CXIII. A COMPARISON OF THE VITAMIN D CONTENTS OF GUERNSEY AND SHORTHORN BUTTER (MILK).

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THE well-known differences in the fat percentages and in the relative carotene and vitamin A contents of the milks of Shorthorn and Guernsey cattle have led us to a comparative investigation of the vitamin D contents of these milks.

EXPERIMENTAL.

(1) *Source of milk.*

Five Guernsey cows and five Shorthorn cows were allowed to run together on the same pasture from about the middle of April until the middle of June, 1935. From the first of May (the last cow joined the group on April 30th) until the end of the experiment the cows received in addition to the pasture an average of 9.6 lb. of concentrates, 24 lb. of mangels and 6 lb. of oat and vetch silage per cow daily in the Guernsey group and 5½ lb. of concentrates, 25 lb. of mangels and 6 lb. of silage similarly per cow in the Shorthorn group. Supplementary feeding to this extent is not common during May and June, but the additional foods were given in this case because the pasture was short and heavily stocked. The difference in the intake of concentrates was partly due to the higher milk yield of the Guernseys and partly to the higher fat content of their milk. They received 4 lb. of cubes for each gallon of milk over 15 lb. per cow per day, while the Shorthorns, producing milk poorer in solids, were given 3½ lb. for similar quantities. The concentrates consisted mostly of cubes compounded from maize meal, middlings, rice meal, decorticated groundnut cake meal, maize gluten feed, linseed cake meal, undecorticated cotton cake, sugar cane molasses, with small additions in the case of two Guernseys and one Shorthorn cow of mixtures of slightly different composition.

All the milk of the cows in the two groups was measured and collected each morning and evening over the period from June 11th, a.m. to June 13th, p.m. both dates inclusive. The fat percentage was estimated twice daily for each cow. From the mixed milks of each group, 6 pints were kept from the evening milk and 9 pints from the morning. The collected milk of each group was separated on the morning of the 14th and churnings of the two creams were done on the 18th of June. The butters were melted at 60°, filtered, and the butter fats were stored at -2°.

Table I gives the ages, dates of calving of the cows, the number of lactation, the date when turned out to pasture and the average daily weight and the fat content of the milk yielded during the 3-day collection period.

Table I. *Ages, dates of calving, number of lactation, dates of turning out to pasture, milk and fat yields of experimental cows.*

Breed	Name	Wt. kg.	Age (years and months)	Days since calving	No. of lacta- tion	Turned out to pasture	Average daily yield June 11th-13th		
							Milk kg.	Fat kg.	Fat %
Guernsey	Fanny 2	447.7	4, 1	117	2	12. iv. 35	16.25	0.735	4.52
	Peace 2	541.1	8, 2	93	6	18. iv. 35	10.77	0.511	4.74
	Rosey 6	449.5	5, 3	79	3	29. iv. 35	16.33	0.807	4.94
	Valven 2	352.0	2, 8	139	1	12. iv. 35	13.61	0.670	4.92
	Venus 1	450.4	7, 2	106	5	12. iv. 35	21.50	0.888	4.13
	Average	448.1	5, 6	107	3	—	15.69	0.722	4.60
Shorthorn	Flora 16	586.0	5, 7	184	3	18. iv. 35	12.36	0.441	3.57
	Flora 21	495.3	4, 7	87	2	29. iv. 35	14.67	0.454	3.09
	Lily 3	526.2	8, 6	82	6	30. iv. 35	11.83	0.372	3.14
	Lottie 7	446.3	4, 0	288	1	18. iv. 35	13.08	0.455	3.48
	Wistaria 4	551.1	6, 5	163	4	9. iv. 35	14.59	0.568	3.90
	Average	521.0	5, 10	161	3	—	13.31	0.458	3.44

(2) *Biological tests on butter fats.*

The Guernsey and Shorthorn butter fats were tested for their vitamin D content in protective experiments on rats. The technique of these tests has been previously described [Kon and Henry, 1935]. The butters were tested at one level of intake, 0.3 g. of butter fat per rat per day (with the exception of Sundays), on groups of 12 rats and compared with one level of the international standard for vitamin D (V.D. 10) and with negative controls.

The results of the tests are given in Table II.

Table II. *Bone ash % of rats receiving Shorthorn and Guernsey butter fats.*

Negative controls				0.2 Unit Standard				0.3 g. Shorthorn butter fat				0.3 g. Guernsey butter fat			
Rat	Sex	Litter	Ash %	Rat	Sex	Litter	Ash %	Rat	Sex	Litter	Ash %	Rat	Sex	Litter	Ash %
4787	♀	1	29.0	4788	♀	1	42.1	4789	♀	1	39.8	4790	♀	1	44.8
4791	♂	1	24.9	4792	♂	1	45.8	4793	♂	1	38.9	4794	♂	1	36.3
4795	♂	2	29.0	4796	♂	2	47.3	4797	♂	2	38.5	4798	♂	2	38.1
4807	♂	3	27.6	4808	♂	3	43.2	4809	♂	3	38.1	4810	♂	3	40.0
4811	♂	3	24.6	4812	♂	3	46.4	4813	♂	3	36.9	4814	♂	3	36.5
4815	♀	4	25.5	4816	♀	4	44.6	4817	♀	4	33.9	4818	♀	4	39.1
4819	♀	4	29.1	4820	♀	4	39.4	4821	♀	4	32.2	4822	♀	4	35.1
4823	♀	5	26.1	4824	♀	5	48.1	4825	♀	5	45.3	4826	♀	5	39.1
4827	♀	5	27.6	4828	♀	5	45.4	4829	♀	5	33.8	4830	♀	5	42.3
4831	♀	6	32.4	4832	♀	6	46.1	4833	♀	6	42.2	4834	♀	6	48.3
4835	♀	6	29.2	4836	♀	6	48.0	4837	♀	6	43.3	4838	♀	6	46.2
4839	♀	7	30.2	4840	♀	7	45.0	4841	♀	7	42.5	4842	♀	7	39.6
Average			27.93				45.12				38.78				40.45
S.E.M.			±0.67				±0.73				±1.18				±1.19

Guernsey butter has produced a slightly better calcification than the same level of Shorthorn butter, the difference being 1.67 percentage units. This difference is, however, not statistically significant, as its standard error of the mean (calculated from differences between litter-mate pairs on both treatments) is 1.24 and the probability of it being due to chance accidents of sampling 1/5 according to "Student's" *t* test [Fisher, 1932].

The gains in percentage of ash over the negative controls were as follows:

Substance tested	Gain	S.E.M.*
0.3 g. level of Guernsey butter fat	+12.52	±0.93
0.3 g. level of Shorthorn butter fat	+10.65	±1.18
0.2 unit of International Standard	+17.19	±1.06

* In order to take advantage of litter-mate comparisons, the standard errors of the mean are calculated from means of individual differences within pairs and not from differences of means.

From these gains the vitamin D content of the butter fats has been calculated in International Units by means of the equation

$$\text{Gain in ash \%} = 26.33 + 16.6 \log \text{dose (I.U.)}.$$

The method of calculation has been fully described in an earlier paper [Kon and Henry, 1935].

The following values were obtained:

Guernsey butter fat 0.35 I.U. per g.¹
Shorthorn butter fat 0.28 I.U. per g.

They are lower than those usually obtained by us for summer butters [Kon and Henry, 1935].

As already pointed out, the difference between these two values is not statistically significant. The following calculations have been carried out on the actual values, but the statistical limitations of this procedure are realised. The average fat yield of the experimental Guernsey cows works out at 0.722 kg. per cow per day and the corresponding figure for Shorthorns is 0.458 kg. (Table I). On the assumption that the total vitamin D activity of the milk is concentrated in the fat, a Guernsey cow secreted daily on the average 253 I.U. and a Shorthorn cow only half this amount, namely, 128 I.U. This comparison is, however, hardly fair, as the Shorthorn cows were more advanced in lactation than the Guernseys by some 8 weeks and were yielding relatively less milk. When the calculations are carried out per kg. of milk the difference between Guernseys and Shorthorns narrows to 16 I.U. against 10, still an appreciable difference. The effect of the stage of lactation on the vitamin content of milk has not been investigated so far beyond the colostral stage and it is difficult to say whether the slight difference between Shorthorn and Guernsey butters (if at all real) was due to such an effect. Whether or not the present findings of a lack of serious difference in the antirachitic activities of Guernsey and Shorthorn butters would be confirmed at other seasons, or under different nutritional conditions, is a matter of conjecture. If they are provisionally accepted as valid, the relative activities of the milks of the two breeds could be simply expressed by the ratio of the fat percentages.

A comparison of the present results with those obtained by Hess *et al.* [1932] when feeding irradiated yeast to cows of various breeds shows an interesting difference. These authors found that on the same intake of antirachitic material the vitamin D concentration in the butter fat was the higher the lower the total yield of fat.

The relationship does not obtain in the present case and it is significant that cows on pasture are likely to obtain the antirachitic factor not only by ingestion but also through the direct action of the sun. The relative contributions from these two sources are now under investigation in our laboratory.

¹ These values express the antirachitic potencies of the butters as measured by the feeding of butter fat to rats. In making use of these figures the reservations made by Kon and Booth [1933; 1934, 1, 2] should be kept in mind.

SUMMARY.

1. The vitamin D contents of Guernsey and Shorthorn butter fats produced on pasture under similar conditions of feeding and management have been compared. The Guernsey butter fat was found to contain 0.35, the Shorthorn 0.28 I.U. of vitamin D per g., but the difference is not statistically significant.

2. It is suggested that if the relationship is generally valid the relative vitamin D activities of the milks of the two breeds could be simply expressed by the ratio of the fat percentages.

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REFERENCES.

- Fisher (1932). Statistical methods for research workers, 4th ed., p. 112.
(Oliver and Boyd.)
Hess, Light, Frey and Gross (1932). *J. Biol. Chem.* **97**, 369.
Kon and Booth (1933). *Biochem. J.* **27**, 1302.
—— — (1934, 1). *Biochem. J.* **28**, 111.
—— — (1934, 2). *Biochem. J.* **28**, 121.
—— and Henry (1935). *Biochem. J.* **29**, 2051.