XXI. THE CHEMICAL COMPOSITION OF THE MILK OF COWS RECEIVING COD-LIVER OIL.

(PRELIMINARY PAPER.)

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SINCE the butter from the milk of cows fed on cod-liver oil was found to be much higher in antirachitic properties than that from cows on ordinary diet [see Golding and Zilva, 1928], and since rickets is a calcium deficiency disease, it was thought that if any of the mineral salts were affected by the feeding of cod-liver oil the most probable ones would be the calcium salts, and that it was therefore desirable to estimate the amounts of the total and diffusible calcium present in the milk of cows receiving cod-liver oil in comparison with that of cows on a normal diet.

General husbandry.

The experiments were carried out over a period of five months, from January until July 1927 and the details of management of the cows is given elsewhere [Golding and Zilva, 1928]. The four cows from which samples were taken were Flora I and Winsome II, which both received cod-liver oil, and Flora II and Clara, who received similar rations, *i.e.* hay, silage, mangolds and concentrates, but in lieu of cod-liver oil were given small quantities of arachis (pea-nut) oil.

From January 18 until April 22, the cows remained in the byre and covered yard; from April 22 until June 14 they were out at grass during the day time, but were brought in at night; from June 14 onwards they remained out both day and night.

Quantities of cod-liver oil.

The quantities of cod-liver oil and arachis oil fed daily were as follows:

<i>To Flora I:</i>	2 oz. cod-liver oil from Jan.	6-11
	4 " "	" 11-29
	6 " "	" 29-Feb. 26
	8 " "	" Feb. 26-May 3
	7 " "	" May 3- 6
	6 " "	" 6-10
	5 " "	" 10-16
	4 " "	" 16-July 6

<i>To Winsome II:</i>	$\frac{1}{2}$ oz. cod-liver oil from Jan.	6- 8
	2 " " "	8-11
	4 " " "	11-29
	6 " " "	29-May 3
	5 " " "	May 3-16
	4 " " "	16-June 7
	2 " " "	June 7-July 17

<i>To Flora II:</i>	2 oz. arachis oil from Feb.	12-24
	4 " " "	24-Mar. 5
	6 " " "	Mar. 5-13
	8 " " "	13-28

<i>To Clara:</i>	2 oz. arachis oil from Feb.	12-24
	4 " " "	24-Mar. 6
	6 " " "	Mar. 6-13
	8 " " "	13-30
	2 oz. cod-liver oil from May 4 onward.	

Chemical analysis.

The following estimations were made.

(1) *Titrateable acidity* by means of $N/9$ NaOH.

(2) *Time of coagulation by rennet.* The method for the determination is one which has been in use in this laboratory for several years and is known to give consistent results. 250 cc. of milk are coagulated in an insulated metal vessel with 3 cc. of rennet, and the time of coagulation, measured in minutes and seconds by means of a stop watch, is taken from the moment when the rennet is added until coagulation begins.

The base of the insulated vessel is fitted with an agate nozzle with an opening of 1 mm. in diameter through which the milk flows until coagulation takes place.

It is hoped to publish shortly more details of this method and the nature of the results obtained.

(3) *Total calcium.* The total calcium was precipitated as oxalate from the ash of a weighed quantity of milk (approx. 25 g.), converted into sulphate and the liberated oxalic acid titrated with $N/10$ permanganate.

(4) *Diffusible calcium.* To determine the amount of diffusible calcium, 25 cc. of milk were dialysed against 150 cc. of 3 % saline for 18 hours. At the end of this time the calcium in the diffusate was estimated by the method described under total calcium, except that the potassium permanganate used was $N/50$. The determinations were made in duplicate and corrections were applied for variations in the porosity of the capsules.

(5) *Total phosphorus.* This was also estimated on the ash of a known weight of milk. The phosphate was precipitated as ammonium phospho-

molybdate, which was converted into magnesium pyrophosphate and weighed as such.

(6) *Total nitrogen*. This was determined by Kjeldahl's method, using 5 cc. of milk.

(7) *Ash*. A known weight of milk was ignited at a low temperature (Argand burner), and the ash determined by weighing until a constant weight was obtained.

(8) *Fat*. The fat was determined by Gerber's method.

Table I.

Cow	Date	Quantity and kind of oil daily	Titrat-able acidity N/9 NaOH	Rennet test, minutes and seconds	Total calcium %	Diffusible calcium g. in 25 cc.	% total calcium diffused	Phos-phorus %	Nitrogen %	Ash %	Fat %
Flora I	7. i. 27	—	0.140	—	0.129	0.00956	—	0.100	—	0.83	4.25
Flora I	18. i. 27	4 oz. C.L.O.	0.155	4' 28"	0.130	0.0109	32.37	0.103	0.49	0.82	3.70
"	1. ii. 27	6 " "	0.120	5 5	0.137	0.0107	30.63	0.097	0.61	0.85	3.35
"	1. iii. 27	8 " "	0.134	5 0	0.129	0.0106	32.18	0.090	0.61	0.79	3.55
"	30. iii. 27	8 " "	0.123	7 15	0.126	0.0101	—	0.096	0.51	0.80	3.35
"	3. v. 27	8 " "	0.100	12 30	0.128	—	—	0.118	0.60	0.86	4.50
"	6. vii. 27	4 " "	0.129	7 35	0.123	0.0086	—	0.097	0.53	0.84	—
Winsome II	18. i. 27	4 " "	0.174	2 20	0.127	0.0100	30.59	0.112	0.49	0.81	3.75
"	2. ii. 27	6 " "	0.155	3 25	0.137	0.0094	26.58	0.099	0.64	0.86	3.65
"	2. iii. 27	6 " "	0.134	5 5	0.144	0.0089	23.89	0.091	0.55	0.80	4.30
"	31. iii. 27	6 " "	0.148	3 35	0.154	0.0093	—	0.125	0.54	0.79	4.10
"	3. v. 27	6 " "	0.140	4 30	0.155	—	—	0.118	0.60	0.86	4.50
"	6. vii. 27	2 " "	0.153	4 0	0.121	0.0088	—	0.104	0.54	0.80	—
Flora II	1. ii. 27	None	0.170	2 25	0.117	0.0109	35.97	0.100	0.63	0.78	4.50
"	1. iii. 27	4 oz. arachis	0.168	2 30	0.119	0.0103	33.71	0.093	0.61	0.73	4.20
"	30. iii. 27	None, but 8 oz. from 13 to 28. iii. 27	0.148	3 55	0.126	0.0104	—	0.113	0.56	0.78	5.15
Clara	2. ii. 27	None	0.175	2 20	0.116	0.0093	31.06	0.099	0.66	0.79	4.90
"	2. iii. 27	4 oz. arachis	0.178	2 5	0.111	0.0088	30.83	0.098	0.55	—	4.80
"	31. iii. 27	None, but 8 oz. from 13 to 30. iii. 27	0.158	2 30	0.123	0.0099	—	0.110	0.54	0.69	5.10

The results which were obtained are shown in Table I. In nearly every case except those of the rennet test and fat, the determinations were carried out in duplicate and the mean results only are given.

It is obvious that insufficient experiments have been made to justify any very definite statement of differences in the composition of some constituents of the milk, but certain results are somewhat striking and the general tendency of the effect of the feeding of the cod-liver oil in the quantities given may be summarised as follows.

(1) The acidity of the milk as determined by titration with N/9 NaOH is less when the cows are receiving cod-liver oil, although the differences are not greater than those which are sometimes found in comparing the milk of individual cows.

(2) The time of coagulation of the milk by rennet is increased very considerably.

(3) The total percentage of calcium is distinctly higher in the milk of the cows receiving cod-liver oil.

(4) Insufficient results were obtained to show definitely the relationship between the amount of the calcium which was diffusible and the total amount: moreover, as the specific gravity had not been determined experimentally, this was calculated from the fat and total solids, the amount of milk in the diffusion thimbles estimated from this theoretical value, and the figures given determined from these results. As far as can be judged from these inadequate data, it seems possible that the percentage of total calcium which is diffusible is less when cod-liver oil is fed than otherwise. If this were the case, it would help to explain the increase in the time of coagulation by rennet.

(5) The total phosphorus content appears to be unaffected.

(6) The total nitrogen content appears to be unaffected.

(7) The ash content is higher in the case of the cod-liver oil cows.

(8) The fat content is lower. This result is amply confirmed by the daily fat tests of the milk of individual cows.

An interesting variation which has been effected in the composition of the milk is the marked increase in the percentage calcium content.

The calculation of the total amount of calcium in the milk given on each occasion when an analysis was made showed that the increase in the percentage of calcium was not due to diminution in the yield of milk.

Since the foregoing work was carried out, the author has found that similar increases in the calcium content of the milk of lactating goats are shown in some of the results of Hart, Steenbock, Kletzein and Scott [1927]. The authors have not drawn attention to this point as they were investigating calcium assimilation and came to the conclusion that when the unsaponifiable fraction of cod-liver oil was fed in capsules it was not effective in improving calcium assimilation, but that when fed as a solution in corn oil it resulted in marked improvement in calcium assimilation.

Further, however, they stated that the corn oil itself had no effect, but if the figures which they quote are considered their results show that, in the case of Animal 1, the calcium in the milk increased from 0.128 to 0.133 % when a quantity of the unsaponifiable fraction corresponding to 40 cc. of cod-liver oil was fed daily in capsules (January 28–February 25); when an equivalent of 80 cc. was fed daily (February 25–March 18) this further increased to 0.146 % and when an equivalent of 80 cc. was fed in solution in 15 cc. of corn oil the percentage was still further raised to 0.148 % (March 18–April 29).

Similar results were obtained with Animal 2, whereas Animal 3 showed a diminution in calcium content of the milk under similar conditions.

With respect to the corn oil in which the cod-liver oil was fed, the authors state that it did not improve calcium assimilation, but from the single result given, it appears that it does cause an increase in the percentage calcium content of the milk, the milk of Animal 4 showing a percentage of 0.131 % when no corn oil was given (June 3 to July 1) which increased to 0.150 % when 15 cc. corn oil were fed (July 1 to 28).

It must be pointed out that these figures do not in any way contradict the conclusions of the authors (Hart, Steenbock, Kletzein and Scott) which are based on complete metabolism experiments, but seem to confirm our finding in that in two cases out of three the percentage of calcium in the milk of the lactating goats was considerably increased when cod-liver oil was added to their ration.

The results which have already been tabulated and discussed are based on analyses of evening milk; another series of calcium estimations was made on samples of morning milk of the same four cows and the results are shown in Table II.

Table II.

Cow	Date	Additional ration	Titrateable acidity	% total calcium
Flora I	24. ii. 27	6 oz. cod-liver oil	0.128	0.133
"	4. iii. 27	8 " "	—	0.135
"	18. iii. 27	8 " "	0.130	0.129
Winsome II	24. ii. 27	6 " "	0.148	0.141
"	4. iii. 27	6 " "	—	0.138
"	18. iii. 27	6 " "	0.154	0.145
Clara	24. ii. 27	2 oz. arachis oil	0.180	0.117
"	4. iii. 27	4 " "	—	0.114
"	18. iii. 27	8 " "	0.183	0.117
Flora II	24. ii. 27	2 " "	0.155	0.118
"	4. iii. 27	4 " "	—	0.121
"	18. iii. 27	8 " "	0.171	0.120

It is seen that these results confirm those which have been already given, showing a distinctly higher percentage calcium content in the milk of the two cows which were receiving cod-liver oil in comparison with those two cows which were receiving arachis oil.

SUMMARY.

The analyses of the milk of cows which have been fed on well-balanced diets to which varying quantities of cod-liver oil and arachis (pea-nut) oil have been added, indicate that the addition of the cod-liver oil results in changes in the chemical composition of the milk, particularly an increase in the percentage of total calcium.

Insufficient results have as yet been obtained to justify any very definite conclusions, but in view of the fact that the butter made from the milk of cows fed on cod-liver oil is known to be much more strongly antirachitic than butter from the milk of cows which have not received cod-liver oil; the results already obtained seem to be of sufficient interest to warrant further analyses.

No suggestion is as yet put forward as to the source of the extra calcium which is found in the milk.

This paper is only of a preliminary nature and further work is now in progress.

In conclusion I wish to thank H. Hallett and A. Wagstaff for help in carrying out the necessary analyses.

REFERENCES.

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