XXIII. ON METHODS OF DETERMINING THE DEGREE OF CALCIUM DEFICIENCY OF SHEEP.

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THE effect of lime and cod-liver oil supplements on the growth and serum-Ca of sheep fed on a Ca-deficient diet has been previously discussed by two of us [Auchinachie and Fraser, 1932].

The present communication records further experiments on the same subject in which Ca-balance determinations over a period were combined with blood analyses and clinical observations on the sheep. It emphasises the possible fallacies which may arise from any single method of investigation.

Four groups of five young sheep of similar age and size were fed the following basal ration:

Cooked maize	...	...	...	1 part	} 2 lbs. per diem.
Crushed oats	...	...	...	1 "	
Bran	...	...	...	1 "	
Dried distiller's grains	...	...	...	1 "	
Turnips	...	...	...	to 10 lbs.	per head daily
Oat straw	...	...	...	to $\frac{1}{2}$ lb.	"
Water and salt (NaCl) <i>ad lib.</i>					

This was supplemented in the various groups as under:

Group I. Nothing.

II. 2.5 ml. cod-liver oil per head daily.

III. 2.2 lbs. of chalk per 100 lbs. concentrates.

IV. 2.2 lbs. of chalk per 100 lbs. concentrates + 2.5 ml. cod-liver oil per head daily.

The relative intakes of CaO on these diets are shown in Table I. Experimental feeding began on 27th October and continued until 24th May, a period of 209 days. The feeding pens were inside, covered, concrete-floored and bedded with moss litter.

RESULTS.

Clinical.

The growth curves of the four groups are shown in Fig. 1. For a period of 84 days there was no differentiation in growth-rate. Subsequently growth-rate diminished in the basal group. In confirmation of previous work, a combined supplement of chalk and cod-liver oil had a greater effect on growth than either chalk or cod-liver oil alone; but in contrast to earlier findings the beneficial effect of chalk alone was greater than that of cod-liver oil alone [Auchinachie and Fraser, 1932].

Table I. *Results of balance experiments with individual sheep.*

Group	Ration and average daily intakes (g.)	Average daily excretions						Average daily balances		
		Nitrogen		CaO		P ₂ O ₅		N	CaO	P ₂ O ₅
		Urine g.	Faeces g.	Urine g.	Faeces g.	Urine g.	Faeces g.			
I	Basal N, 12.18; CaO, 2.10; P ₂ O ₅ , 7.20	4.32	3.79	0.0045	2.02	0.60	5.22	4.07	0.08	1.38
II	Basal + C.L.O. N, 14.57; CaO, 2.48; P ₂ O ₅ , 8.43	4.97	4.62	0.0044	1.46	3.38	2.84	4.98	1.02	2.21
III	Basal + Chalk N, 12.18; CaO, 7.20; P ₂ O ₅ , 7.20	4.46	3.58	0.0049	6.18	0.04	6.54	4.14	1.02	0.62
IV	Basal + Chalk + C.L.O. N, 15.56; CaO, 8.16; P ₂ O ₅ , 7.89	6.91	4.12	0.0211	6.82	1.04	6.07	4.53	1.32	0.78

Chalk and cod-liver oil together raised the CaO balance to the highest level, namely 1.3 g., and decreased P₂O₅ retention.

The relative excess of P₂O₅ retained in groups I and II raises an interesting problem. In group I with calcium in equilibrium there was a daily retention of 1.38 g. of P₂O₅. Obviously this could not have been deposited in the bones in the normal manner as calcium phosphate, and yet osseous tissue is the only large storage depot for phosphorus in the body. There is no evidence in the experimental data to elucidate this point, but further investigations are in progress along these lines.

Blood analysis. The results of periodical serum-Ca and blood-inorganic P determinations are shown in Table II. The changes in plasma-phosphatase have been previously recorded [Auchinachie and Emslie, 1933].

Table II.

Group	October		December		February		March		April	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
1. Basal	11.55	4.77	9.41	5.88	7.01	8.40	6.29	7.50	6.36	7.59
2. Basal + C.L.O.	11.75	5.19	10.61	5.46	10.52	6.54	10.00	8.17	10.44	7.03
3. Basal + CaCO ₃	11.68	5.28	10.73	5.12	9.00	5.01	7.75	8.93	7.31	7.12
4. Basal + CaCO ₃ + C.L.O.	11.04	5.27	10.98	5.77	10.91	6.10	10.96	7.99	11.60	7.43

The figures show that serum-Ca fell to extremely low figures in groups I and III, but that C.L.O. retained it at a comparatively normal level.

DISCUSSION.

The effect on growth of lime and cod-liver oil supplements to a Ca-deficient ration here recorded confirm previous work. The results of the blood analysis and balance determinations require further discussion.

Consideration of Table II shows that the average serum-Ca figure for group I had fallen by April to 6.36 mg., an abnormally low figure. That for group III was 7.31 mg., also abnormally low. On the contrary the figure for group II, 10.44 mg., was within the normal range and only slightly lower than the figure for group IV, 11.60 mg.

The logical interpretation of these figures is that the calcium deficiency of the basal ration lowered the serum-Ca of the basal group; that a chalk supplement had little effect in raising it, but that cod-liver oil, either alone or in combination with chalk, restored it to a normal level. It might therefore be assumed that cod-liver oil was more effective than chalk in restoring a normal calcium balance, and

that cod-liver oil alone was almost as effective in this respect as when combined with chalk.

This interpretation of the results of serum-Ca determinations does not correspond with the growth differentiation of the groups or with the findings of the balance experiments. Consideration of Table I shows that a chalk supplement was as effective as cod-liver oil in increasing the positive CaO balance, and that chalk and cod-liver oil in combination were more effective than either alone; results which are in general conformity with the growth differentiation of the four groups.

The indications of the state of calcium metabolism obtained respectively from blood analysis and balance determinations do not agree. The same is true of phosphorus metabolism. Thus in April the figures for blood-inorganic phosphorus were closely similar in all four groups (Table II), whereas there were wide differences in P_2O_5 retention (Table I) at the same period.

While the serum-Ca figures are averages for all five sheep in each group, the balance determinations were done on single sheep and are not therefore strictly comparable. A more reliable guide is to consider the serum-Ca figures for the individual sheep from blood samples drawn at approximately the same dates as those on which their calcium balances were determined (Table III).

Table III.

Group	Index no. of sheep	Av. daily balance g. CaO	Serum-Ca mg./CaO ml.
I. Basal	78	0.08	5.85
II. " + C.L.O.	27	1.02	10.70
III. " + Chalk	49	1.02	8.35
IV. " + " + C.L.O.	35	1.32	12.00

From Table III it is again apparent that cod-liver oil had a relatively greater effect in raising the level of serum-Ca than in increasing calcium retention. It is also evident that a chalk supplement has a more favourable influence on calcium retention than the serum-Ca figures would suggest.

On the other hand the association of calcium equilibrium with a serum-Ca figure of half the normal value is unexpected. It probably results from minimum Ca excretion owing to the low serum-Ca level. It follows that balance determinations alone might, under certain conditions, give a misleading indication of normality.

From the results recorded in this communication it is evident that growth rate and clinical observation remain the soundest method of determining the effects of supplements added to a calcium-deficient ration. Blood analysis, particularly, cannot be accepted as a satisfactory substitute, until the factors governing serum-Ca level are more perfectly understood. A calcium dietary deficiency undoubtedly lowers the serum-Ca level, at least in sheep, but the effect of different factors in again raising it is not necessarily correlated with their effect on growth or on calcium retention.

SUMMARY.

1. Supplements of chalk or cod-liver oil, fed to sheep on a calcium-deficient ration, resulted in improved growth rate and health, a combined supplement of chalk and cod-liver oil being more effective than either alone.

2. The arrest of growth in the basal, Ca-deficient group was due partly to decreased food consumption and partly to inefficient utilisation of the food consumed.

3. The Ca-deficient ration lowered the serum-Ca, while supplements of chalk or cod-liver oil tended to retain it at a normal level. In this respect cod-liver oil was more effective than chalk.

4. Balance determinations showed that the basal group was in calcium equilibrium; that both chalk and cod-liver oil produced a positive Ca balance; and that their effects were additive.

5. The effect of cod-liver oil in retaining a normal serum-Ca level was much greater than its effect in increasing Ca retention or rate of growth.

REFERENCES.

- Auchinachie and Fraser (1932). *J. Agric. Sci.* **22**, 560.
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