The Role of Carotene in Ovine Rickets

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IT is well known that rickets, usually attended by a check in growth rate, frequently occurs in weaned lambs grazing on green feed during the late autumn and winter (1, 2, 3, 4, 5). From the widespread distribution of the more severe outbreaks, which can be detected by the onset of lameness and the more or less typical stiffness in gait of affected animals, it is reasonable to assume that the occurrence of the disease in its mild form, not attended by any easily recognised symptom, is widespread throughout New Zealand.

The known causes of rickets were either an inadequate Ca. or P. uptake or a deficiency of vitamin D. The observations recorded by the earlier workers on ovine rickets (2, 3, 4, 6, 7) indicated that the amounts of both Ca. and P. in rickets producing greenfeeds were more than sufficient to meet the requirement of the grazing lamb. Apart from the possibility of an inadequate uptake of vitamin D from the feed and solar ultraviolet irradiation of the animal there appeared to be involved some positive rachitogenic factor associated with greenfeed. The presence of this factor first suggested by Fitch (2) seemed probable when it was shown by Fitch and Ewer (3) that a flock of lambs on winter greenfeed became rachitic even though they received a vitamin D supplement in excess of their normal daily requirement. In this trial each animal was given cod liver oil approximately equivalent to 300 i.u. vitamin D per diem which was about twice the daily requirement indicated by the work of Andrews and Cunningham (6).

A further investigation into the problem of the rachitogenic factor has been recently conducted at Wallaceville. Since little information was available in the literature on the subject the initial stages of the present project were devoted to an investigation into the vitamin D content of pastures and green feeds at different seasons and stages of growth.

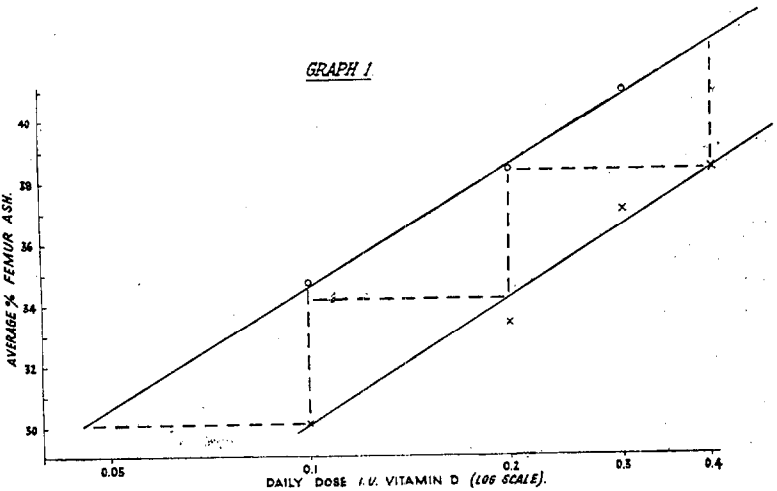
It soon became apparent that the amount of vitamin D in pasture bore no relation to the season of the year in which the sample was taken, but it was noted that those feeds in which appreciable amounts of the vitamin were present contained a considerable proportion of dead leaves. It seemed likely, therefore, that in any pasture sample vitamin D was present in the dead brown fraction and that the green growing leaves contained practically none.

From a permanent pasture sample containing a high proportion of dead leaves was separated out a fraction consisting of "pure" green leaves and another fraction consisting of dead brown tissue only. As was expected the fat soluble extract from the dead leaves assayed at a high level of vitamin D, namely 80 i.u./100g. dry matter.

The green leaf extract not only failed to produce any vitamin D response in the rat but actually depressed bone calcification to a level appreciably lower than that resulting from an absence of vitamin D supplement. The only explanation of this result was that the green leaves contained a rachitogenic factor.

In a study of the effects of a green leaf extract upon bone calcification in the rat it was shown that when a fixed dose of the factor was administered with varying amounts of vitamin D, the response to each dose of the vitamin was depressed to a similar extent. For example, the results of one trial represented in the first graph indicate that the vitamin D potency was approximately halved in each case:

GRAPH 1

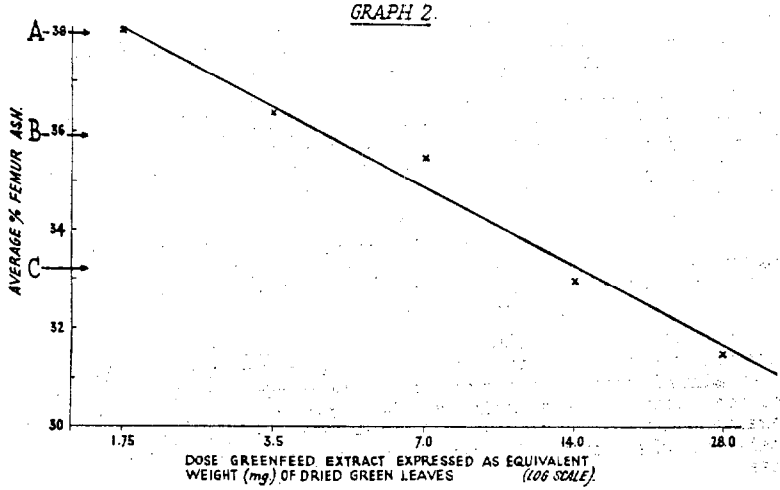


In this graph percentage bone ash is plotted against the logarithm of the daily dose of vitamin D administered to the rat on the McCollum 3143 rachitogenic ration. The upper line represents the normal log dose response relationship, while the lower line indicates a response line depressed by the green feed extract. It can be seen that the percentage bone ash response to 0.1 i.u. Vitamin D was depressed by the rachitogenic factor to a level expected from a dose of 0.05 i.u. Vitamin D. Similarly the same rose of the greenfeed extract depressed the bone ash response to higher D doses to levels normally produced by half the D doses.

The bone calcifying response to a fixed dose of vitamin D was found to decrease with increasing doses of the rachitogenic factor in the manner represented in graph II.

"A" represents the normal bone ash response to .15 i.u. vitamin D per diem; "B" represents the response to .10 i.u. vitamin D; and "C" represents the response to .75 i.u. vitamin D.

GRAPH 2.



Each point "X" on the graph represents the response to a fixed daily dose of 0.15 i.u. of D plus the amount of greenfeed extract indicated.

The data from this experiment and those obtained from a series of similar trials were analysed statistically by Dr. Carter, and the results indicated a linear relationship between response and the logarithm of the dose.

Following these trials, a method was devised whereby an approximate assessment of the rachitogenic factor content of greenfeeds or greenfeed concentrates could be made. Thus it became possible to estimate the rachitogenic factor content of greenfeed at different stages of growth and to follow the fate of the factor through the series of processes involved in any attempt at its isolation.

The results presented in the following table indicated that the highest concentration of the rachitogenic factor in greenfeed oats could be expected after two or three weeks of rapid growth and before there was any appreciable degree of stalk development.

TABLE I.

Stage at which oat crop sampled		Rachitogenic factor content in arbitrary units per gram dry matter
4 days' growth	Young shoots 1-3in. high	200
14 days' growth	Crop 5-6in. high	320
23 days' growth	Crop 7-9in. high	320
35 days' growth	Crop 9-12in. high—considerable stalk development	100
50 days' growth	Crop coming into ear	50

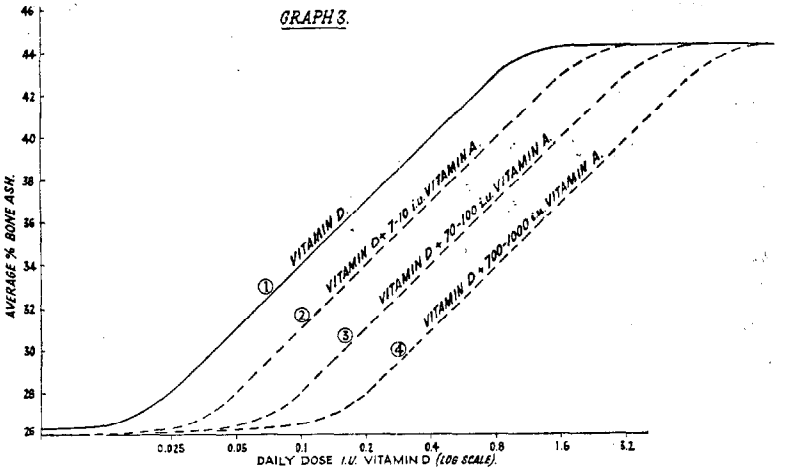
In an attempt to isolate the factor, a crop of green oats was cut and dried after three weeks' rapid growth and the mixture of substances making up the fat soluble extract was fractionated chromatographically. The active principle was found to be concentrated in that band of the chromatogram coloured red by the presence of carotene. From the mixture of compounds making up this band a number of crystalline substances were separated and purified, and one of these, carotene itself, proved to be the rachitogenic factor.

The rachitogenic factor is more properly vitamin A, to which carotene is largely converted during the process of absorption from the gut, for it has been demonstrated that carotene and vitamin A when dosed in equivalent amounts have identical rachitogenic properties. As far as can be judged from the results already obtained in a series of trials, in which the rachitogenic action of pure vitamin A acetate is being fully investigated, the depressing effects of vitamin A on bone ash response to vitamin D in the rat can be summarised approximately by the relationships indicated in the hypothetical graph III.

Line (1) represents the typical bone ash response of rats on the McCollum ration to vitamin D. Lines 2, 3 and 4 show the bone ash depressing effects of vitamin A doses. When the test animals receive no vitamin D the bone ash level produced by the ration is not greatly depressed by a large vitamin A dose. Similarly, at the other end of the scale, a high A intake does not appreciably depress the bone ash response to a vitamin D dose which is much greater than the normal daily requirement.

Although there is a marked bone ash depressing effect over the linear range, the slope of the log D dose response line does not appear to be appreciably affected by a vitamin A supplement.

The addition of 7-10 i.u. vitamin A per diem to the diet approximately halves the vitamin D potency (Line 2). Lines (3) and (4) indicate that the vitamin D potency is reduced to about one-quarter by



70-100 units of A, and to approximately one-eighth by 700-1000 units of A. It is also plain that increasing doses of vitamin A increase the daily D requirement of the animal. The increasing rachitogenic effect with increasing vitamin A dosage appears to continue in this manner until amounts of the order of 3-4000 i.u. vitamin A daily are reached and further increases beyond this level produce the complicating toxic symptoms of hypervitaminosis A manifest by unthriftiness and growth failure in the test animals. Under these conditions bone ash responses become erratic. It is an interesting fact that the bone ash depressing effect of any vitamin A dose can be completely prevented by increasing the vitamin D intake but no increase in vitamin D can alleviate the toxic effects of hypervitaminosis A. Apparently the two effects are quite distinct.

If relationships similar to those described in this paper for the rat hold in the case of sheep, it seems that, when the vitamin D status or intake of the animal falls to a level which is less than adequate, the rachitic tendencies of the animal could be appreciably increased by only a moderate vitamin A uptake. The carotene absorbed and converted to vitamin A would need to be large in amount, in order to produce rickets in a lamb which receives much more than its normal daily D requirement.

The amounts of carotene present in crops of greenfeed oats at varying stages of growth and grown in different seasons of the year, are shown in Table II:—

No. of days growth	Crude Carotene Content—Microgram/gram Dry Matter: Spring crop	Summer crop	Autumn crop	
3- 4	555	505	675	
6- 7	616	495	683	
10-11	742	594)	653)	
13-14	682	604)	775)	Leaf
17-18	701	586)	—)	development
20-21	Crop	574)	782)	stage
24-25	destroyed	574)	775)	
27-28		531)	—)	
31-32		501	709	
40-41		477	493)	Rapid
46-47		418	346)	stalk
53-54		178	198)	growth
60		124	99)	

Unfortunately the spring oats were destroyed by cattle during the third week of growth but a sufficient number of determinations were obtained to indicate that its carotene content was similar to that of the autumn crop. The lower carotene values obtained for the corresponding growth stages of the summer crop may have been due to some check in growth rate caused on two occasions by flooding of the paddock in exceptionally heavy rains. These results showed that the carotene content of the oat plant was highest between 10 and 30 days growth during the leafy stage and before there was any marked stalk development.

The carotene contents of perennial ryegrass and white clover cut during an autumn growth flush were determined as 708 and 722 microgram/gram dry matter respectively.

These figures are sufficient to indicate that the carotene contents of good quality greenfeeds are extremely high and such that sheep grazing upon them will have a daily carotene intake equivalent to 1-2 million i.u. vitamin A. This estimate does not take into account a high rate of carotene synthesis by the micro-organisms of the ovine ileum and caecum indicated by the recent work of McGillivray (8) at Massey College.

The importance of vitamin A in the development of ovine rickets has been indicated by a field trial conducted at Wallaceville. A flock of 15 wether lambs divided by random selection into three groups each of 5 sheep were run together from 12th May to the 12th August on a permanent pasture of a type on which rickets development would not normally be expected.

Each animal of group 1 received daily 125,000 i.u. pure Vitamin A.

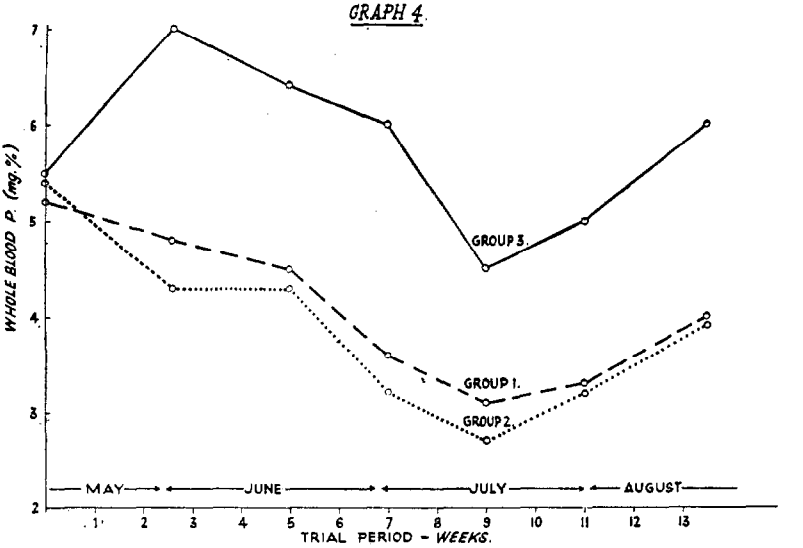
Each animal of group 2 received daily 250,000 i.u. pure Vitamin A.

The animals of group 3 received no supplement.

Graph 4 depicts the average change in blood P level for each group throughout the trial.

If a blood P level of less than 5mg% can be indicative of the onset of rickets it is obvious that the rachitogenic effects of the vitamin A doses were operative very soon after the beginning of the trial. X-ray examination of the sheep towards the end of the trial indicated that 7 out of the 10 animals receiving a vitamin A supplement were rachitic

GRAPH 4.



and this diagnosis was confirmed by examination of the bones at slaughter. No sign of rickets was detected in any of the control group animals.

To date the results of an investigation into the carotene absorption by sheep grazing on greenfeed indicate that only a small proportion of the intake is absorbed and converted to vitamin A, in which case it seems that the major causative factor in ovine rickets is a deficiency or near deficiency of vitamin D intensified by a moderate vitamin A uptake.

The observed variation in the rachitogenic potency of different greenfeeds and of the same feed, grown in different years and the fact that some animals are much more resistant to the disease than others in the same flock may be due to a number of causes some of which are listed as follows:—

(1) Variation in the amounts of carotene and vitamin D absorbed from the ingested pasture. This will depend not only on the relative amounts of green and dead leaf tissue present but also on the amounts of carotene and vitamin D they contain and upon the availability of each constituent to the grazing animal.

(2) Variations in the amount of vitamin D synthesised by ultraviolet irradiation of the animal during the greenfeed grazing period. Not only does the intensity of the ultraviolet to which the animal is exposed alter with changes in solar ultraviolet emission, meteorological and topographical conditions and altitude of the sun, but the amount of vitamin D thus made available must depend on the skin area of the sheep not shielded by the fleece from the sun's rays. The exposed skin areas vary with the breed and even within a breed individual differences in this respect can be marked.

(3) Variations in the body stores of both vitamins A and D when the lambs are first turned on to winter greenfeed. It is known that sheep in common with other animals are able to store considerable amounts of these vitamins. Vitamin D reserves are probably built up in most animals during the summer months as the result of a high rate of solar synthesis both of the feed and the exposed body surfaces and these reserves may be sufficient to protect most animals from avitaminosis D during the winter. Again because of extensive fleece coverage the sheep is probably at a disadvantage compared with other farm animals in this respect and it seems reasonable to suppose that its vitamin D reserves are comparatively low at the beginning of winter.

(4) Variations in the Ca. and P. levels and ratio in the feed are factors known to have appreciable effects in rickets development.

Since it is obvious from these considerations that no reliable assessment can be readily made as to whether or not a winter greenfeed will produce rickets in grazing lambs and since the incidence of the disease on such feed is high in New Zealand, it would seem sound practice to administer to all lambs grazing on good winter pasture or greenfeed the single and inexpensive massive dose of calciferol (1,000,000 i.u. vitamin D) shown by Ewer and Bartrum to be entirely effective in preventing ovine rickets and maintaining a good growth rate throughout the winter.

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Discussion

Mr. ANDREWS: Is Dr. Grant prepared to say anything about the toxic effects of high carotene intakes?

Dr. GRANT: A daily dose of vitamin A equivalent to the amount of pasture carotene consumed by the greenfeed grazing animal is sufficient to produce marked toxic symptoms. It has been shown however, that the amount of carotene in greenfeed absorbed and converted to vitamin A within the animal body is not more than 5% of the total intake. This quantity is far below the level which would result in hypervitaminosis A.

Dr. FILMER: The work of Fitch and Ewer indicated that there were marked differences between the rickets producing properties of different types of greefeeds. If Calciferol is dosed to all hoggets 80% of it may be wasted.

Dr. GRANT: Differences in rachitogenicity of greenfeeds can probably be accounted for by differences in the availability of carotene in the feeds. For instance in one feed 1% of the carotene present might be available. In another 2% of the carotene might be absorbed. Though only small fractions of the total intakes are absorbed in both cases the difference in the amounts utilized would be large.

The administration of calciferol to all lambs during the winter cannot be recommended. Only in those lambs which are being wintered on good quality greenfeed is an outbreak of rickets likely to occur. The problem seems to be confined mainly to the stud breeder who, in an attempt to maintain maximum growth rate in his young rams either grows winter greenfeed for their consumption or grazes these animals on the best of his pastures. In such cases drenching with calciferol early in the winter will insure against a likely outbreak of the disease and help to maintain a good growth rate.

Professor COOP: What is the effect of management practices such as feeding of hay? This I know is not common in the North Island but I believe it is good animal husbandry.

Dr. GRANT: Certainly hay contains appreciable amounts of vitamin D but since ovine rickets is primarily a disease which occurs in animals grazing good quality greenfeed it might be difficult to induce lambs to eat the hay unless they were taken off the more palatable diet. Some such practice might be adopted.

Mr. JEBSON: How can one differentiate between rickets and unthriftiness in winter?

Dr. GRANT: Healthy lambs which develop moderate to severe rickets may become markedly unthrifty as a result. They will also exhibit some degree of lameness or stiffness in gait from which the disease can be readily diagnosed. Those animals which are only mildly affected may not show obvious signs of unthrift.

A marked degree of unthriftiness unaccompanied by lameness is probably not the result of rickets though subsequently rickets and lameness may appear in these already unthrifty animals.