CLXVII. THE EFFECTS OF COD-LIVER OIL ON THE CALCIUM AND PHOSPHORUS METABOLISM OF THE LACTATING ANIMAL.

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THE demands of the modern dairy animal for calcium and phosphorus are great. The desirability of maintaining a positive balance of these elements has been emphasised recently by Orr [1923], Erf [1923] and Henderson and Magee [1926]. Large amounts of these minerals leave the body in the milk and, where pregnancy and lactation are concurrent, the requirements of the foetus must also be supplied if both the cow and her calf after birth are to be maintained in good condition. The retention of adequate amounts of these minerals is thus a matter of physiological as well as of economic importance. Unfortunately in modern practice the feeding of concentrated foodstuffs, while supplying an abundance of the organic constituents of the diet, will not ensure a sufficient supply of the inorganic elements, since their content of these is, in general, low.

Cod-liver oil is a well-recognised stimulant of calcium and phosphorus assimilation as has been shown by McCollum *et al.* [1922] with rats and by Husband, Godden and Richards [1923] with growing pigs. It therefore seemed of interest to determine whether cod-liver oil would affect the calcium and phosphorus metabolism of the lactating animal in the same way. With that object in view, the following experiments were carried out.

METHODS.

Animals. Goats were used. The procedure was similar to that described by Henderson and Magee [1926].

Feeding. The food consisted of two portions, one of grain and one of hay, the former being made up from a mixture of the following amounts of cereals: 72 lb. ground maize, 28 lb. oatmeal, 14 lb. linseedmeal.

Before the experiment began the animal was fed to satisfaction under the conditions of the experiment and, at the end of a week, a knowledge of its appetite had been obtained. Suitable amounts of grain and hay were then decided upon and the ration was kept at this level throughout the experiment. During the experimental period the oil was mixed with the grain ration and

fed in two approximately equal parts in the morning and evening. Distilled water was given *ad lib.* in each experiment.

As far as possible, each experiment was divided into three periods of 16 days' duration, the excreta being collected daily and analysed every 4 days by the following methods:

calcium: precipitation as oxalate and titration with *N*/10 potassium permanganate.

phosphorus: the Pemberton-Neumann method as modified by Richards and Godden [1924].

iodine: a modification by Leitch and Henderson [1926] of Fellenberg's titrimetric method.

milk-fat: ether extraction in a Soxhlet apparatus.

Exp. 1. The experiment began 257 days after parturition and was limited to two periods, a pre-period of 16 days and an experimental period of 20 days, there being no post-period owing to the onset of oestrus.

Diet: 450 g. grain and 900 g. hay per day containing 5.93 g. CaO and 6.39 g. P_2O_5 . During the experimental period 10 cc. cod-liver oil were given daily.

Results:

Average daily excretion and balance in g.

	Weight of faeces	Output of CaO				Balance of CaO
		Milk	Faeces	Urine	Total	
Pre-period	1223	1.26	3.65	0.24	5.15	+0.78
Experimental period	1080	1.43	3.20	0.35	4.98	+0.95

		Output of P_2O_5				Balance of P_2O_5
Pre-period	—	2.40	3.84	0.05	6.29	+0.10
Experimental period	—	2.38	3.69	0.04	6.11	+0.28

During the oil-period there was an increased retention of calcium in spite of the increased output in both milk and urine. This was accounted for by the decreased output in the faeces, the average daily weight of which was reduced during the oil-period. The balance of phosphorus was also increased, but this was due to diminished excretion in milk and urine, as well as in the faeces.

The average daily milk volume was 550 cc. in the pre-period and 560 cc. in the oil-period. The percentage of calcium (Fig. 1) showed a definite increase immediately after the introduction of the oil, while the phosphorus and fat contents fell as the volume increased.

Exp. 2. The experiment began on the 23rd day of lactation. There were three periods, each of 16 days' duration.

Diet: 300 g. grain and 900 g. hay per day containing 8.49 g. CaO and 6.19 g. P_2O_5 . During the experimental period 10 cc. cod-liver oil were given daily.

*Results:**Average daily excretion and balance in g.*

	Weight of faeces	Output of CaO				Balance of CaO
		Milk	Faeces	Urine	Total	
Pre-period	1066	1.79	7.19	0.05	9.03	-0.54
Experimental period	995	1.46	6.95	0.05	8.46	+0.03
Post-period	1063	1.24	7.14	0.05	8.43	+0.06

		Output of P ₂ O ₅				Balance of P ₂ O ₅
		Milk	Faeces	Urine	Total	
Pre-period	—	2.38	4.01	0.02	6.41	-0.22
Experimental period	—	1.80	4.74	0.02	6.56	-0.37
Post-period	—	1.56	4.57	0.02	6.15	+0.04

The balance of calcium showed a marked improvement during the oil-period because of reductions in the amounts in milk and faeces. In the post-period the faecal excretion increased, but the amount in the milk continued to fall with the result that the balance became more positive. There was no corresponding improvement in the phosphorus balance in the oil-period for here the faecal excretion reached its maximum. In the post-period the amounts in the milk and faeces fell and the balance became positive. The average daily weight of faeces was reduced during the oil-period and rose again in the post-period.

Fig. 1. Volume and percentage composition of milk.

— . — . Vol. Fat
 - - - - - P₂O₅ - - - - - CaO

The milk volume fell throughout the experiment, but the percentages of calcium and fat showed little variation except for a slight fall in the case of calcium in the post-period. The phosphorus content fell definitely from an average of 0.242 % in the pre-period to 0.223 % and 0.222 % in the oil- and post-periods respectively.

Exp. 3. The goat had been lactating for 162 days when the experiment began. There were two periods of 16 days and one of 18 days. The extension of the post-period was necessary because the animal developed diarrhoea and the intake was reduced by making the first two analyses over 5-day periods

and giving only 4 days' food. The figures for these 5-day collections were not used in calculating the average excretions and balances.

Diet: 750 g. grain and 1100 g. hay per day containing 9.93 g. CaO and 9.91 g. P_2O_5 . During the experimental period 15 cc. of cod-liver oil were given daily.

Results:

Average daily excretion and balance in g.

	Weight of faeces	Output of CaO				Balance of CaO
		Milk	Faeces	Urine	Total	
Pre-period	1782	1.15	8.95	0.92	11.02	- 1.09
Experimental period	1405	1.19	7.80	0.71	9.70	+ 0.23
Post-period	1488	0.92	7.73	0.26	8.91	+ 1.02

		Output of P_2O_5				Balance of P_2O_5
		Milk	Faeces	Urine	Total	
Pre-period	—	2.06	9.12	0.04	11.22	- 1.31
Experimental period	—	2.03	7.87	0.04	9.94	- 0.03
Post-period	—	1.80	6.56	0.02	8.38	+ 1.53

In the pre-period the balance of calcium was negative in each analysis, but after the introduction of the oil it became positive with one exception. The diminution in excretion occurred in the faeces, the average daily weight of which again showed a reduction in the oil-period. The balance of phosphorus showed a similar improvement in the oil-period. In the post-period the diarrhoea produced large negative balances of both minerals and the succeeding analyses showed positive balances.

Fig. 2. Volume and percentage composition of milk.

— · — Vol. Fat
 - - - - - P_2O_5 - - - - - CaO

During the oil-period the milk volume was slightly lower than in the pre-period, and in the post-period it was much more so owing to the diarrhoea (Fig. 2). When this passed off the volume rose, but never regained its former level. In the oil-period the percentage of calcium increased steadily, but reached its maximum in the post-period when the volume was at its minimum.

The phosphorus content also increased in the oil-period but, after a slight fall, rose much higher in the post-period. The percentage of fat showed wide variations and also reached its maximum in the post-period when the volume was lowest.

Exp. 4. The special objective of this experiment was to determine whether the increases in the calcium content of the milk in the oil-period of Exps. 1 and 3 were related to the stage of lactation. The animal was the same as in Exp. 1 and the experiment began on the 5th day of lactation. There were three periods of 16 days. Only the milk was analysed.

Diet: 500 g. grain per day with hay *ad lib.* 10 cc. of cod-liver oil were given daily during the experimental period.

Results: the average daily volume for the three periods was, pre-period 928 cc., oil-period 771 cc. and post-period 454 cc. The calcium content was unaffected by the oil, the average figures for the three periods being 0.220 %, 0.223 % and 0.265 %. The phosphorus percentage also showed little change during the oil-period: pre-period 0.335 %, oil-period 0.337 % and post-period 0.365 %. The average fat content was lowest in the oil-period, falling from 5.98 % in the pre-period to 5.56 % in the oil-period, and rising to 7.22 % in the post-period. It will be noted that all three constituents rose markedly in the post-period as the volume fell. In this experiment the iodine content was also estimated with the following results for each analysis:

Pre-period	13,	13,	13,	11 γ %.	Av. 12.5,
Oil-period	17,	17,	17,	19 γ %.	Av. 17.5,
Post-period	16,	15,	14,	13 γ %.	Av. 14.5,

where

$$\gamma = 0.000001 \text{ g.}$$

The average total amounts of iodine secreted in the milk per day were: pre-period, 116 γ ; oil-period, 135 γ ; post-period, 66 γ . Thus the feeding of 10 cc. of cod-liver oil which contained 80 γ of iodine increased both the percentage and the total iodine contents of the milk.

Exp. 5. Kelly [1925] has shown that with growing pigs the addition of small quantities of iodine as potassium iodide to the diet results in an increased retention of nitrogen and phosphorus, but has less definite effects on that of calcium. This suggested that the beneficial effects of cod-liver oil might be attributable to its high iodine content. An experiment similar to Exp. 3 was accordingly carried out in which the same animal was given an equal volume (15 cc.) of olive oil plus an equivalent amount of iodine (120 γ) as potassium iodide. The experiment began on the 229th day of lactation; the pre-period and experimental periods were of 16 days' and the post-period of 12 days' duration.

Diet: 400 g. grain and 1200 g. hay per day containing 20.52 g. CaO and 9.85 g. P₂O₅. During the experimental period 15 cc. of olive oil and 120 γ of iodine as potassium iodide were mixed with the grain every day. The large calcium intake was due to an unusual amount of clover in the hay.

*Results:**Average daily excretion and balance in g.*

	Weight of faeces	Output of CaO				Balance of CaO
		Milk	Faeces	Urine	Total	
Pre-period	1319	0.99	17.77	0.12	18.88	+ 1.64
Experimental period	1290	0.81	19.00	0.09	19.90	+ 0.62
Post-period	1243	0.59	20.35	0.08	21.02	- 0.50

		Output of P ₂ O ₅				Balance of P ₂ O ₅
Pre-period	—	1.81	5.57	0.03	7.41	+ 2.44
Experimental period	—	1.41	8.39	0.03	9.83	+ 0.02
Post-period	—	0.89	9.50	0.03	10.42	- 0.57

The balance of both calcium and phosphorus fell greatly during the experimental period as a result of greatly increased excretion in the faeces. The average daily weight of faeces fell throughout the experiment, but there were increases in the percentages of both calcium and phosphorus. In the post-period assimilation remained poor and the balances were, on the average, negative.

The milk volume fell continuously throughout from a daily average of 437 cc. in the pre-period to 326 cc. in the experimental period and 225 cc. in the post-period. During the experimental period the percentage of calcium rose steadily and remained high in the post-period, the average percentages being 0.227 %, 0.248 % and 0.264 % in the respective periods. The percentage of phosphorus also reached its maximum in the experimental period, the average figures for the period being 0.414 %, 0.434 % and 0.394 %. The fat percentage remained steady in the pre-period and in the first half of the experimental period, but rose to its maximum in the second half of that period and fell again in the post-period. The average iodine contents for the periods were: pre-period, 17 γ %; experimental period, 23 γ %; post-period, 22 γ %. The average daily total iodine secreted in the milk was the same in the pre- and experimental periods, but fell markedly in the post-period.

Exp. 6. Exps. 1-4 indicated that the fat content of milk was not definitely affected by cod-liver oil in the amounts given, and in Exp. 5 the feeding of olive oil and potassium iodide gave an increased fat content in the second half of the experimental period. Further, the feeding of iodine in either organic or inorganic form produced definite increases in the iodine content of the milk. It was therefore decided to determine the effects of olive oil alone on the composition of the milk, in order to find whether the effects observed in Exps. 4 and 5, *viz.* increase in iodine and fat contents, could be attributed to the oil *per se*.

The animal used was the same as in Exps. 1 and 4 and the experiment was of the same length as Exp. 5. It began on the 69th day of lactation and only the milk was analysed.

Diet: 400 g. grain per day with hay *ad lib*. In the experimental period 15 cc. of olive oil were given per day.

Results: the milk volume rose throughout the experiment, the average daily figures being: pre-period, 537 cc.; experimental period, 605 cc.; post-period, 729 cc. The average percentages of calcium and fat were highest in the oil-period, but the average phosphorus percentage fell throughout; the average iodine content and total iodine were both at a minimum in the oil-period. These figures are given in the following table.

	CaO %	P ₂ O ₅ %	Fat %	Iodine γ %
Pre-period	0.245	0.387	6.19	16
Experimental period	0.251	0.337	6.90	13
Post-period	0.234	0.362	6.33	14

DISCUSSION.

Each of the cod-liver oil experiments shows a reduction in the average daily weight of faeces during the oil-period as compared with the pre- and post-periods. That this was not due to progressive constipation as a result of confinement is shown by the rise in the post-period in one case to within a few g. of the pre-period amount. This fall in the weight of faeces was accompanied by an increase in the percentage of calcium immediately after the introduction of the oil. In Exps. 2 and 3 the average percentage of calcium in the faeces was higher in the oil-period than in the pre-period and in Exp. 1 it fell by only a very slight amount.

In each of these experiments, however, there was an improved balance of calcium during the oil-period. In Exps. 1 and 3 this was completely accounted for by the reduction which took place in the faecal excretion; for in these there was an increased excretion in the milk while changes in the urinary excretion were comparatively slight. In Exp. 2 the amount of calcium in the faeces was also reduced, but the improved balance was not entirely due to this, for the amount in the milk was also falling.

This improved balance of calcium as a result of feeding cod-liver oil agrees with the work of Hart, Steenbock and Hoppert [1921], who found that with both lactating and dry goats the amount of calcium in the faeces was reduced.

The phosphorus balances, on the other hand, did not show a similar improvement. In Exps. 1 and 3 the increased retention was almost wholly the result of a reduction in the faecal excretion. The second experiment, however, showed no such improvement, as the faecal excretion was at its maximum in the oil-period; but it may be noted here that this animal was at a different stage of lactation from the others and showed other differing results.

The results with regard to calcium and phosphorus balances are not in agreement with those of Meigs [1926], who found no improvement in the retentions of either of these minerals or of nitrogen after feeding 100 cc. daily to cows.

Numerous experiments have been made in attempts to increase the concentration of a particular constituent of the milk by feeding excessive amounts of it in the diet, but these have generally failed [Lauder and Fagan, 1914;

Denis, Sisson and Aldrich, 1922; Mattick and Wright, 1925]. Exps. 1 and 3, however, show that, where the absorption of calcium was increased by feeding cod-liver oil to animals in the later stages of lactation, the calcium content of the milk was increased. The benefits of increased calcium assimilation would thus be shared by both mother and offspring, since some of the extra calcium absorbed would be available for the young. It is of interest in this connection, as shown by Telfer and Crichton [1924], that even small changes in the mineral composition of goat's milk may affect the nutrition of young kids. In the case of the animals in early lactation (Exps. 2 and 4), there was no such increase in the calcium content of the milk.

Iodine, however, differs from the other mineral constituents of milk in the ease with which its concentration may be increased by feeding. This increase has been shown to occur when iodine is given either in what is presumably organic combination in cod-liver oil, or in inorganic form as potassium iodide. These findings confirm the results of earlier experiments at this Institute [Orr and Leitch, 1927]. Preliminary trials by dialysis and ultra-filtration also showed that, of the total normally occurring in goat's milk, about 80 % was in ionic form and that, when the increase took place after either cod-liver oil or potassium iodide, the relative proportions of ionic and colloidal iodine remained unaffected.

The effects of cod-liver oil on the fat content were much more indefinite. Exp. 1 showed a reduced percentage in the oil-period but with the rise in volume the average daily total secretion of fat remained the same. In the second experiment changes in the fat content were insignificant, while in the third it varied inversely as the volume. In Exp. 4 it was lowest in the oil-period, but in this experiment with the fall in volume in the post-period there was a marked rise in the fat content. These results are not in agreement with those of Drummond *et al.* [1924] and Golding, Soames and Zilva [1926], who concluded that cod-liver oil produced a reduction in the yield of milk-fat which was greater than might be attributed to the natural decline with advance of lactation; they are rather in agreement with those of Taylor and Husband [1922], who stated that, after feeding rations high in each of the constituents, protein, carbohydrate and fat, the percentage composition of milk was determined by its rate of secretion and that diet had no direct influence on it, but affected it only indirectly through changes produced in the daily volume.

The single experiment in which cod-liver oil was replaced by an equal volume of olive oil and an equivalent amount of iodine as potassium iodide did not show that the beneficial effects of cod-liver oil could be reproduced by such a change. Although the addition of small amounts of potassium iodide to a cereal ration fed to growing pigs produced increased balances of nitrogen and phosphorus [Kelly, 1925], it must be emphasised that the amounts of iodine were greatly in excess of those which had previously been given in cod-liver oil by Husband, Godden and Richards [1923]. In this experiment

and in the one in which olive oil was given alone there was an increase in the fat content of the milk in each case in the second half of the experimental period. These results are complicated by the fact that in one case the volume was falling and in the other rising and it does not seem possible to ascribe the effect to either the iodine or the oil. The olive oil experiment, however, shows that the increase in the iodine content in Exp. 5 must have been due to the potassium iodide and not to any effect of the oil *per se*.

SUMMARY.

1. The feeding of cod-liver oil to goats improved the balance of calcium by reducing the amount excreted in the faeces. The effects on phosphorus excretion were variable.
2. In the case of animals in late lactation, the percentage and total amounts of calcium in the milk were increased by feeding cod-liver oil.
3. The amount of iodine in the milk was increased by feeding either cod-liver oil or potassium iodide.
4. The fat content of the milk showed no definite changes as a result of feeding cod-liver oil.
5. In a single experiment in which cod-liver oil was replaced by olive oil plus an equivalent amount of iodine as potassium iodide, the effects of the former were not reproduced.

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