19.6	307	103	103
	Mixture 3	21.6	358	144	128
	Mixture 5	19.6	336	115	109
4	Control	19.4 <sup>a</sup>	288 <sup>a</sup>	95 <sup>a</sup>	97 <sup>a</sup>
	Mixture 6	21.3 <sup>b</sup>	356	141 <sup>b</sup>	143
5	Control	18.6 <sup>a</sup>	302 <sup>a</sup>	94 <sup>a</sup>	98 <sup>a</sup>
	Mixture 7	17.5 <sup>b</sup>	299	82 <sup>b</sup>	90

#### DISCUSSION

The present experiments have established that a mixture of the ten amino acids essential for growth in the rat is effective for stimulating wool growth, and have confirmed (Reis and Tunks 1978) that methionine is a vital component of the mixture. The abomasal infusion of 1-3 g/day cyst(e)ine supplementing rations similar to that given here stimulates wool growth (Reis 1979), and the effect is probably due mainly to the provision of cysteine as substrate for the synthesis of wool proteins. Equivalent amounts of methionine may also provide cysteine via the transsulphuration pathway (Reis 1979). In view of the small amount of methionine in wool proteins, and its complete absence from the matrix proteins (see Reis 1979), the requirement for methionine as a substrate for the synthesis of wool proteins would be small.

The observation that methionine cannot be effectively replaced by cysteine in Experiments 1 and 3 indicates that methionine must be influencing wool growth by a mechanism other than acting as a source of cysteine. These experiments provide the first demonstration of a situation in which the provision of additional cysteine failed to stimulate wool growth significantly. It may be postulated that, even when cysteine is supplied, without sufficient methionine to perform some specific function, the machinery for synthesis of wool proteins cannot function at a high rate. Specific effects of methionine on wool growth are most probably related to the formation of S-adenosyl-L-methionine. This compound is a methyl donor for many important reactions which may influence wool growth (see Reis 1979). It is also an intermediate in the biosynthesis of the polyamines, spermidine and spermine, which appear to have a role in nucleic acid and protein synthesis, especially in actively dividing tissues (Tabor and Tabor 1976; Williams-Ashman and Canellakis 1979).

The relative importance of cyst(e)ine and methionine in controlling the rate of wool growth would be best determined during experiments in which the animal's total supply of amino acids was controlled, by giving all nutrients either post-ruminally or parenterally.

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