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Studies in Mineral Metabolism XXXII.

The Effect of Different Forms of Sulphur in the Diet upon the Growth and Wool Production of Sheep.

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INTRODUCTION.

SHEEP wool contains about 4 per cent. of sulphur, hence for continued wool production sheep must receive in their diet sufficient sulphur in a form that can be utilized by the animal. Furthermore, the sulphur of wool is present as cystine which obviously raises the question whether the cystine necessary for wool production should be present as such in the diet or whether it can be synthesized in the animal body from sulphur-containing compounds. The source of the cystine contained in wool has become a controversial question and several articles have appeared recently on this matter (Rimington and Bekker, 1932; Speakman and Hirst, 1931 and 1932; Henrici, 1932, etc.). The absence of a reliable method for the determination of cystine in vegetation has further involved the question whether sheep do or do not synthesize cystine in their bodies. An exact determination of the cystine intake of sheep is, of course, necessary to establish definitely whether the cystine content of the food is greater than that of the wool produced. If it is not, then the controversy as to whether cystine can be produced from other sulphur-containing compounds, be it in the intestine or after absorption, has definitely been settled one way. Rossouw and Jorden (1934) have successfully evolved a method for the determination of cystine in vegetation and provisionally it seems that generally the cystine content of pasture is on an average high enough to provide the total cystine required for the production of wool, but that, unless the animal can synthesize cystine, this product may become a limiting factor in increased wool production. For instance, from the analyses of mixed pasture grasses as eaten, the following values for cystine have been obtained: ·07, ·05, ·085 grammes per 100 grammes dry grass; or in other words the cystine present in the diet of sheep on pasture would account for the cystine present in 3·5 lb. of scoured wool or approximately 7·0 lb. of grease wool. Many classes of sheep produce more than this quantity of wool annually, and the cystine required must be accounted for by a greater consumption of grasses or by synthesis of cystine in the animal body or perhaps both sources.

It was anticipated that the experiment to be described would also throw light on the relative efficacy of cystine and other sulphur-containing compounds when fed to groups of sheep in equivalent amounts and so provide information on the relation between the cystine content of the diet and wool production.

Another important aspect of the question of feeding sulphur or sulphur-containing compounds to sheep was brought forward by Steyn (1931, 1932) and more recently by Seddon (1933) and Pierce (1933), which deals with the effect of sulphur in the diet on the increase in weight of sheep. Although Steyn's experiment was not devised as a nutritional study, (Steyn, 1934), some remarkable changes in weight were brought about in the animals receiving a daily supply of sulphur, which seemed to suggest that sulphur might have a beneficial effect on the weight of sheep. However, on subjecting Steyn's results to a statistical study, it was found that no significant differences existed in the weight increases of the groups receiving their daily dose of sulphur when compared with the increase in weight of the control group. It would seem, therefore, that Steyn's results do not warrant the conclusion that markedly beneficial effects as regards increases in both body-weights and wool yields were obtained by feeding sulphur to Merino wethers and ewes. Seddon (1933) found no visible effect on the weight or the wool yield of sheep, entirely grazed, and receiving 10 gm. of sulphur thrice weekly. Pierce (1933) gave mature wethers and ewes only 2 gm. of sulphur daily, but could discover no beneficial effect of the sulphur on weight increase and wool production when his sheep were kept on a maintenance ration or one slightly above maintenance. Steyn (1934) stated that his work was not directly comparable with that of Seddon and Pierce. However, prominence was undoubtedly given to the possible nutritional value of sulphur by Steyn's work, and it was, therefore, thought that the experiment to be described would also throw light on the effect of feeding sulphur elementary or combined—on the weight and wool production of sheep with advantage.

The objects of the experiment therefore were (1) to determine the relative effects of cystine and other compounds containing sulphur in the ration of sheep on weight increase and wool production, and (2) to determine whether any advantage as regards weight increase and wool production accrued from the feeding of cystine to sheep.

A full discussion of the literature on sulphur metabolism is given by Kellermann (1934) and will not be considered here.

EXPERIMENTAL DETAILS.

Sixty Merino wethers, fifteen to twenty months old, were selected for uniformity of breed, type of wool, build and weight. These animals were divided into six uniform groups of ten each and placed in a gravelled paddock 30 by 12 yards with boxes for individual feeding along three sides of the enclosure. On the fourth side, in a shed, was a hay rack where the animals had free and continual access to teff hay. The sheep were placed daily in the

feeding boxes from 6.30 until 9.30 a.m., when each was given the basal ration consisting of half a pound of crushed yellow maize and half a pound of green feed—lucerne, maize stalks, or maize ensilage during about 3 months in winter when no green feed was available. The animals invariably cleared up the crushed maize, although some of the green feed was not infrequently left over, as explained later in this article.

On an average 2½ lb. of hay were consumed daily per head, but obviously in this case, unlike the maize and green feed, group-feeding was practised and the hay supplied *ad lib.* The daily protein intake per head was approximately 80 gm. which, according to the Wolff-Lehmann feeding standards given by Henry and Morrison for growing sheep—wool breeds—is ample for rapid growth and weight increase. The total digestible nutrients are also slightly higher than the requirements of growing sheep of this type for production purposes. In short, the daily consumption of feed was above maintenance, as is also evident from a glance at the weight increase of the control group, or all the groups during the first year of the experiment when no supplement was given.

The six groups of animals were placed on the basal ration in December, 1931. As it was intended to carry out certain wool studies which will be reported on in due course in another article, it was thought best to place the animals for a full year on the basal ration only, without giving the supplements of sulphur or compounds containing sulphur. The sheep were weighed monthly, inspected daily for clinical symptoms of disorders, shorn at the beginning of the experiment, again 12 months afterwards, and finally in December, 1933, at the conclusion of the investigation. The wool was kept on each occasion for further study and report on its quality. During the last year of the experiment, i.e. from December, 1932, until December, 1933, the sheep were dosed daily, except Sundays, with the quantities of supplements given below:—

Group I received no supplement.

In Group II each animal was given ·45 gm. cystine daily.

In Group III each was given ·7 gm. of a mixture of ·2 gm. K_2SO_4, ·3 gm. $MgSO_4$ and ·2 gm. $CaSO_4$ daily.

In Group IV the daily supplement was ·36 gm. KCNS.

Group V received 5 gm. sulphur each daily.

In Group VI each sheep was given ·12 gm. sulphur daily.

The quantities of supplements except in the case of Group V were calculated to contain the same amount of sulphur as that contained in ·45 gm. of cystine given to each animal in Group II. Group V received the amount of sulphur daily which gave the best response in Steyn's experiments.

RESULTS.

(*a*) *Food Consumption.*—As already stated, hay was given *ad lib.* If any maize (½ lb.) was left over it was invariably cleared up, i.e. the following day. Some of the green feed was left on occasions and especially during the latter portion of the experiment,

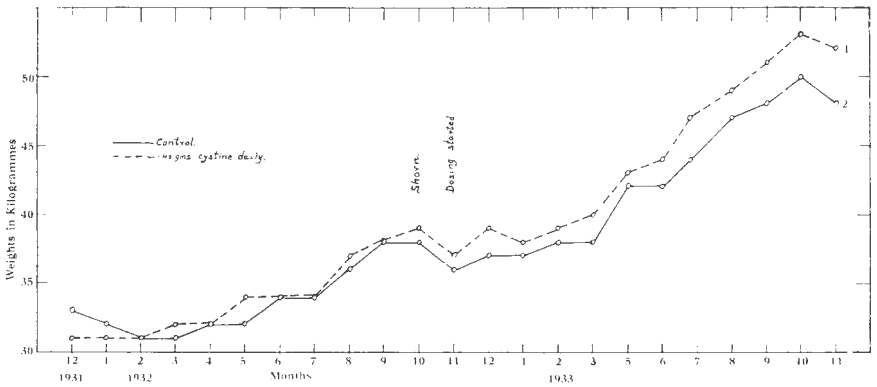

Fig. 1.

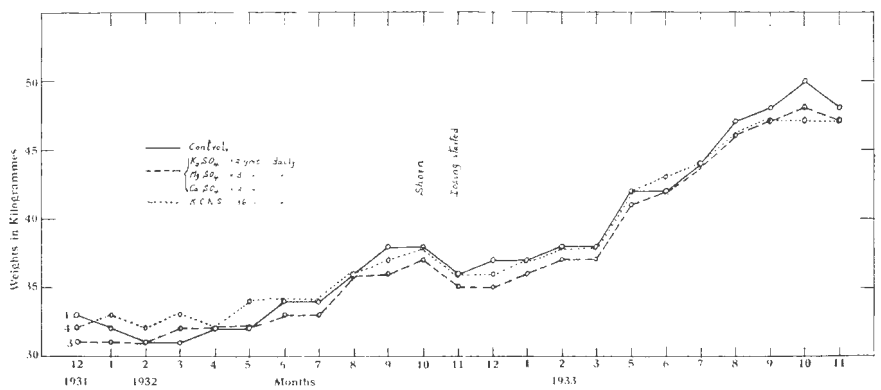

Fig. 2.

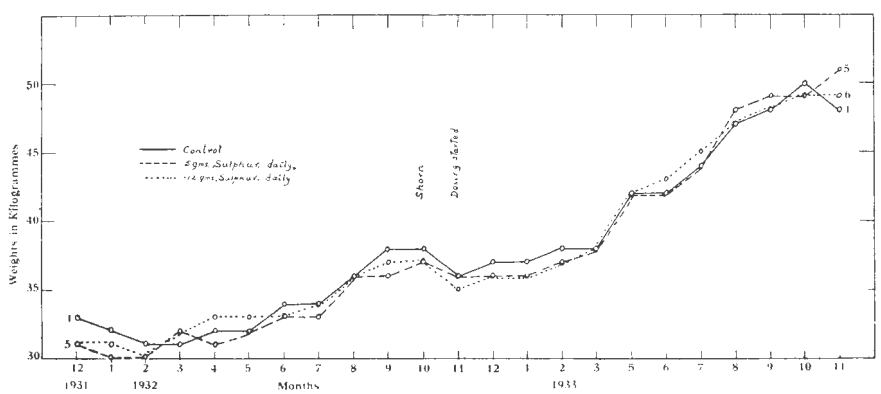

Fig. 3.

i.e. during the rainy season, only about half of the green feed was consumed as it was often cut and placed in the feeding boxes while still wet when the sheep showed less than the usual inclination to consume it. However, as green feed was added not to enrich the ration in any particular food constituent, for the ration was quite adequate in protein and in the other nutrients, but to provide the animals with some fresh, green material, it is most unlikely that the irregular consumption of the green feed during the latter part of the experiment is of any significant interest, especially as it did not favour any particular group or individuals in a group. The green feed was weighed back regularly and the quantity left throughout the experiment was negligible—never more than ·5 lb. a fortnight per sheep—except during the last two months of the experiment when ·25 lb. green feed was the least quantity consumed by any one sheep daily. The daily consumption of green feed per sheep in the different groups for the last two months of the experiment was ·27 lb., ·35 lb., ·37 lb., ·25 lb., ·35 lb., and ·35 lb. for Groups I, II, III, IV, V and VI respectively.

(*b*) *Weight Increase.*—The following curves give the weight increases of the individual groups of sheep compared with the control group in each case. See Figures 1, 2 and 3.

Discussion.—A glance at figures 1, 2 and 3 reveals that no significant differences existed in the relative weights of the groups throughout the experiment. The group receiving cystine showed greater increase in weight than the controls but the difference is not significant. It must be remembered that the absence of difference in weight during the last year of the experiment when the supplements were fed, is made more significant by the fact that no difference in the weights of the groups was registered during the first year of the experiment when all the groups received the unsupplemented basal ration only.

(*c*) *Wool Production.*—As already mentioned, the sheep were shorn in December, 1931, and November, 1932, respectively, at the beginning and end of the pre-period and again in December, 1933, at the conclusion of the experiment. For details in regard to wool quality the reader is referred to a later article by Bekker *et al.* For the present, only the average weights of greasy wool produced by each group will be given in kg.

Groups.	Weight Wool, October, 1932. (327 days growth).	Weight Wool, October, 1933. (384 days' growth).
I Controls.....	3·2 Kg.	5·5 Kg.
II (Cystine).....	3·3 Kg.	5·7 Kg.
III (Sulphates).....	3·0 Kg.	5·0 Kg.
IV (KCNS).....	3·3 Kg.	5·5 Kg.
V (5 gm. S).....	3·1 Kg.	5·4 Kg.
VI (·12 gm. S).....	3·3 Kg.	5·5 Kg.

The individual weights of wool produced and therefore also of the wool yield of the relative groups, when submitted to statistical analysis show no significant differences among the groups and one is forced to conclude that as in the case of weight increase the supplements had no visible effect on the amount of wool produced by the sheep. It is interesting to note that the average of 5·5 kg. wool produced per group in 1933 contained about 300 gm. cystine and that approximately the same amount of cystine was present in the total feed consumed per head during the 384 days required to produce the wool except in the feed of the group receiving a daily supplement of cystine where obviously more cystine was present in the feed than that contained in the wool. In other words, if the exclusive source of cystine had been that contained in the food and if no cystine was synthesized in the animal body, before or after absorption of sulphur-containing compounds, the animals must have utilized practically all the cystine ingested exclusively for wool production. It is hardly conceivable that one hundred per cent. of the cystine contained in the food could have been utilized for wool production and that the cystine requirements of the animal body for growth were negligible.

The determination of cystine in the feed was checked several times and found to be correct; the yellow maize contained on an average 150 mgm. cystine per 100 gm., teff hay 50 mgm. and the green feed used 80 mgm. of cystine. The calculations of the cystine content of the wool produced during the 384 days were made on the scoured wool and gave an average total cystine content per sheep per group of 291·3 gm., the individual values varying between 277 and 299 gm. and being therefore not significantly different.

The question of the sources of the cystine required by sheep for both growth and wool production cannot be answered in the light of the present experiment, although the results warrant the tentative suggestion that the sheep in the experiment described above were apparently not entirely dependent on the cystine contained in their food as there was practically no difference between the total cystine intake and that actually contained in the wool shorn. As regards the probable source of the cystine required other than that contained in the food no satisfactory answer without further work can be given. Further investigations are being carried out at this institute.

(d) Mortality.—The following table gives an analysis of the incidence of disease and of the mortality of the sheep during the course of the experiment:—

No. of sheep.	Group.	Date of disease and period.	Cause.	Result.
4	I Controls	31.3.32 no supplementary feeding	Urinary calculi	Died.
		19.1.33 „ „	„	Recovered.
		31.7.33 „ „	„	„
		4.9.33 „ „	„	Died.
2	II	1.7.33 Cystine supplement.....	„	Recovered.
		28.7.33 „	„	Died.
0	III	Group receiving sulphates.....	No deaths	—
3	IV	25.8.32 (i.e. preperiod) no supplementary feeding	„	Recovered.
		2.9.32 „ „	„	„
		14.7.33 KCNS supplement.....	„	Recovered after operation.
0	V	Group receiving 5 gm. sulphur.....	No deaths	—
2	VI	21.7.33 Sulphur supplement.....	„	Recovered.
		31.10.33 „	„	Died.

Although superficially it would appear that the absence of calculi in Group V might be associated with the sulphur supplement or in Group III with the supplement of sulphates a biometrical analysis of results does not show any significant differences between the groups. The appearance of calculi has often been noticed in other sheep at this Institute and their composition varies widely. It can only be concluded that the occurrence of urinary calculi in the sheep in the experiment under discussion was not associated with supplements of sulphur or its compounds or with their administration.

The animals were tested for intestinal worms from time to time and were found to be practically free throughout the course of the experiment.

SUMMARY AND CONCLUSIONS.

(1) Details are presented of an experiment on the effects of sulphur and of compounds containing sulphur on the food consumption, weight increase, wool yield and disease of young wethers kept on a production ration for two years.

(2) The sheep were fed in individual feeding boxes except for the hay which was given *ad lib.*

(3) The supplements given were dosed daily except Sundays and were given as follows:—

Group I: No supplement.
Group II: Cystine.
Group III: Sulphates.
Group IV: KCNS.
Group V: 5 gm. sulphur.
Group VI: ·12 gm. sulphur.

(4) No significant effects of the supplements on the sheep could be detected after 12 months' supplementary feeding nor did the experimental groups differ significantly from the control group at the end of the experimental period.

(5) The supplement of cystine had no effect on the quantity of wool produced.

(6) The mortality and disease due to the presence of urinary calculi were apparently not associated with the supplements.

(7) The analyses of the wool will be reported on in due course.

The authors are indebted to Mr. A. P. Malan, M.Sc., for a statistical analysis of the results.

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APPENDIX.

In the light of the foregoing results an earlier experiment at Onderstepoort on the rôle of cystine and sulphur in the nutrition of sheep may be mentioned. Due to the inadequacy of the number of sheep in that experiment the publication of the results was delayed until the results of the present investigation became known and is now presented as an addendum.

Five groups of four-tooth Merino wethers consisting of 6 sheep each were shorn and placed on the following ration in July, 1930:—

0·5 lb. of veld hay.
1·0 lb. of crushed maize.
rain water *ad lib.*
8 gm. of common salt.

The sheep were kept on a ground floor paddock where the above ration per sheep was given in a common trough. Group feeding proved quite successful. The grain was fed in the morning and the hay in the evening.

The animals in the various groups were each dosed the following supplement daily except Sundays:—

Group I: Controls were not dosed.

Group II: 3 gm. of sulphur, which is approximately equivalent to the sulphate content of 2½ lb. of natural pasture.

Group III: 3 gm. of sulphur.
0·5 gm. of cystine, which is the cystine required for the production of 8 lb. of wool annually.

Group IV: 3 gm. of sulphur.
20 gm. of blood meal (3 per cent. cystine according to Brailsford Robertson; Pamphlet 16, 1929), which would contain the equivalent of 0·5 gm. of cystine.

Group V: 3 gm. of sulphur.
100 c.c. of wool hydrolysate containing amongst other things 0·5 gm. of cystine.

The sheep were weighed each month. The different groups showed steady gains throughout the twelve-month experimental period. The order of the average gains in weight per sheep for the groups is given in Table I.

TABLE 1.—AVERAGE GROUP GAINS IN LB.

Group.	Initial weight.	Final weight.	Total gain.	Daily gain.
I Controls	97	117	20	·06
II Sulphur	93	109	16	·04
III S + Cystine	96	121	25	·07
IV S + bloodmeal	94	112	18	·05
V S + wool hydrolysate	95	106	11	·03

From the initial weights it will be seen that the sheep were fully grown and in good condition when the trial started.

The hydrolysate was obtained by boiling scoured wool for about 8 hours in 20 per cent. hydrochloric acid neutralizing, filtering and concentrating the solution so that ·5 gm. of cystine was contained in 50 c.c. of solution. The gains in weight in the separate groups were not significant. The last group (receiving hydrolysate) was not thrifty and the animals disliked the hydrolysate intensely and were adversely affected by it. These animals showed purging from time to time and loss of appetite. Two sheep in this group died before

the conclusion of the experiment. The administration of wool hydrolysate without the elimination of some of its impurities is inadvisable. The cystine dosed in Group II was prepared from wool at this Institute. Rather disappointing increases in weight were obtained in Groups II and IV, but this will be commented on in the discussion.

The unscoured wool weights are given in Table 2:—

TABLE 2.—WEIGHTS OF WOOL IN LB.

Group.	Nos.	Fleece weight.	Average group weight.
I.....	26825	10·3	
	26834	9·0	
	26833	11·6	9·85
	26816	10·2	
	26812	9·2	
	26819	8·8	
II.....	26806	10·1	
	27841	6·5	
	26808	9·0	9·43
	26852	9·0	
	26836	11·0	
	26807	11·0	
III.....	26839	9·0	
	26817	9·5	9·17
	26831	9·0	
IV.....	26840	10·1	
	26829	10·4	
	26835	died	8·92
	26810	8·4	
	26838	9·8	
	26842	5·9	
V.....	26822	6·8	
	26814	9·3	
	26828	11·2	8·30
	26815	died	
	26837	8·3	
	26826	died	

The differences of average fleece weights of the various groups are not significant and do not call for further comment. All the wool was particularly clean, exhibiting a very desirable colour.

DISCUSSION.

As was previously stated, disappointing results were obtained in Groups II and IV. In each of these groups there was one sheep, however, that became abnormally emaciated, causing the group average to be seriously affected. In order to illustrate more accurately the final results obtained, an average of all weighings of each sheep is plotted in their respective groups. No. 26807 in Group II and No. 26842 in Group IV occupy a position on the chart very much lower than any of the other sheep in either of these groups. The median drawn as shown in Figure 1 is a truer indication of the actual group standing.

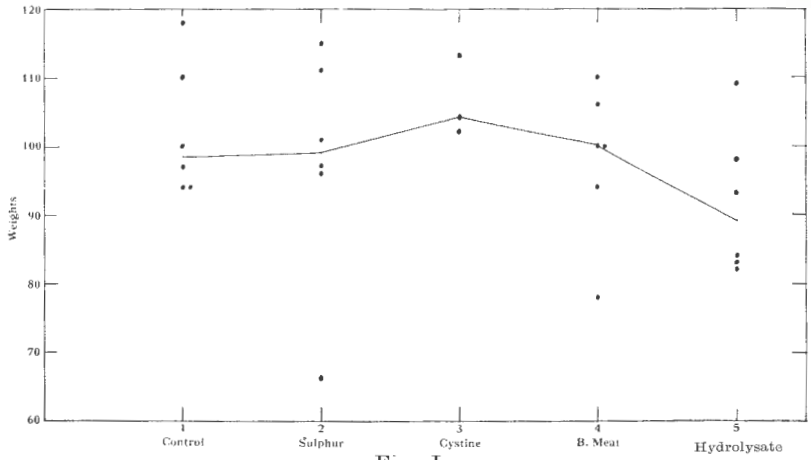

Fig. I.

Due to cystine scarcity towards the close of the experiment the number of sheep in Group II was reduced to three, selected at random and the daily dose of cystine halved.

This experiment, although inconclusive without further work, suggested that neither cystine nor sulphur when given under the conditions mentioned, would have beneficial effects on the body-weight or wool production of sheep. This indication is borne out fully by the results of the investigation reported on in the main body of the present publication.