53. The Vitamin D Content of English Butter Fat throughout the Year

By K. M. Henry and S. K. Kon, *From the National Institute for Research in Dairying, University of Reading*

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Wilkinson [1939] has studied extensively the seasonal variation in the vitamin D content of Danish and Scotch butters. Equally extensive data for American butters have been reported by Bechtel & Hoppert [1936]. No comparable measurements have been published for English butter with the exception of the few figures quoted by Mattick [1938] and by Campion, Henry, Kon & Mackintosh [1937].

We have therefore undertaken an examination of the vitamin D content of butter churned at intervals from the milk of the typical south of England herd of cattle kept at the National Institute for Research in Dairying. For reasons fully set out in the accompanying paper [Booth, Henry & Kon, 1942] and in our earlier publications [Kon & Booth, 1933; 1934 *a, b*] we are of the opinion that the customary method of measuring the vitamin D content of butter fat by feeding it to rats receiving high Ca low P rachitogenic diets leads to errors due to the antirachitic action of the fat itself. This fat effect would be especially noticeable with low potency butters. To avoid such spurious measurements we have used in our tests the non-saponifiable residue of butter. On several occasions tests on the butter itself were done at the same time.

EXPERIMENTAL

1. *Butters.* The butters were churned at approximately monthly intervals from March 1940 to March 1941, from the bulk milk of the Institute's herd which during that time consisted on the average of 6 (from 3 to 9) Guernseys and 41 (from 39 to 44) Shorthorns in various stages of lactation. The feeding and management of the cows was as described by Golding, Mackintosh & Mattick [1932], the cows were out by day throughout the year and out day and night from 26 April to 18 November 1940.

The fat was rendered at a temperature not exceeding 60° and was filtered. It was stored at 0° until required. Saponification was by the short method as described by Kon & Booth [1933]. The non-saponifiable residue was extracted 6 times with ether. After washing 4 times with water most of the ether was distilled off. A weighed amount of olive oil was then added and enough ether to give a clear solution. The ether was then removed as completely as possible by gently heating *in vacuo* and the olive oil solution was made up with olive oil to the requisite dilution. First grade olive oil which was proved to be a satisfactory solvent for vitamin D was used. The dilution was generally so adjusted that the daily dose was contained in 4 drops (77 mg.) from a standard pipette. A fresh batch was prepared every fortnight and was stored in the ice chest. The butter fat itself was fed from standardized pipettes at a temperature of about 40°.

2. *Rats.* All comparisons were on littermates. Details of technique will be found in our previous paper [Booth *et al.* 1942].

3. *Experimental technique.* Six separate prophylactic tests [Kon & Henry, 1935] were done. In each, two or three levels of the International Standard for vitamin D were fed and a group of negative controls was included. As a rule only one level of each butter or non-saponifiable residue was given. The potencies were calculated from the best fitting

line relating, in each experiment, the gain in ash content over negative controls to the logarithm of the dose of standard. When more than one level of butter was fed the values were calculated by the average slope method [Irwin, 1937].

4. *Results.* The results are given in Tables 1 and 2 and Fig. 1. It was subsequently found that the standard (VD 21) used in the third experiment had deteriorated. This involved the May, August, September and October butters. Of these only the August sample was available in quantities sufficient for a repeat test. When this was carried out

Table 1. *Assay of the antirachitic activities of butter fats and their non-saponifiable residues; the bone ash contents of the experimental rats*

	No. of animals		% bone ash	Standard error of mean
	♂	♀		
Exp. I, June 1940				
Negative controls	6	6	28.7	±0.77
Standard (v.d. 20): {0.05 i.u.	6	6	32.0	±0.45
{0.10 i.u.	6	6	37.0	±0.89
Butter 21-22 Mar. 1940: {N.S.R. 0.9 g. fat	6	6	33.3	±0.67
{0.3 g. fat	6	6	35.1	±1.34
Exp. II, Aug. 1940				
Negative controls	5	6	32.3	±0.67
Standard (v.d. 20): {0.10 i.u.	5	6	42.0	±0.86
{0.20 i.u.	5	6	46.0	±0.49
Butter 1-2 June 1940: N.S.R. 0.5 g. fat	5	6	42.3	±0.86
{N.S.R. {0.3 g. fat	6	5	45.2	±0.83
{0.6 g. fat	6	5	48.5	±0.80
Butter 7-8 July 1940: {0.15 g. fat	6	5	42.7	±0.81
{0.30 g. fat	6	5	48.2	±0.60
Exp. III, Oct. 1940				
Negative controls	6	6	31.0	±0.87
Standard* (v.d. 21): {0.075 i.u.	6	6	31.9	±0.68
{0.15 i.u.	6	6	32.7	±0.64
Butter 29 Apr.-2 May 1940: N.S.R. 0.6 g. fat	6	6	38.0	±1.08
Butter 3-4 Aug. 1940: N.S.R. 0.3 g. fat	6	6	36.7	±0.82
Butter 31 Aug.-1 Sept. 1940: N.S.R. 0.4 g. fat	6	6	47.1	±1.24
Butter 29-30 Sept. 1940: {N.S.R. 0.6 g. fat	6	6	42.0	±1.02
{0.3 g. fat	6	6	39.1	±1.31
Exp. IV, Feb. 1941				
Negative controls	6	6	30.0	±0.62
Standard (v.d. 22): {0.075 i.u.	6	6	32.7	±1.00
{0.15 i.u.	6	6	37.3	±0.65
{0.30 i.u.	6	6	44.2	±0.82
Butter 3-4 Aug. 1940: N.S.R. 0.3 g. fat	6	6	35.8	±0.81
Butter 11-12 Nov. 1940: N.S.R. 0.9 g. fat	6	6	32.7	±0.62
Butter 10-11 Dec. 1940: N.S.R. 1.0 g. fat	6	6	34.6	±0.67
Butter 8-9 Feb. 1941: N.S.R. 1.0 g. fat	6	6	34.2	±1.22
Exp. V, Apr. 1941				
Negative controls	6	6	27.5	±0.59
Standard (v.d. 23) {0.075 i.u.	6	6	30.3	±0.53
{0.15 i.u.	6	6	35.2	±0.76
{0.30 i.u.	6	6	42.8	±0.74
Butter 29-30 Dec. 1940: {N.S.R. 1.0 g. fat	6	6	29.2	±0.51
{0.3 g. fat	6	6	30.0	±0.49
Herring body oil A: {N.S.R. 3 mg. oil	6	6	36.2	±0.70
{3 mg. oil	6	6	36.7	±1.44
Exp. VI, July 1941				
Negative controls	6	6	29.1	±0.67
Standard (v.d. 23): {0.075 i.u.	6	6	33.2	±0.91
{0.15 i.u.	6	5	37.0	±0.76
Butter 8-9 Mar. 1941: N.S.R. 1.0 g. fat	5	6	33.6	±0.61

* Defective standard, cf. above.

Table 2. *Seasonal variation in the vitamin D content of butter fat measured on the fat itself or on its non-saponifiable residue*

Period of collection	i.u. vit. D/g. fat on		Ratio of activity N.S.B. : fat	Period of collection	i.u. vit. D/g. fat on		Ratio of activity N.S.B. : fat
	N.S.B.	Fat			N.S.B.	Fat	
21-22 Mar. 1940	0.07	0.29	1 : 4.1	29-30 Sept. 1940	0.35	0.50	1 : 1.4
29 Apr.-2 May 1940	0.22	—	—	11-12 Nov. 1940	0.09	—	—
1-2 June 1940	0.21	—	—	10-11 Dec. 1940	0.10	—	—
7-8 July 1940	0.55	0.86	1 : 1.6	29-30 Dec. 1940	0.07	0.25	1 : 3.6
3-4 Aug. 1940	0.38	—	—	8-9 Feb. 1941	0.09	—	—
31 Aug.-1 Sept. 1940	0.97	—	—	8-9 Mar. 1941	0.08	—	—

the gain in ash content caused by the non-saponifiable residue of this butter proved to be almost identical in the two tests. We therefore felt justified in using the line of response obtained in Exp. 4 for the evaluation of the three remaining butters assayed in the previous experiment. The butter collected at the end of August and beginning of September produced a high degree of calcification and for it the calculation involves considerable extrapolation.

Fig. 1. The seasonal variation of the vitamin D content of English butter fat in relation to change in sunshine. — Average daily hours of sunshine (Reading). - - - - i.u. vitamin D/g. fat measured on the non-saponifiable residue of butter fat. ○ i.u. vitamin D/g. fat measured on butter fat.

In the fifth experiment an examination of a herring body oil before and after saponification was included. The saponification was carried out exactly as for butter, and Table 1 shows that it caused no loss of antirachitic potency.

The vitamin D potency of the butters remained at an almost constant low level of 0.07-0.1 i.u./g. during the winter months. In spring and summer it rose steeply to a maximum of 1.0 i.u./g. at the end of August. Sunshine records were obtained for Jealotts Hill, 9 miles E.N.E. of Shinfield, and for Sutton's Seed Trial Grounds at Reading $3\frac{1}{2}$ miles N.N.E. of Shinfield. The two sets of records were similar but only those for the nearer place are quoted here (Fig. 1). On the whole there was good correlation between the vitamin D content of butter and the amount of sunshine to which the cows were exposed [Campion *et al.* 1937; Bechtel & Hoppert, 1936; Wilkinson, 1939]. In particular, August was sunnier than July and this may partly account for the very high value for the butter collected at the end of August. As mentioned above, errors of extrapolation should also be taken into account for this sample.

On all four occasions when it was fed, butter fat proved more potent than the non-saponifiable residue prepared from it. It is noteworthy that the difference between the two was not constant but that it varied according to the initial potency of the butter. Thus winter butter (March and December 1940) lost on saponification some three-quarters of its potency, whereas the corresponding loss for summer butter (July and September 1940) was only of the order of one-third; Kon & Booth [1934*a*] had already noticed a similar variation. This is certainly what one would expect in view of the fact that fat

itself is antirachitic for the rat [Booth *et al.* 1942], for with butters low in true vitamin D relatively more fat would be fed. In comparing our values with those of other authors it should be remembered that they are expressed per g. of butter fat and not of butter. On the whole, our figures for English butters are, allowing for differences in technique, of the same order as those reported for butters from other countries [e.g. Wilkinson, 1939; Bechtel & Hoppert, 1936].

SUMMARY

1. The vitamin D potencies of the fat of butters churned at approximately monthly intervals from the milk of the Shinfield herd have been measured between March 1940 and March 1941.
2. The assay was done by the prophylactic bone ash method against the international standard on groups of 11 or 12 rats, all comparisons being between littermates.
3. For the tests the butter was saponified and the non-saponifiable residue was fed to avoid the antirachitic effect of fat itself. On four occasions simultaneous determinations were also made on the untreated fat.
4. The values for the non-saponifiable residue varied from less than 0.1 i.u./g. fat in the months November-March to 0.55 i.u./g. in July and 0.97 i.u./g. in August. The latter figure may be too high.
5. The values for untreated fat were invariably higher than those measured after saponification, the difference being much more marked in winter than in summer.

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