METABOLISM OF CYSTINE BY MERINO SHEEP GENETICALLY DIFFERENT IN WOOL PRODUCTION

II.* THE RESPONSES IN WOOL GROWTH TO ABOMASAL INFUSIONS OF L-CYSTINE OR DL-METHIONINE

By A. J. Williams,† G. E. Robards,† and D. G. Saville†‡

[Manuscript received 30 June 1972]

Abstract

The effects of daily abomasal infusions of L-cystine (2 g) or DL-methionine (2·5 g) on the production of wool and its content of sulphur were compared in Peppin Merino ewes from paired flocks selected for high (Fleece Plus) or low (Fleece Minus) fleece weight.

The six ewes of the Fleece Plus flock produced 16% more wool than those of the Fleece Minus flock, when the ewes of both flocks were offered a basal diet of 800 g of ground and pelleted lucerne hay daily. Abomasal infusions of L-cystine or DL-methionine increased the rate of wool growth in the two flocks, but an interaction between treatment and genotype ($P < 0 \cdot 05$) resulted from a greater response to the amino acids in sheep from the Fleece Plus flock (55 *v.* 15%). The infusion of DL-methionine caused a greater increase ($P < 0 \cdot 05$) in wool production than that due to the infusion of an equivalent quantity of L-cystine (41 *v.* 32%).

Both amino acids increased the sulphur content of the wool ($P < 0 \cdot 05$) with no difference in the response due to either amino acid. The wool grown by sheep in the Fleece Plus flock contained 10% less sulphur, and this difference between the flock means was independent of treatments.

I. Introduction

The high content of cystine in keratin relative to the low concentration of this amino acid in the dietary plant material led Marston (1955) to suggest that wool growth may be limited by the availability of cystine to sheep. Extensive experimental evidence (Reis and Schinckel 1963, 1964; Reis 1967; Dryden, Wickham, and Cockrem 1969) now indicates that wool growth does respond to both cystine and methionine, provided that deamination of the amino acids in the rumen is prevented. It is unlikely that abomasal supplements of any one amino acid, other than cystine and methionine, influence wool growth with normal roughage diets (Reis 1970). If the mechanism through which methionine influences wool growth involves trans-

* Part I, *Aust. J. biol. Sci.*, 1972, **25**, 1259–68.

† New South Wales Department of Agriculture, Agricultural Research Station, Trangie, N.S.W. 2823.

‡ Present address: Veterinary Research Station, Glenfield, N.S.W. 2167.

sulphuration (Du Vigneaud 1952), the responses in wool growth to abomasal infusions of methionine would be expected to be no greater than those due to abomasal infusions of equivalent quantities of cystine. However, the relative responses in wool growth to the infusions of equivalent amounts of these two amino acids have not been established.

In view of the importance of the sulphur amino acids to the rate of wool growth, the genetic differences among Peppin Merinos in both wool production and efficiency of conversion of feed into wool may be due to variation in some aspects of the supply of utilization of the sulphur amino acids. In two flocks, selectively bred for either high or low clean fleece weight, there were no differences in the entry rate of cystine (Williams, Leng, and Stephenson 1972). Thus, the cause of the differences in efficiency of conversion of food to wool between these selection flocks (Dolling and Moore 1960; Ahmed, Dun, and Winston 1963) was unlikely to be that different amounts of sulphur amino acids are available to the sheep from the same diet.

As wool production has responded to selection (Dun 1958; Brown and Turner 1968), changes in the utilization of the available sulphur amino acids for the growth of wool have probably occurred. However, in this situation the opportunity for expression of genetic merit may differ between the flocks, resulting from the availability of the sulphur amino acids being more limiting for wool growth in the high-fleece-weight flock. If this occurs, phenotype may no longer adequately measure genotype, and genotype $\times$ environment interactions may arise (Williams 1966; Piper and Dolling 1969).

The objectives of an experiment, the results of which are reported in this paper, were twofold. Firstly, the hypothesis was tested that wool production of Merino sheep from genetically high- and low-producing flocks was equally limited by the availability of sulphur amino acids. Secondly, the relative responses in wool production to abomasal infusions of equivalent amounts of L-cystine and DL-methionine were compared.

II. Materials and Methods

Six ewes, born in 1965 and 2 years old at the commencement of the experiment, were selected from each of two single-character selection flocks. The structure of and the selection criteria in the Fleece Plus and Fleece Minus flocks, which had been maintained at the Agricultural Research Station, Trangie, since 1951, have been described by Dun (1958). Briefly, each is a closed flock, and replacement sheep for breeding are selected solely on high (Fleece Plus) or low (Fleece Minus) clean fleece weight. The mean values for clean fleece weight and body weight at shearing when the sheep were aged 15–16 months are given in Table 1 for both the whole flocks and the selected experimental sheep. The method of Carter and Clarke (1957) was used to measure the density of follicles in skin samples located immediately posterior to a clipped patch on the shoulder, and collected a week before the experiment commenced (Table 1). The abomasum of each ewe was fistulated and fitted with a rubber cannula (Jarrett 1948) several months before the start of the experiment.

Throughout the experiment, all sheep were fed 800 g daily of ground and pelleted lucerne hay. The treatments imposed were:

(1) Daily abomasal infusion of 1 litre of water (control);

(2) Daily abomasal infusion of 1 litre of solution (pH 1–2) containing 2 g L-cystine (Koch–Light; puriss);

(3) Daily abomasal infusion of 1 litre of solution containing $2\cdot5$ g DL-methionine ($2\cdot6$ g of methionine—Pfizer Pty. Ltd., feed grade, 98%).

The solutions were infused using disposable plastic blood transfusion sets, adjusted to deliver the volume of solution during 20–24 hr.

Table 1

MEAN VALUES FOR CLEAN FLEECE AND BODY WEIGHT AT 15–16 MONTHS OF AGE, AND FOLLICLE DENSITIES IN THE SELECTED SHEEP BEFORE THE EXPERIMENT

Values in parentheses are the standard deviations

Flock	No. of ewes	Fleece weight (kg)	Body weight (kg)	Follicle density (No. per mm²)
Fleece Plus				
Whole flock	39	3·7 (0·4)	36·6 (3·9)	—
Selected ewes	6	3·4 (0·3)	36·7 3·5)	63·1 (4·8)
Fleece Minus				
Whole flock	36	2·0 (0·4)	38·3 (3·6)	—
Selected ewes	6	2·2 (0·2)	39·0 (1·7)	45·1 (11·1)

The experimental design adopted was a 3 × 3 balanced latin square (Cochran and Cox 1962) in which treatments were imposed during three periods, each of 6 weeks, and separated from previous periods by 1 week (Fig. 1). The analysis from this design estimated separately the direct and residual effects (i.e. those due to a treatment in a previous period) of the environmental treatments.

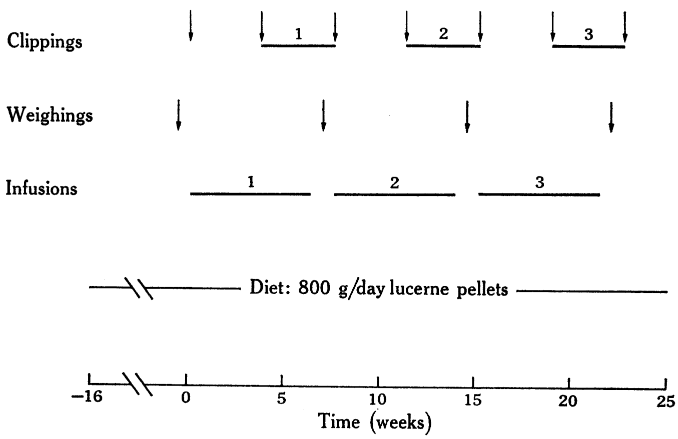

Fig. 1.—Time schedule for periods of infusion in relation to times of clipping the wool from shoulder areas.

Clean wool production per unit area of skin was estimated by periodically clipping the wool from a patch (100 cm²) located on the left shoulder of each ewe, and by measurement of the unstretched area of this patch at each clipping. The greasy wool was cleaned by successive washings in light petroleum (b.p. 60–80°C), alcohol, and water. The relationship of the clipping dates to the periods of infusion is shown in Figure 1. Data for the average daily wool production per unit area of skin, derived from the final 3-weekly clipping in each period, were analysed. If the cells in the fibre require 5–7 days to progress from the follicle bulb to a clipping height above the skin surface (Downes and Sharry 1971), the analysed data would represent the wool growth during the final 3 weeks of each infusion period. Samples of wool from these final clippings were combusted in an oxygen flask to determine sulphur content by the method outlined by Reis and Schinckel (1963).

Each ewe was weighed twice before the infusions commenced in period 1. Ewes were reweighed twice during the week between periods 1 and 2, and similarly between periods 2 and 3. Finally, the ewes were weighed on the fourth and sixth day after the termination of infusions in period 3. These weights were corrected for the weight of greasy fleece. The change in body weight for each ewe during each period was calculated from the difference between the mean fleece-free body weights at the beginning and end of a period.

III. Results

The infusion of each of the amino acids resulted in significant direct and residual effects on the rate of wool growth (Table 2). Although both amino acids substantially increased the rate of wool growth relative to that when no amino acid was given, the response to DL-methionine (41%) was greater than the response (32%) to L-cystine ($P < 0\cdot05$) (Table 3). The rate of wool growth differed between the flocks ($P < 0\cdot05$), and there was a significant interaction between the flocks and the direct effects of treatments (Tables 2 and 3).

Table 2

SUMMARY OF ANALYSIS OF VARIANCE FOR WOOL PRODUCTION, SULPHUR CONTENT, AND CHANGE IN BODY WEIGHT

Source of variation	Degrees of freedom	Mean squares: Wool production (μg cm^{-2} day^{-1})	Mean squares: Sulphur content (%)	Mean squares: Body weight change (kg per 6 weeks)
Series	1	165	0·033	0·1213
Sequences within series	4	3889*	0·034	0·740
Periods within series	4	486	0·017	6·088*
Adjusted direct effects	2	9420*	0·481*	0·080
Adjusted residual effects	2	779*	0·022	1·191
Error (*a*)	4	96	0·016	0·288
Flocks	1	38875*	1·037	0·001
Flocks × direct effects (adj.)	2	3419*	0·049	0·493
Flocks × residual effects (adj.)	2	49	0	0·477
Error (*b*)	13	821	0·017	0·286

* $P < 0\cdot05$.

Both amino acids increased the sulphur content of the wool by similar amounts, and there were no significant residual effects of the treatments (Table 2). The sulphur content of the wool grown by Fleece Plus ewes was less ($P < 0\cdot05$) than that from Fleece Minus ewes. No interactions between flocks and the direct or residual effects of the treatments for the sulphur content of the wool (Tables 2 and 3) were significant.

At the commencement of the first period, the mean body weight of the Fleece Plus ewes was $33\cdot0\pm0\cdot8$ kg, and was not significantly different from that of the Fleece Minus ewes ($35\cdot0\pm0\cdot8$ kg). Body weights increased during the first and second period, but decreased slightly during the third period, resulting in a significant effect of periods in the analysis (Table 2). Neither treatments nor flocks had any significant effect on the change in body weight (Tables 2 and 3).

TABLE 3

DIRECT EFFECT MEANS FOR WOOL PRODUCTION PER UNIT AREA, SULPHUR CONTENT OF WOOL, AND CHANGES IN BODY WEIGHT OF EACH FLOCK DURING EACH TREATMENT

Flock	Treatment	Wool production ($\mu g\ cm^{-2}\ day^{-1}$)	Sulphur content (%)	Body weight change (kg per 6 weeks)
Fleece Plus	Control	743	2·99	0·30
	2 g L-cystine	1114	3·45	1·18
	2·5 g DL-methionine	1190	3·53	0·97
Fleece Minus	Control	639	3·48	1·13
	2 g L-cystine	714	3·77	0·21
	2·5 g DL-methionine	757	3·74	1·08
Standard error of mean differences among treatments		21	0·06	0·35
Standard error of mean differences between flocks		79	0·08	0·31

IV. DISCUSSION

In addition to demonstrating the efficacy of the sulphur amino acids in stimulating the rate of wool growth, the present experiment provided statistical evidence that the response in wool growth rate was greater with abomasal infusions of DL-methionine. Hence, methionine apparently had a specific effect on the rate of wool growth, separate from its role of supplying cystine by transulphuration (Du Vigneaud 1952). The results of Williams and Leng (1972) indicated that daily abomasal infusions of 2·5 g DL-methionine increased the rate of entry of cystine into the body pool of cystine by approximately 60%. However, a greater proportion than this of the infused methionine may be available as cystine within the follicle, as extensive transulphuration of methionine in this tissue was demonstrated by Downes, Sharry, and Till (1964). Whether the specific influence of methionine on the rate of wool growth was exerted through one or more of its various functions as detailed by Reis (1967), or simply as an amino acid limiting for protein synthesis within the bulb of the follicle, remains uncertain. Cystine may be the amino acid primarily limiting protein synthesis in the follicle, with methionine availability becoming critical when the limitation due to cystine was removed. Thus, the infusion of methionine would increase the availability of both amino acids for protein synthesis, whereas when cystine only is infused protein synthesis may be less, due to a limited availability of methionine. In view of the findings of Reis (1967), that increasing amounts of methionine can reduce the rate of wool growth, a general conclusion concerning the relative abilities of these two amino acids in stimulating wool growth cannot be given until the dose–response curve for each amino acid has been accurately determined.

Selective breeding based on clean fleece weight has been effective in producing large differences between these two flocks in this trait. Assuming that this difference is largely due to genetic differences in wool production per unit area (Dun 1958), the superiority of the selected Fleece Plus sheep was less marked on the basal diet than when the sheep were grazing. A similar result was obtained by Dolling and Moore (1960) who suggested that the greater difference at pasture was due to differences in feed intake. However, Williams and Miller (1965) were unable to detect differences in intake between rams from the two flocks used in the present study.

The difference between the flocks in wool production per unit area increased considerably when equivalent quantities of cystine or methionine were infused into the abomasum. Thus the wool production of Fleece Plus ewes was apparently more restricted by the availability of sulphur amino acids when the sheep were consuming the basal ration. The present results and, presumably, the interactions between genotype and dietary environment for wool production (Williams 1966; Piper and Dolling 1969), indicate that genetic differences exist for the sensitivity of the follicle population to the supply of sulphur amino acids. As the mechanisms involved in the stimulation of wool growth by these amino acids are uncertain, the cause of the difference in sensitivity is unknown, and cannot be decided from the present results.

The results from this experiment confirm that the sulphur content of wool is variable and can be influenced by both diet (Reis and Schinckel 1963) and genotype (Piper and Dolling 1966; Reis *et al.* 1967). In contrast to the differential effect of cystine and methionine infusions on the rate of wool growth, the two amino acids caused similar changes in the sulphur content of the wool, which would indicate a greater synthesis of both high- and low-sulphur keratins due to methionine than to cystine.

As there was no interaction between genotype and treatments for the sulphur content of the wool, the observation of Reis and Williams (1965) that the variation in sulphur content of wool was greater for phenotypically high wool producers apparently did not result from a genetic relationship between these two traits. Although the lower sulphur content of wool from Fleece Plus ewes was probably associated with the more efficient utilization of the sulphur amino acids for wool growth, it does not appear that this is a simple relationship as envisaged by Gillespie and Reis (1966). If this relationship arises from the effects of mass action, as suggested by those authors, it is unlikely that there would be a genetic difference when the sulphur content was measured at its maximum value.

Abomasal infusions of 2 g of L-cystine or 2·5 g of DL-methionine resulted in the growth of wool containing a maximum concentration of cystine (Reis 1967), and it was therefore probable that the cystine contents of the wool from the two flocks during the infusions in the present experiment reached their maximum values. The genetic difference in sulphur content of the wool appears to be a fundamental difference, but the cause remains to be investigated experimentally.

Reis and Schinckel (1963) and Reis (1967) presented results suggesting that abomasal infusions of the sulphur amino acids may slightly increase the body weight of sheep fed a maintenance ration. The results of the present experiment do not confirm their findings. If the availability of the sulphur amino acids influences the efficiency of energy utilization, short-term changes in body weight are not precise-enough criteria on which to assess this effect.

V. Acknowledgments

The capable assistance given by Mr. R. J. Winston in the handling of the sheep is gratefully recorded. The financial assistance of the Australian Wool Board is also acknowledged.

VI. References

Ahmed, W., Dun, R. B., and Winston, R. J. (1963).—The efficiency of conversion of feed to wool in Merino flocks selected for or against fleece weight. *Aust. J. exp. Agric. Anim. Husb.* **3**, 269.

Brown, G. H., and Turner, H. N. (1968).—Response to selection in Australian Merino sheep. II. Estimates of phenotypic and genetic parameters for some production traits in Merino ewes and an analysis of the possible effects of selection on them. *Aust. J. agric. Res* **19**, 303.

Carter, H. B., and Clark, W. H. (1957).—The hair follicle group and skin follicle population of Australian Merino sheep. *Aust. J. agric. Res.* **8**, 91.

Cochran, W. G., and Cox, G. M. (1962).—"Experimental Designs." 2nd Ed. (John Wiley and Sons, Inc.: New York.)

Dolling, C. H. S., and Moore, R. W. (1960)—Efficiency of conversion of food to wool. 1. Correlated response to selection for high or low clean wool weight per head. *Aust. J. agric. Res.* **12**, 580.

Downes, A. M. (1961).—On the mechanism of incorporation of [^{35}S]cystine into wool. *Aust. J. biol. Sci.* **14**, 109.

Downes, A. M., and Sharry, L. F. (1971).—Measurement of wool growth and its response to nutritional changes. *Aust. J. biol. Sci.* **24**, 117.

Downes, A. M., Sharry, L. F., and Till, A. R. (1964).—The fate of intradermal doses of labelled amino acids in the skin of sheep. *Aust. J. biol. Sci.* **17**, 945.

Dryden, G. M., Wickham, G. A., and Cockrem, F. (1969).—Intravenous infusion of cystine and wool growth of Romney sheep. *N.Z. Jl agric. Res.* **12**, 580.

Dun, R. B. (1958).—The influence of selection and plane of nutrition on the components of fleece weight in Merino sheep. *Aust. J. agric. Res.* **9**, 802.

Du Vigneaud, V. (1952).—"A Trail of Research in Sulphur Chemistry and Metabolism." (Cornell University Press: New York.)

Gillespie, J. M., and Reis, P. J. (1966).—The dietary-regulated biosynthesis of high-sulphur wool proteins. *Biochem. J.* **98**, 669.

Jarrett, I. G. (1948).—The production of rumen and abomasal fistulae in sheep. *J. Coun. scient. ind. Res. Aust.* **21**, 311.

Marston, H. R. (1955).—In "Progress in the Physiology of Farm Animals." (Ed. J. Hammond.) Vol. 2. Ch. 11. (Butterworths Scientific Publications: London.)

Piper, L. R., and Dolling, D. H. S. (1966).—Variation in the sulphur content of wool of Merino sheep associated with genetic differences in wool producing capacity. *Aust. J. biol. Sci.* **19**, 1179.

Piper, L. R., and Dolling, C. H. S. (1969).—Efficiency of conversion of food to wool. 4. Comparison of sheep selected for high clean wool weight with sheep from a random control group at three levels of dietary protein. *Aust. J. agric. Res.* **20**, 561.

Reis, P. J. (1967).—The growth and composition of wool. 4. The differential response of growth and of sulphur content of wool to the level of sulphur-containing amino acids given per abomasum. *Aust. J. biol. Sci.* **20**, 809.

Reis, P. J. (1970).—The influence of abomasal supplements of some amino acids and sulphur-containing compounds on wool growth rate. *Aust. J. biol. Sci.* **23**, 441.

Reis, P. J., and Schinckel, P. G. (1963).—Some effects of sulphur-containing amino acids on the growth and composition of wool. *Aust. J. biol. Sci.* **16**, 218.

Reis, P. J., and Schinckel, P. G. (1964).—The growth and composition of wool 2. The effect of casein, gelatin, and sulphur-containing amino acids given per abomasum. *Aust. J. biol. Sci.* **17**, 532.

Reis, P. J., and Williams, O. B. (1965).—Variations in the sulphur content of wool from Merino sheep on two semi-arid grasslands. *Aust. J. agric. Res.* **16**, 1011.

Reis, P. J., Tunks, D. A., Williams, O. B., and Williams, A. J. (1967).—A relationship between sulphur content of wool and wool production by Merino sheep. *Aust. J. biol. Sci.* **20**, 153.

Williams, A. J. (1966).—The efficiency of conversion of feed to wool during limited and unlimited feeding of flocks selected on clean fleece weight. *Aust. J. exp. Agric. Anim. Husb.* **6**, 90.

Williams, A. J., and Leng, R. A. (1972).—The effect of abomasal infusions of L-cystine and DL-methionine on the entry rate of cystine in sheep. *Proc. Aust. Soc. Anim. Prod.* **9**, in press.

Williams, A. J., Leng, R. A., and Stephenson, S. K. (1972).—Metabolism of cystine by Merino sheep genetically different in wool production. I. Comparison of the entry rates of cystine in sheep from flocks selectively bred for high and low fleece weight. *Aust. J. biol. Sci.* **25**, 1259–68.

Williams, A. J., and Miller, H. P. (1965).—The voluntary feed intake of sheep genetically different in wool production. *Aust. J. exp. Agric. Anim. Husb.* **5**, 385.