CCXLII. VARIATIONS IN THE CAROTENE AND VITAMIN A VALUES OF THE MILK FAT (BUTTER) OF CATTLE OF TYPICAL ENGLISH BREEDS.

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EARLIER experiments have shown that the colour and vitamin A content of butter can be maintained at the high levels usually regarded as peculiarly characteristic of summer even in the winter period of stall feeding by the addition of artificially dried grass to the ration [Gillam *et al.*, 1933; Watson *et al.*, 1933]. Similar results have also been obtained with A.I.V. silage [Watson *et al.*, 1934]. As an extension of these experiments it was decided to obtain data on the variation of the carotene and vitamin A contents with season, breed and stage of lactation of the milk fat of cows kept under typical English conditions of feeding and management. Studies of some of these variations in the milk of English cows have previously been made using either colorimetric or biological methods of assay [Dann, 1933; Booth *et al.*, 1933; 1934], but at the time this investigation was commenced, no comprehensive study of the variations in the carotene and vitamin A contents of milk, using the more precise spectrophotometric methods of assay, had been made. Since then, however, thorough investigations of the fat of milk produced under American conditions of management have been carried out [Baumann *et al.*, 1934], using the spectrophotometric method developed earlier [Baumann & Steenbock, 1933].

Details of experiment.

For the experiment, two cows were selected from each of the main dairy breeds—Shorthorn, Ayrshire, Friesian and Guernsey. All the cows calved during October or November 1934, and seven of them were part of a large herd, receiving therefore uniform management and feeding. The remaining cow, an Ayrshire—Heather Lass—belonged to a neighbouring herd in which “outwintering” of cattle is the usual practice.

The particulars concerning the cows were as follows:

Breed	Name	No. of calves previous to experiment	Calving date
Shorthorn	Annette	3	31. x. 34
	Cherry	1	13. x. 34
Ayrshire	Ninna	7	22. xi. 34
	Heather Lass	4	28. xi. 34
Friesian	Dairymaid	4	20. x. 34
	Pearl	3	3. x. 34
Guernsey	Eva	2	6. x. 34
	Nellie	2	28. x. 34

During October, November and December, the cows received a winter ration of hay, kale and concentrates, the kale being fed at the rate of 30–40 lb. per day. From January 1935 to the end of April the kale was replaced by mangolds. The cows were turned out in April, but owing to a wet cold spell, there was very little available grass. During May and June, the cows had access to a plentiful supply of good quality pasture. In July, as a result of a drought, the quality and quantity of the grass fell off rapidly, and after the second week the fields were bare and burnt, and supplementary feeding with hay and concentrates was necessary.

Collection of butter samples. Immediately following calving, the colostrum was collected, as completely as was possible practically, during the first 2–3 days. With some difficulty, butter was prepared from the material after it had settled for a few days. At monthly intervals thereafter, the total milk from an afternoon and following morning's milking was collected, separated, allowed to "ripen" and then churned. The butter was then immediately clarified and the butter fat stored in a refrigerator until the necessary determinations could be carried out. In the later stages of lactation, it was necessary to collect two days' supply of milk in order to obtain sufficient for the making of the butter.

Determination of carotene and vitamin A in the butters. Samples (20–25 g.) of the dry filtered butter fats were saponified and the carotene and vitamin A determined in the unsaponifiable matter by absorption spectra methods, following the technique previously described [Gillam, 1934]. The gross absorption of the unsaponifiable matter in chloroform at 455–460 $m\mu$, less a constant 6% correction for xanthophyll, was converted into absolute carotene values by comparison with the standard absorption of pure β -carotene, $E_{1\text{ cm.}}^{1\%}$ 463 $m\mu$ (chloroform) = 2200 [Gillam, 1935]. The absorption at 328 $m\mu$ (Hilger E_3 quartz spectrograph), after correction for that due to carotenoids, was similarly converted into percentages of vitamin A by making use of the value of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ = 1600 for the purest vitamin A distillates [Carr & Jewell, 1933]. The values so obtained are relative and, provided that vitamin A is not concentrated further, are absolute also. (Karrer & Morf [1933] have reported a value of $E_{1\text{ cm.}}^{1\%}$ = 1700.)

Discussion of results.

The results of the examination of the butters of each cow throughout the whole period of lactation are shown graphically in Figs. 1 and 2. In considering the data, it must be emphasized that only two cows of each breed were used, and these exhibit marked individual variations, particularly in regard to carotene content, the vitamin A values showing closer agreement. As a result, the variations in values in the different breeds are only significant when the differences are very marked. This is shown clearly in contrasting the Shorthorn and Ayrshire groups. In a previous experiment [Watson *et al.*, 1934] the relative positions of these two breeds were the inverse of those of the present investigation. These variations, however, do not affect the general trend of the results. It will be seen that the butters show the normal decrease in their vitamin A and carotene values as the winter proceeds, reaching a minimum about the end of March [Gillam *et al.*, 1933]. With the advent of spring and fresh grass, the values rise sharply and continue until they reach the high values characteristic of summer butters. An interesting example of this well-known dependence of the quality of the butter on the supply of carotene-rich food is shown in the butters from the Ayrshire cow, Heather Lass. Whilst all the other cows in the experiment were stall-fed on similar rations, this cow was "outwintered", i.e. allowed access to pasture

throughout the winter. The results are shown in the higher carotene values of the resulting butter and more particularly in the vitamin A values, which

Fig. 1. Variation in the carotene content of butter fat during lactation of the cow. Oct. to March, stall-feeding. April, grazing but grass scarce. May and June, grass good and plentiful. July and Aug., drought; grass scarce and poor.

(All cows calved one month pr or to date of first sample.)

are consistently much higher than those of the other Ayrshire cow while this was stall-fed (Fig. 2).

Fig. 2. The variation of the vitamin A content of butter fat during lactation of the cow. Oct. to March, stall-feeding. April, grazing but grass scarce. May and June, grass good and plentiful. July and Aug., drought; grass scarce and poor.

(All cows calved one month prior to date of first sample.)

Average values for typical English milks.

The carotene and vitamin A values of the milk fats from the individual cows have been collected and summarized in Table I together with the average values for each breed.

Table I. *Summarized values for all four breeds.*

Breed		Gross average value	Winter average	Summer average	Minimum values	Maximum values	Vitamin A/carotene ratio
Shorthorn	Carotene	0.27	0.25	0.29	0.09	0.54	2.5 : 1
	Vitamin A	0.68	0.58	0.84	0.36	1.24	
Ayrshire	Carotene	0.36	0.27	0.41	0.11	0.69	2.4 : 1
	Vitamin A	0.85	0.66	1.18	0.30	1.42	
Friesian	Carotene	0.40	0.35	0.47	0.11	0.85	2.25 : 1
	Vitamin A	0.90	0.61	1.21	0.52	1.50	
Guernsey	Carotene	0.92	0.73	1.14	0.42	1.97	0.80 : 1
	Vitamin A	0.75	0.54	0.95	0.37	1.42	

NOTE 1. All values are expressed in mg./100 g. fat. (For bases of calculation, cf. p. 1729.)

NOTE 2. The samples were divided into winter and summer samples arbitrarily, October-March being called "winter" and April-September being called "summer".

The Ayrshire values are a little higher than would otherwise be the case owing to the fact that one cow—Heather Lass—had access to fresh winter grass.

The Shorthorn values are lower than were expected from previous work. This is shown by the figures given later in this paper (Table IV) for larger groups of Shorthorn cows taken at different stages of lactation. The average values here are 0.50 mg./100 g. of carotene and 1.01 mg./100 g. of vitamin A. These values are not directly comparable with the values in Table I which include a large number of winter samples. On account of the fact that the gross growth-promoting activity of the butter fats is due to two different entities (vitamin A and carotene), the relative amounts of which vary both from breed to breed and, to a less extent, from cow to cow, a better appreciation of the relative values of the fats is obtained by expressing the total vitamin A activity in terms of biological units calculated from the known amounts of carotene and vitamin A present. The results are expressed arbitrarily in β -carotene units (γ per g.) on the basis of equal potencies for pure vitamin A and pure β -carotene [Moore, 1933]. The resulting values have the advantage of presenting a relative set of figures on a uniform basis for the four breeds; it is not claimed, however, that the same values would be found by actual biological test.

Table II. *Calculated growth-promoting activities of the butter fats in γ per g. (β -carotene units).*

Breed	Gross average	Summer average	Winter average	Maximum	Minimum
Shorthorn	9.5	11.3	8.3	17.8	4.7
Ayrshire	12.1	15.9	9.3	20.8	4.1
Friesian	13.0	16.8	9.6	23.2	6.9
Guernsey	16.7	20.9	12.7	31.7	8.8

These figures make the Guernsey appear the best of the four breeds as far as the gross content of vitamin A-active substances in the milk fat is concerned, a result which is not consistent with the finding that Shorthorn and Guernsey butters have approximately identical biological activities throughout the year [Booth *et al.*, 1934]. In this connection, it has recently been shown that pure

carotene fed in vitamin A-free butter fat is apparently 2-3 times less effective than when fed in cottonseed oil [Kraybill & Shrewsbury, 1936], a result which may explain the observation that the vitamin A of butter is several times more effective biologically than the carotene [Booth *et al.*, 1934]. Thus it would seem that carotene is not utilized at all efficiently from butter fat by the rat, and this would tend to lower the apparent gross vitamin A activity (as determined biologically) of Guernsey butters, which owe more of their activity to carotene than to vitamin A. Thus, though Guernsey butters commonly contain more of the vitamin A-active substances than butters of other breeds, the question whether the human organism, unlike that of the rat, can absorb the carotene as effectively as the vitamin A must still remain open.

Colostrum.

The carotene and vitamin A values of the colostrum milk (bulk samples collected during the first two days after calving) are markedly greater than those of normal milks, thus serving the purpose of providing the calf with a highly concentrated food in the first few days of life. The data on colostrum compared with milks from the same cows a month after calving are shown in Table III. The colostrum values, having been obtained on samples collected over two days, are not as high as those obtained on the first day's colostrum only.

Table III. *Carotene and vitamin A of colostrum.*

Date	Cow	Breed	Carotene mg./100 g. fat	Vitamin A mg./100 g. fat
Oct. 20	Dairymaid	Friesian	3.40	1.24
Nov. 20	"	"	0.38	0.52
Nov. 22	Ninna	Ayrshire	4.60	2.96
Dec. 22	"	"	0.18	0.37
Nov. 30	Heather Lass	"	4.21	3.13
Dec. 30	"	"	0.35	0.44
Oct. 28	Nellie	Guernsey	3.61	1.13
Nov. 28	"	"	0.91	0.67
Oct. 13	Cherry	Shorthorn	2.90	3.51
Nov. 13	"	"	0.28	0.48
Oct. 31	Annette	"	3.80	1.37
Nov. 30	"	"	0.35	0.68

The high vitamin A value of cow's colostrum has previously been demonstrated biologically by Dann [1933] in England, and spectroscopically by Semb *et al.* [1934] in America. The latter authors have shown that the high potency of the colostrum milk persists for only a few days after the calf is born before dropping to a more normal value, a fact which is fully confirmed by the present results.

End of lactation effect.

The graphs (Figs. 1 and 2) show the variation in carotene and vitamin A content of the fat of each of the cows in the experiment throughout the whole of lactation. The marked decrease in these values towards the end of lactation, which coincided with the known scarcity of grass occasioned by the drought of July-August 1934, made it necessary to carry out experiments to decide whether the drop in quality of the milk was in fact due to a low-carotene diet, or was a genuine end of lactation effect. In order to decide between these two alternatives, bulked fat from 5 groups of 6 cows, all on the same diet, and at the same season

but at different stages of lactation, was examined in the early winter of 1935. The following results were obtained, and show clearly that there is no obvious drop in the carotene and vitamin A of milk fat as the cow nears the end of lactation. This is in agreement with the results obtained by Treichler *et al.* [1935].

Table IV. *Carotene and vitamin A values of milk fat of Shorthorn cows at different stages of lactation.*

Sample	Weeks after calving	Date	Carotene mg./100 g. fat	Vitamin A mg./100 g. fat
A	10	27. xi. 35	0.46	1.10
D	15	28. xi. 35	0.45	0.95
B	19-23	29. xi. 35	0.58	1.06
E	29	20. xi. 35	0.54	0.93
C	30	29. xi. 35	0.46	1.03
Averages			0.50	1.01

SUMMARY.

The carotene and vitamin A values of the milk fats (butter) of each of eight cows have been determined spectrophotometrically at monthly intervals from the day of calving to the end of lactation. Four groups of two cows were used, each group representing a typical English breed (Shorthorn, Ayrshire, Friesian and Guernsey), and all were treated alike, being stall-fed during the winter and pastured in spring and summer under typical English conditions of management. The results show that individual variations among cows of the same breed are large but that, apart from the abnormally high values of colostrum, the carotene and vitamin A values of the butters are much more dependent on diet than on stage of lactation. Maximum, minimum and mean values for each breed are recorded.

Comparison of the summations of carotene plus vitamin A values places the breeds, in order of vitamin A activity of the butters, Guernsey > Friesian > Ayrshire > Shorthorn. The results of this and previous work indicate that the differences between butters of the last three breeds are scarcely significant.

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