X. THE SOURCE OF VITAMIN D IN SUMMER MILK¹

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THOUGH it is well known and generally accepted that the vitamin D content of cow's milk is subject to seasonal variation, and that it is higher in summer than in winter, no agreement has so far been reached as to the exact causes of the difference. The problem at issue is whether the increase observed in summer is due to the properties of the pasture compared with those of foods used in winter, to the direct action of the sun on the cow, or to a combination of the two factors. In the opinion of Chick & Roscoe [1926] the antirachitic value of milk depends principally upon the degree of insolation of the cow, but fresh green food is said to be a contributory factor. The superior calcifying potency of butter produced by cows exposed to sunlight as compared with butter yielded by cows fed in the dark is mentioned in a brief report by Dutcher & Honeywell [1927]. After extensive tests Steenbock *et al.* [1930] have reached a different view, namely that "the well recognized superior quality of summer-produced milk and butter fat must . . . have its primary origin in other factors than sunlight acting directly upon the cow".

More recent evidence based either on the vitamin D content of milk produced in winter by cows receiving artificially dried grass [Watson *et al.* 1933] or on the rickets-preventing action of dried grass when fed to rats [Anonymous, 1933] or calves [Bechdel & Borland, 1934] would allow one to infer that grass itself cannot serve as a source of vitamin D.

Such conflicting evidence has led us to carry out the experiments described below.

EXPERIMENTAL

(1) *Plan of experiment*

The experiment was carried out in 1936 between 1 May and 30 June, that is at a time when, under our conditions, the actinic activity of the sun is high and grass is plentiful.

Eight Shorthorn cows were used. Six of them were known to give positive reactions to the agglutination test for contagious abortion and two of these had calved prematurely. The other two cows—Linda 5 and Lottie 5—had given a negative result to the agglutination test. The grouping of the cows is given in Table I. The treatment was as follows:

Group 1. Winter housing and feeding conditions.

Lottie 8 and Flora 16. Kept indoors day and night in a well-ventilated and lighted shed. All the light came into the shed through glass. The cows were not exposed to direct sunlight and received a normal winter ration consisting of 25 lb. of pulped mangolds, 3 lb. of dried sugar-beet pulp (together equal to about

¹ Read before the Biochemical Society, 17 October 1936 [Campion *et al.* 1936].

Table I. *Distribution of cows on experiment*

Name of cow	Lactation	Days since calving	Colour percentage			Treatment	
			Red	White	Roan	Before experiment	On experiment
Lottie 8	2	74	100	—	—	Kept indoors from 18. ii onwards	Normal winter ration indoors
Flora 16	4	92	90	10	—	Kept indoors from 20. iii onwards	
Carlet 3	5	140	—	—	100	Kept indoors from 14. iii onwards	Normal winter ration outdoors with no grass
Lottie 7	2	50	10	30	60	Kept indoors from 12. iii onwards	
Linda 5	2	168	90	10	—	With herd	Summer ration on pasture with the herd
Lottie 5	3	110	80	20	—	With herd	
Campion 3	2	11	90	10	—	Kept indoors from 24. iv onwards	Summer ration fed indoors with 1 cwt. cut grass per cow daily
Pippin 1	4	87	100	—	—	Kept indoors from 20. iii onwards	

45 lb. of mangolds), 15 lb. of vetch and oat silage and 5 lb. of seeds hay daily for maintenance and a concentrate mixture consisting of flaked maize 3 parts, dried brewer's grains 3 parts, crushed oats 1 part, decorticated groundnut cake 1 part and linseed cake 1 part, fed according to milk yield. Throughout the period the yield of this pair of cows declined gradually in a normal manner.

Group 2. Winter feeding conditions and direct exposure to summer sun- and sky-shine.

Carlet 3 and Lottie 7. This pair was kept in a small fenced yard made partly in the open and partly under a Dutch barn, and received the same production mixture and maintenance ration as the above pair. The maintenance ration was fed under the Dutch barn out of direct sunlight between 9 and 10 a.m. and at about 5 p.m., and was usually quickly consumed. It was noticed that on finishing the morning allowance of maintenance ration the cows would lie down in the shade, so on and after 18 May they were shut out in the open part of the yard from 12 noon (B.S.T.) until they were fetched in for milking at about 3 p.m. This was done on all days when it was not raining hard, and so the conditions were similar to those of the cows on grass which had to graze for some part of the day in direct sunlight. From 1 to 3 May this pair was only put into the yard during the daytime (from after the morning milking until the evening milking); thereafter they were in the yard day and night except for milking (6 a.m. to 9 a.m. and 3 p.m. to 5 p.m.). During the time they were in the fenced yard care was taken that no grass or greenstuff grew within reach of the cows. The milk yield of both cows declined gradually in a normal manner throughout the period.

Group 3. Normal summer conditions.

Linda 5 and Lottie 5. This pair of cows was running with the herd for the duration of the experiment, and the grazing was changed to fresh pastures as and when conditions made it necessary. The quality of the pasture was good, but for brief periods the grass was short, and this necessitated an extra amount of exercise in the light while the cows were grazing. In all fields there was ample shade. From 28 April and throughout the period of the experiment this pair of cows was on pasture day and night. Up to 5 May, in addition to pasture they received an allowance of a mixture of concentrates consisting of flaked maize

3 parts, dried grains 3 parts, crushed oats 1 part, decorticated groundnut cake 1 part and linseed cake 1 part, and from 6 May to the end of the experiment the concentrates consisted of a mixture of maize germ cake 2 parts and undecorticated cotton cake 1 part. The milk yield of Linda 5 declined slowly during the period, whereas the milk yield of Lottie 5, which was higher than that of Linda 5 at the beginning, declined more rapidly. This cow received a larger allowance of concentrates throughout the period.

Group 4. Summer feeding conditions but no direct exposure to sun- or sky-shine.

Pippin 1 and Campion 3. These two cows were kept indoors under the same conditions as group 1 but received a known amount of freshly cut grass for the maintenance ration. The amount of cut grass given per head was increased gradually from 46 lb. on 1 May to 112 lb. on 19 May and then remained constant at that rate. The daily quantities from 1 May to 19 May are shown below:

1 May	46 lb.	6 May	67 lb.	11 May	75 lb.	16 May	100 lb.
2 "	46 "	7 "	64 "	12 "	96 "	17 "	102 "
3 "	56 "	8 "	70 "	13 "	96 "	18 "	98 "
4 "	56 "	9 "	75 "	14 "	95 "	19 "	112 "
5 "	65 "	10 "	75 "	15 "	100 "		

In addition to the cut grass, these cows received the same mixture of concentrates as the cows in group 3. They were thus under summer conditions except that they had no direct sun- and sky-light and little exercise.

The milk yield of Pippin 1 declined more rapidly than usual during the experimental period. Campion 3 calved prematurely on 20 April and her milk yield declined at a normal rate.

The estimate of 112 lb. green grass daily as the consumption of a cow is supported by recent work in America using Holstein and Jersey cows, which has since been published [Woodward, 1936]. Therein it is concluded that a cow's capacity for grass is about 150 lb. of immature green grass per day. The cows were on a plentiful supply of grass. While it was impossible to get any accurate estimate of the grass eaten by a Shorthorn cow under herd conditions at this Institute where the amount of grass available gradually decreases until the cows are put on new pasture, it is believed that the amount of cut grass fed to the cows indoors (112 lb. daily) approximates to the average consumed by the cows outdoors. This belief is supported by the estimations of the vitamin A and carotene contents of the milks of the experimental groups, reported on p. 84.

The fresh grass was cut before noon every day and fed during the succeeding 24 hours except at the weekend when a double amount was cut on the Friday and fed on Friday and Saturday, and a single lot cut on Saturday to be fed on Sunday afternoon and Monday morning.

The grass was cut from a field intended for mowing to give a crop of hay and only the areas of shortest grass were cut. Towards the end of the experimental feeding period some of the grasses were in flower and by this time also there was an increasing amount of clover present in the herbage.

After cutting, the grass was put into sacks for transport to the farm buildings, where it was emptied and spread thinly (to avoid heating) on a cold concrete floor in a dark room until fed.

(2) *Weather conditions*

May was very dry with only 7 days with a precipitation of 0.2 mm. or more, and a total rainfall of 22 mm. Many days though rainless were dull, and the two meteorological stations nearest to Shinfield, Ascot Heatherwood and Warfield

reported respectively daily averages of 6.65 and 6.47 hours of bright sunshine. June was very wet; it had 15 days with a precipitation of 0.2 mm. or more and a total rainfall of 96 mm. The two above-named stations recorded respectively 6.46 and 6.13 hours of bright sunshine. The second half of June was, in Shinfield, much more sunny than the first with some 10 sunny days.

(3) *The vitamin A and carotene contents of the milks (butters) of the four groups of cows*

As a check on the feeding and to make sure of the normal behaviour of the cows, estimations of the carotene and vitamin A contents of the milks were

Fig. 1. Vitamin A and carotene contents of butter fats from cows on various treatments.
 - - - - Group 1, winter housing and feeding conditions. - · - · - Group 2, winter feeding conditions. Direct exposure to sun- and sky-shine. — Group 3, normal summer conditions.
 · · · · Group 4, summer feeding conditions but no direct exposure to sun- or sky-shine.
 ↓ Cows turned out to grass.

carried out at frequent intervals. Butters were churned once a week from the milks of the experimental cows. Fig. 1 presents the results of estimations

expressed in Moore units and carried out according to the method described by Booth *et al.* [1933]. It will be seen that, with the exception of group 3 which had gone out to pasture with the herd before the experiment was started, the other groups showed at the beginning levels of carotene and vitamin A typical of the stall-feeding season. The two groups fed on winter rations (groups 1 and 2) continued at this low level throughout the experiment. On the other hand the butter of group 4, receiving freshly cut grass, rose rapidly to the same level as the butter of the pasture-fed cows.

(4) *The vitamin D content of the milks (butters) of the four groups of cows*

The milk yield and fat percentage of individual cows were measured at each milking. Two samples of butter were churned from the pooled milk of each group of cows for rat-feeding tests. The first churning was carried out on milk collected between the morning milking of 16 June and the evening milking of 18 June, both dates inclusive. For the second, milk yielded between the evening milking of 27 June and the morning milking of 30 June was used. The butters were melted at 60° and filtered, and the butter fats were stored at -2°. The vitamin D contents of the butters were assessed in prophylactic tests on rats by the technique described by Kon & Henry [1935]. The first churning of each group was used for the first fortnight and was then replaced by the second churning. All 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 15 rats and compared with 3 levels of the I.S. for vitamin D (V.D. 12) and with negative controls. There were only 10 rats on the 0.2 unit level of the standard. All comparisons were between litter-mates. The results of the tests are given in Table II.

Table II. *Bone ash percentage of the rats on various treatments*

Litter		Negative controls		I.S.								Butters															
				0.05 unit		0.1 unit		0.2 unit		Group 1		Group 2		Group 3		Group 4											
				Sex	Ash %	Sex	Ash %	Sex	Ash %	Sex	Ash %	Sex	Ash %	Sex	Ash %	Sex	Ash %										
1	♀	29.6	♀	32.4	♀	37.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2	♀	29.0	♀	36.0	♀	40.9	♂	39.3	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
3	♀	26.0	♀	30.7	♀	36.4	♀	43.9	♀	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
4	♀	29.0	♀	33.8	♀	33.2	♀	42.5	♀	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
5	♀	30.8	♀	37.0	♀	39.2	♂	41.5	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
6	♀	27.7	♀	32.2	♀	39.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
7	♀	27.1	♀	31.0	♀	38.7	♂	38.8	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
8	♀	30.7	♀	35.9	♀	41.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
9	♀	27.8	♀	36.1	♀	39.8	♂	43.0	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
10	♀	26.5	♀	29.7	♀	33.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
11	♀	29.0	♀	31.8	♀	37.2	♂	43.9	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
12	♀	24.9	♀	27.6	♀	36.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
13	♀	24.3	♀	27.7	♀	33.4	♂	41.1	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
14	♀	26.2	♀	28.0	♀	38.6	♀	38.0	♀	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
15	♀	27.6	♀	30.7	♀	34.0	♂	39.1	♂	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Av.	8♀ 7♂	27.75	8♀ 7♂	32.04	8♀ 7♂	37.28	2♀ 8♂	41.11	8♀ 7♂	35.77	8♀ 7♂	43.69	8♀ 7♂	39.01	8♀ 7♂	31.19											
S.E.M.		±0.51		±0.82		±0.72		±0.70		±0.57		±0.76		±0.90		±0.39											

The butters of the two groups of cows which were allowed direct access to sun- and sky-shine have produced much better calcification than the butters produced indoors. The difference is so obvious that it is superfluous to give statistical proof of significance. It will also be noticed that, under the same conditions of exposure to light, winter feeding has produced more potent butters

than summer feeding. The difference is smaller than in the first comparison, but is statistically highly significant. (The probability of the difference being due to chance accidents of sampling is, for butters produced indoors and outdoors, less than 1:2500 when calculated from differences between litter-mate pairs according to "Student's" [1908] procedure using Table XXV of Pearson [1930].

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

Substance tested	Gain
0.05 unit of I.S.	+ 4.29
0.10 "	+ 9.53
0.20 "	+ 13.36
0.3 g. level of butter fat of group 1	+ 8.02
0.3 g. " 2	+ 15.94
0.3 g. " 3	+ 11.26
0.3 g. " 4	+ 3.44

From these gains the vitamin D contents of the butter fats have been calculated in I.U. 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 & Henry, 1935].

For the calculation of the ratio of dosages each butter fat was compared with the two nearest levels of I.S. Thus the responses of 0.05 and 0.1 I.U. were used for the butter fats of groups 1 and 4 and the responses of 0.1 and 0.2 I.U. were used for the butter fats of groups 2 and 3. Arithmetical means of pairs of results obtained in this way were taken as final values.

The following values were obtained:¹

Butter fat of group 1 (winter ration, kept indoors)	0.27 I.U. per g.
" 2 (winter ration, kept outdoors)	0.88 "
" 3 (summer ration, on pasture)	0.46 "
" 4 (summer ration, fresh grass, kept indoors)	0.15 "

When the activities of butters produced on the same ration out of doors and indoors are compared it is found that, in the case of "summer" butters, the butter produced on pasture is $\frac{0.46}{0.15}$ or three times as potent as the butter produced indoors. For "winter" butters the ratio works out at $\frac{0.88}{0.27}$, that is also approximately 3:1. In other words direct exposure of cows to sun- and sky-shine has resulted in a threefold increase in the antirachitic potency of their butter as compared with the butter of cows similarly fed, but given access only to light which has passed through ordinary glass.

Table III gives the milk and fat yields of the experimental cows and also the amounts of vitamin D secreted by the different groups, calculated on the assumption that the total vitamin D activity of the milk is concentrated in the fat.

The calculations are carried out on average values for milk and fat yield for the whole experimental period. As the experimental butters were churned from two 3 days' collection periods towards the end of the experiment it should be said here that the relative milk and fat yields of the groups of cows during the collection periods were very similar to the averages used in the calculations. As

¹ The values for groups 1 and 3 agree well with the normal values for winter and summer Shorthorn butters produced at this Institute [Kon & Henry, 1935]. The value for group 2 is higher than any obtained here so far, whilst that for group 4 is the lowest.

Table III. *Average daily yields of milk, fat and vitamin D of the experimental cows for the period 1 May to 30 June*

Group	Cow	Milk kg.	Fat kg.	Fat %	Vitamin D I.U.	
					Total	Per kg. of milk*
1. Winter ration, kept indoors	Lottie 8	13.42	0.420	3.13	—	—
	Flora 16	13.07	0.396	3.03	—	—
	Average	13.24	0.408	3.08	110	8.3
2. Winter ration, kept out of doors	Carlet 3	8.02	0.252	3.14	—	—
	Lottie 7	16.03	0.461	2.88	—	—
	Average	12.02	0.356	2.96	313	26.0
3. Summer ration, on pasture	Linda 5	14.60	0.537	3.68	—	—
	Lottie 5	14.97	0.560	3.74	—	—
	Average	14.78	0.548	3.71	252	17.0
4. Summer ration, fresh grass, kept indoors	Pippin 1	8.54	0.307	3.59	—	—
	Campion 3	11.06	0.390	3.53	—	—
	Average	9.80	0.348	3.55	52	5.3

* These values were given wrongly in the preliminary report [Campion *et al.* 1936]. By mistake the figures were halved.

the butters were churned from the pooled milks of each group only average group values are given for vitamin D yields.

When these are compared it will be seen that direct exposure to sun- and sky-shine has caused a cow on winter ration to secrete daily some 200 I.U. of vitamin D more than a cow similarly fed indoors. On summer ration and fresh grass the difference is also about 200 I.U. It is interesting to note that the winter ration, whether fed outdoors or indoors, has yielded more vitamin D than the similarly fed summer ration. In both cases the difference in the daily secretion of a cow is some 60 I.U. Though such good agreement is, no doubt, fortuitous, the fact that the cows secreted more vitamin D coupled with the higher concentration of the factor in the respective butters shows that the winter diet was a better source of vitamin D than the summer ration. The winter diet included, as already stated, 5 lb. of seeds hay per cow daily. Hay is known to acquire some antirachitic properties in the process of curing [Steenbock *et al.* 1925; Smith & Briggs, 1933]. The superiority, as far as the vitamin D is concerned, of a diet including hay over one containing more than double its equivalent of fresh grass shows again the inefficiency of fresh grass as a source of the antirachitic factor for the cow. It may be safely concluded that, in the present experiments, the direct exposure of the cows to sun- and sky-shine has been responsible for all, and pasture for none, of the increase in the vitamin D potency of milk which takes place in summer.

SUMMARY

1. Eight Shorthorn cows were divided into four groups of two and placed on the following treatments: 1st group kept indoors on winter ration which included 5 lb. of hay per cow daily; 2nd group kept outdoors exposed to sun- and sky-shine, given the same ration as group 1; 3rd group out on pasture with the herd; 4th group kept indoors but given 1 cwt. daily per cow of freshly cut grass, and the same summer maintenance and production ration as given to group 3. The experiment lasted from 1 May to 30 June 1936.

2. Butters were churned for feeding tests in the middle and at the end of June. Prophylactic tests were carried out on groups of 15 rats and the activities of the butter fats were compared with those of doses of the i.s. for vitamin D.

3. It was found that butter fats produced outdoors and indoors on the summer ration had potencies of 0.46 and 0.15 i.u. per g. respectively (ratio of potencies 3:1), whilst the figures for similar butters produced on the winter ration were 0.88 and 0.27 i.u. per g. respectively (ratio of potencies also 3:1).

4. The average total amount of vitamin D secreted daily by a cow was as follows: 1st group 110 i.u., 2nd group 313 i.u., 3rd group 252 i.u. and 4th group 52 i.u. The yields per kg. of milk were 8.3, 26.0, 17.0 and 5.3 i.u. respectively.

5. The cows (whether fed outdoors or indoors) on the winter ration which contained hay secreted more vitamin D than the corresponding cows on summer feed consuming a plentiful supply of fresh grass.

6. It is concluded from the experiments that the direct exposure of the cow to sun- and sky-shine contribute all, and the pasture none, of the increase in the vitamin D potency of milk which takes place in the summer.

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