Sulphur as a nutrient for Merino sheep

1. Storage of sulphur in tissues and wool, and its secretion in milk

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1. Estimates of sulphur retention by Merino sheep during growth, pregnancy and wool production, and of S excretion during lactation were obtained to derive estimates of the S requirements of Merino sheep.

2. The S content of fifty Merino sheep was related by weighted regression analysis to age, live weight and (age × live weight). The quantity of S stored was calculated for varying rates of live-weight gain in sheep differing in age and live weight.

3. The S contents of twenty-five samples of foetus, foetal membranes and uteruses were determined. Relationships between S content and days from mating were calculated, and differentiated to give daily net changes in S content during pregnancy.

4. Milk production of twenty-seven Merino ewes was estimated on eight to ten occasions at approximately weekly intervals to the 84th day of lactation. The average S content was 472 mg/l; it increased on average by 1·0 mg/l per d of lactation. The mean S content of ninety-seven samples of wool averaged 34 g S/kg clean dry wool.

5. It was concluded that wool represented the major demand for S by the Merino since approximately 70 g S were secreted in producing 2 kg clean wool, 50 g S in a lactation yielding 100 l milk and 8 g S in giving birth to a single lamb.

Ruminants cannot survive on diets deficient in sulphur. When such diets are given to sheep, feed intake and production decline (Thomas, Loosli, Williams & Maynard, 1951), serum sulphate concentrations are depressed (Weir & Rendig, 1954), and lactic acid accumulates in the reticulo-rumen (Whanger & Matrone, 1965). S requirements of sheep are not adequately defined and were not discussed by the Agricultural Research Council (1965). The requirement for S is determined by the quantity of S retained during growth, gestation, lactation and in wool production, the inevitable losses of S by the sheep, and the efficiency with which ingested S is utilized. In this paper we have presented estimates of S deposition during growth, gestation, lactation and wool production, and in a subsequent paper, estimates will be presented of the efficiency of utilization of S in forage diets.

EXPERIMENTAL

Retention of sulphur during growth

Langlands & Sutherland (1969) slaughtered and minced 107 newly shorn fine-wool Merino wethers and non-pregnant ewes. In the present study fifty of the samples of mince, from twenty-three wethers and twenty-seven ewes, were analysed for S content. The sheep ranged in age from newly born to 110 months and in live weight from 1 to 53 kg.

Retention of S during pregnancy

Twenty-five samples of foetus, foetal membranes and uterus from the pregnant ewes slaughtered by Langlands & Sutherland (1968) and ten uteruses from non-pregnant ewes were analysed for S content.

Detailed descriptions of the ewes, their management and the procedures used to prepare the samples for analysis were given by Langlands & Sutherland (1968).

Secretion of S in milk

The S content of the milk of twenty-seven grazing fine-wool Merino ewes aged from 5 to 8 years, and rearing single wether lambs was estimated on eight to ten occasions at approximately weekly intervals to the 84th day of lactation. Three ewes grazed each of nine paddocks varying in size. Paddocks (1)–(6) inclusive were of *Phalaris tuberosa* L.–*Trifolium repens* L. stocked at 2, 5, 10, 20, 26 or 40 sheep per ha respectively. At all times there was sufficient herbage on paddocks (7), (8) and (9) to allow *ad lib* intake; the pastures were *Lolium perenne* L.–*Trifolium repens*, *Medicago sativa* L. and native species (Roe, 1947) respectively.

The milking technique of Corbett (1968) was used. The ewes were injected intravenously at 09.30 hours with oxytocin, milked by machine until the udder was empty, fitted with an udder cover to prevent suckling and returned to pasture with the lamb. Approximately 4 h later oxytocin was again injected, the ewe was milked until the udder was empty, and a sample of milk was retained for S analysis.

S content of wool and the rate of wool growth

Ninety-seven samples of clean, dry wool were taken from fifty-five fine-wool Merino ewes and wethers and were analysed for S content. The samples were taken at monthly intervals at various times of the year from areas defined by tattoos on the mid-side of the sheep. The sheep were either maintained in pens on lucerne chaff or grazed *Phalaris tuberosa* pastures.

Two-hundred and eighty estimates of daily wool production per sheep, live weight and daily live-weight change averaged over monthly periods were available from experiments of 5 years duration in which non-pregnant fine-wool Merino ewes grazed *Phalaris tuberosa–Trifolium repens* pastures at 2·5, 7·4, 12·4, 17·3, 22·2, 27·2, 32·1 or 37·1 sheep per ha, or native pastures (Roe, 1947) at 1·9, 3·7 or 5·6 sheep per ha. Daily wool production was estimated by the dye-banding technique (Langlands & Wheeler, 1968) and live weights were adjusted for the weight of fleece carried. The sheep were shorn in either October or November.

S determination

Carcasses: preliminary treatment. Samples of minced carcass containing approximately 8 g dry matter were digested in 25 ml concentrated nitric acid and 10 ml of a 2:3 (v/v) mixture of concentrated nitric and 60% perchloric acids containing 0·7 g ammonium metavanadate and 1 g potassium dichromate per l. The digest was

Table 1. *Sulphur content of the carcasses of fifty Merino ewes and wethers*

S content (g)	Live wt (kg)	Age (months)	S content (g)	Live wt (kg)	Age (months)
51·8	31·5	50	43·0	39·9	30
27·7	18·2	6	45·8	27·0	48
39·7	32·7	110	15·5	11·3	1
53·5	34·5	50	11·6	7·6	1
32·6	19·1	6	1·6	1·7	0
33·2	23·6	6	20·9	18·4	2
17·7	12·9	4	29·3	12·9	2
41·9	30·4	50	5·8	3·6	0
31·0	17·2	6	9·0	5·8	1
41·4	24·0	26	13·2	7·0	1
28·9	16·8	6	22·9	12·8	1
55·6	34·9	110	13·2	9·8	2
63·9	35·4	110	3·1	2·7	0
81·8	52·6	18	4·0	2·5	0
50·4	32·4	50	27·1	18·0	4
29·6	16·8	2	20·0	14·4	4
9·0	8·6	1	23·1	15·3	4
9·0	8·4	2	28·2	18·0	4
45·7	29·5	50	23·2	15·9	4
47·6	48·5	110	34·9	25·9	26
15·5	12·0	2	30·9	23·6	26
20·3	15·4	2	31·0	24·0	26
2·4	2·0	0	35·9	27·2	26
0·5	1·1	0	49·4	40·0	18
4·7	3·4	0	2·9	3·1	0

diluted to 250 ml with water, and 10 ml portions were analysed by the procedure described below.

Carcasses, foetal tissues and milk. The carcass samples, 0·2 g dried foetal material or 2 ml milk, were digested slowly in Kjeldahl flasks with 10 ml of the nitric–perchloric digestion mixture described above. After approximately 2 h, 50 ml of a solution containing 90 ml/l of a 2:2:5 (by vol.) mixture of concentrated hydrochloric, orthophosphoric and acetic acids were added and the digest was diluted to 100 ml with water, and filtered when necessary. The digest was allowed to react with a solution containing 80 g barium chloride per l in an automated analytical system, and the resulting turbidity was measured at 625 nm (Mottershead, 1971).

Wool. Wool samples were suspended in succession in solutions of diethyl ether, hot water containing a commercial detergent, and then in hot water and distilled water. The wool was then dried at 100° and the S content was estimated as described by Reis & Schinckel (1963).

RESULTS

Retention of S during growth

The S content of the individual sheep is recorded in Table 1. A multiple regression relationship was calculated between the S content of the sheep (y, g), and age (x, months), live weight (z, kg) and (age × live weight) with each observation weighted inversely to the total weight of dry mince:

$$y = 0{\cdot}351x + 1{\cdot}474z - 0{\cdot}0104xz - 0{\cdot}659 \quad (r^2 = 0{\cdot}96). \qquad (1)$$

Table 2. *Sulphur content of Merino sheep varying in age and live weight, and the daily changes in S content for these sheep when increasing in live weight at various rates*

Live wt (kg)	Age (months)	Total S content (g)	Daily changes in S content (mg) for different rates of live-wt gain (g/d) of: 0	50	100	200
5	0·5	7·5	+10·0	+83	+157	+304
	1·5	7·8	+10·0	+83	+156	+302
15	1·0	22·2	+ 6·0	+80	+153	+299
	4·5	22·9	+ 6·0	+78	+149	+292
25	10·0	37·7	+ 3·0	+71	+140	+277
	40·0	40·4	+ 3·0	+56	+109	+214
35	12·0	51·3	− 0·5	+67	+134	+269
	48·0	50·8	− 0·5	+48	+ 97	+194
45	12·0	64·8	− 4·0	+63	+131	+266
	48·0	60·6	− 4·0	+45	+ 93	+191
55	18·0	77·0	− 7·0	+57	+121	+250
	48·0	70·3	− 7·0	+41	+ 90	+187

Each observation was weighted inversely to the weight of dry mince because the residual variation about the relationship appeared to be a function of the weight of mince. This is to be expected if the error in determining S content per unit weight of mince is constant. We have calculated from equation (1) the S content of sheep varying in age and live weight, and the daily change in S content for such sheep when increasing in live weight at various rates (Table 2). From equation (1) and from the equation for nitrogen content given by Langlands & Sutherland (1969), the N:S ratio at the mean age (20 months) and live weight (19 kg) for these values was estimated to be 19·5.

Retention of S during pregnancy

Individual observations are recorded in Table 3. The mean S content of uteruses from non-pregnant ewes was $63{\cdot}3 \pm 6{\cdot}1$ mg. This value was deducted from the S content of the uteruses of pregnant sheep to give the net S storage in the uterus as a result of pregnancy. Net S storage (Q, mg S) in uterus, membranes and foetus was related to time from mating (p, d) by allometric or polynomial relationships:

$$\text{foetus: } Q = 1{\cdot}182p^{5{\cdot}9} \times 10^{-9} \tag{2}$$

(residual standard deviation ± 533);

$$\text{membranes: } Q = -10{\cdot}74p + 0{\cdot}298p^2 - 0{\cdot}00133p^3 \tag{3}$$

(residual standard deviation ± 194);

$$\text{uterus: } Q = 1{\cdot}329p + 0{\cdot}0295p^2 \tag{4}$$

(residual standard deviation ± 104).

These relationships were differentiated with respect to time to give the daily rate of change in the S content of the gravid uterus (Table 4).

The rate of S storage in the conceptus increased rapidly during the later stages of pregnancy; total S content was primarily determined by S storage in the foetus since the relative S contents of the membrane and uterus were small.

Table 3. *Sulphur content of uteruses*, foetuses and foetal membranes from Merino ewes slaughtered at different stages of gestation*

Uterine S (mg)	Days from mating	Foetal membrane S (mg)	Days from mating	Foetal S (mg)	Days from mating
125	43	36	43	4	48
121	44	39	44	21	54
111	48	48	48	53	63
134	48	61	48	114	71
184	50	92	50	140	73
172	54	92	54	157	76
379	63	205	63	222	77
347	71	172	71	238	80
289	73	179	73	710	98
316	76	306	76	699	99
422	80	377	80	900	101
410	99	312	99	1272	102
447	99	314	99	1366	106
746	102	638	102	1161	106
482	111	606	111	942	111
559	114	820	114	981	112
821	119	895	119	1601	119
669	120	1018	120	2344	121
643	121	502	121	1403	122
754	122	1136	122	1983	122
764	131	665	131	2965	131
545	134	292	134	3083	134
817	135	745	135	4547	135
952	140	453	140	6980	140
668	140	902	140	5873	140

* Values before deducting 63·3 mg, the mean S content of the uterus of non-pregnant sheep (see p. 532).

Secretion of S in milk

Linear relationships between S concentration in milk and day of lactation were calculated for each sheep. The pooled slope, 1·03 ± 0·12 mg/l per d did not differ significantly between sheep. Intercepts differed significantly ($P < 0{\cdot}001$), mean values for individual sheep adjusted to the 47th day of lactation varying from 417 to 523 mg/l. Differences between sheep were not associated with differences between paddocks. The mean value for all the milks analysed was 472 mg/l.

Corbett (1968) reported an average value of 52·1 g protein/kg for a number of milk samples from ewes similar to those used in the present trial. This is equivalent to approximately 7870 mg N/l. The N:S ratio of ewe's milk is therefore approximately 16·7:1.

Secretion of S in wool

Mean wool S content for the ninety-seven samples averaged 34·0 and ranged from 31 to 38 g S/kg wool, and wool growth ranged from 2·8 to 7·9 g clean wool/d. It was not possible with the information available to explain the variability in terms of nutrition, time of year, or efficiency of wool growth. Wool contains approximately 166 g N/kg wool (Simmonds, 1955); the N:S ratio of wool is therefore approximately 4·9:1.

Table 4. *Predicted sulphur content of the gravid uterus and the predicted daily rate of change in S content of the gravid uterus of Merino sheep at different stages of pregnancy*

Days from mating	Predicted S content of gravid uterus (g)				Daily rate of change in S content of gravid uterus (mg)			
	Total	Foetus	Membranes	Uterus	Total	Foetus	Membranes	Uterus
90	1·24	0·40	0·48	0·36	+ 43	+ 26	+11	+ 7
110	2·47	1·31	0·65	0·50	+ 83	+ 69	+ 7	+ 8
125	4·13	2·78	0·72	0·63	+139	+129	+ 1	+ 9
135	5·81	4·38	0·71	0·72	+194	+188	− 3	+ 9
145	8·15	6·68	0·65	0·81	+269	+267	− 8	+10

Relationships between wool grown/d (d, g), live weight (e, kg) and daily live-weight change (f, g) were calculated for each month separately from the 280 observations. Although the slopes differed significantly between months ($P < 0{\cdot}001$), common regression coefficients ($0{\cdot}141e \pm 0{\cdot}013$ and $0{\cdot}022f \pm 0{\cdot}002$) were adopted because the residual standard deviation (1·16 g) was reduced by only 0·07 g when separate coefficients were calculated for each month. The intercepts differed significantly between months ($P < 0{\cdot}001$) and were 3·13, 2·65, 2·59, 1·66, 1·16, 1·34, 1·65, 1·55, 2·02, 2·85, 1·46 and 2·31 for January to December respectively. The intercepts appeared to be correlated with ambient temperature except for the values for November and December, which may have been affected by shearing.

DISCUSSION

Net S requirements for growth, pregnancy, lactation and wool production can be calculated from the estimates presented and show some differences from those reported by other workers.

Hansard & Mohammed (1968) analysed the products of conception of native Louisiana ewes at various stages of pregnancy. At 95 and 140 d, total products of conception for a 40 kg ewe contained 2·9 and 10·2 g S respectively. In the present study S contents at 90 and 145 d were estimated to be 1·2 and 8·2 g respectively. Macy, Kelly & Sloan (1953) reported an average value for cow's milk of 300 mg S/l, with a range from 240 to 360 mg/l. Our average value for ewe's milk was 472 mg/l, but the composition varied at different stages of lactation.

There have been many reports of the S content of wool; recently Reis & Tunks (1968) reported ranges in S contents of 30–39 g S/kg wool for Merino sheep consuming lucerne and oats, 34–42 for sheep on a high-protein diet and 31–39 for grazing sheep. Our average value, 34 g S/kg wool, appears to be similar to values given by Reis & Tunks (1968) for grazing sheep, and for those consuming lucerne. Reis & Tunks (1968) observed that the S content of wool increased with increasing level of nutrition but that differences between sheep were inversely related to the rate and efficiency of wool growth. It is therefore likely that error will be introduced when S secretion in wool is calculated from the average value of 34 g S/kg wool observed in these studies. Unfortunately, our results were inadequate for precise estimation of the S

content of wool at different times of the year or at different levels of nutrition. Some allowance should also be made for S secretion in suint but no estimates of this were derived in the present study.

Several possible reasons can be suggested for the divergencies between our values and those of other workers. Differences between the Merino and breeds selected for meat production might arise because the Merino is slow growing and late maturing and has been specifically selected for wool production, a form of animal production characterized by a high demand for S amino acids. Differences in diet and the difficulties associated with S determination may also be important. Fletcher, Robson & Todd (1963) used three different and apparently satisfactory methods to analyse wool and observed mean values of 35·2, 37·1 and 37·4 g S/kg wool.

The quantities of S utilized at various phases of a sheep's life can be calculated from our results. Wool growth represents the major demand for the Merino. Approximately 70 g S are laid down in producing 2 kg clean wool, 50 g are secreted in a lactation yielding 100 l milk, and only 8 g in giving birth to a single lamb. Approximately 30 g S are laid down in growth by a sheep weighing 25 kg at 12 months of age. The ability with which the sheep can utilize its diet to meet these demands will be examined in a subsequent paper.

REFERENCES

Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock.* No. 2. *Ruminants* London: Agricultural Research Council.
Corbett, J. L. (1968). *Aust. J. agric. Res.* **19**, 283.
Fletcher, J. C., Robson, A. & Todd, J. (1963). *Biochem. J.* **87**, 560.
Hansard, S. L. & Mohammed, A. S. (1968). *J. Nutr.* **96**, 247.
Langlands, J. P. & Sutherland, H. A. M. (1968). *Br. J. Nutr.* **22**, 217.
Langlands, J. P. & Sutherland, H. A. M. (1969). *Br. J. Nutr.* **23**, 603.
Langlands, J. P. & Wheeler, J. L. (1968). *Aust. J. exp. Agric. Anim. Husb.* **8**, 265.
Macy, I. G., Kelly, H. J. & Sloan, R. E. (1953). *Publs natn. Res. Coun., Wash.* no. 254.
Mottershead, B. E. (1971). *Lab. Pract.* **20**, 483.
Reis, P. J. & Schinckel, P. G. (1963). *Aust. J. biol. Sci.* **16**, 218.
Reis, P. J. & Tunks, D. A. (1968). *Proc. Aust. Soc. Anim. Prod.* **7**, 402.
Roe, R. (1947). *Bull. Coun. scient. ind. Res., Melb.* no. 210. p. 26.
Simmonds, D. H. (1955). *Aust. J. biol. Sci.* **8**, 537.
Thomas, W. E., Loosli, J. K., Williams, H. H. & Maynard, L. A. (1951). *J. Nutr.* **43**, 515.
Weir, W. C. & Rendig, V. V. (1954). *J. Nutr.* **54**, 87.
Whanger. P. D. & Matrone, G. (1965). *Biochim. biophys. Acta* **98**, 454.

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