Seasonal Variation of Carotene and Vitamin A in Butter-fat and in Serum

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During the past few years, numerous papers have appeared dealing with the seasonal variation of either the vitamin A activity of butter or the carotene and vitamin A content of blood serum. For instance, Koehn (1939, 1941) showed that the activity of butter-fat followed a definite cycle, decreasing steadily from August to December, and then increasing threefold between January and June. Sen & Sarkar (1942), feeding carotene at the level of 870,000 i.u./day, obtained a butter-fat of potency 21.6 i.u./g. in January. When the animals were transferred to a diet low in carotene, the carotene of the butter-fat dropped at once and the vitamin A *per se* decreased more slowly. An interesting observation made by these workers was that the contribution of the carotene, which was normally about 27% of the total vitamin A activity of the fat, could not be increased above 35% even by feeding green food. Berl & Peterson (1943) found potencies of 22 and 40 i.u./g. of fat in January and July–September respectively.

Most of the data on serum vitamin A are concerned with young or new-born calves, in which the serum vitamin A is very low (Moore, 1942). Early work by Gillam & El Ridi (1935) showed a marked seasonal variation in serum carotene and vitamin A of cows, winter figures being 0.4 mg. and 400 i.u./100 ml. for carotene and vitamin A respectively, and the corresponding summer figures being 1.10 mg. and 900 i.u./100 ml. A recent paper by Braun (1945) gives for pasture-fed cows carotene values which decrease steadily from 1.35 mg./100 ml. in March to 0.3 mg. or less in September, followed by an increase to 1.0 mg. between September and January.

It is becoming apparent that of the ways of assessing vitamin A deficiency, the measurement of serum carotene and vitamin A is the most sensitive. Lewis, Bodansky, Falk & McGuire (1942), using rats as experimental animals, have shown that an intake of only 2 i.u./day is sufficient to maintain normal dark adaptation, and 25 i.u./day will support normal growth, but to maintain serum level of 100–112 i.u./100 ml., which is considered compatible with high liver storage and satisfactory vitamin A nutrition, 50 i.u./day must be supplied.

The results obtained in the present work are reported partly because there does not appear to be

any paper giving both butter-fat and serum results for the same cows at about the same time, partly because of the increasing importance of serum measurements, and partly because the serum values obtained, while agreeing qualitatively with those of Braun (1945), show a different seasonal distribution.

EXPERIMENTAL

Animals and feeding practice

The herd consisted of 70 pure-bred Ayrshire cattle at the farm of J. Bibby and Sons, Ltd., at Puddington, Cheshire. The animals were 4–5 years old and at various stages of lactation. From April until the end of October, they were grazing on permanent pasture of average quality. From November until the middle of December, the animals were out at pasture during the day only, and afterwards they were in stalls continuously until the following April. Stall-feeding consisted of kale, hay and roots with concentrates to maintain milk yield.

The butter samples, prepared from milk from the entire herd, were kindly supplied by Miss R. A. Spence. The blood samples were drawn by the staff of the University of Liverpool Veterinary Field Station, Willaston, by the courtesy of Prof. Wright. They were taken at about monthly intervals from ten cows at random; 25 or 50 ml. of the pooled serum were treated as described below.

Methods

Butter samples of known moisture content were treated using method B described by Morton, Lord & Goodwin (1941). Results were calculated in i.u./g. of fat for both carotene and vitamin A, and the total vitamin A activity was computed as the sum of these amounts.

The samples of serum were treated by the method of Yudkin (1941), followed by saponification and separation of the carotene by passing over a bone-meal column as described by Mann (1943). After removal of the carotene, the vitamin A was eluted using a mixture of equal volumes of light petroleum (40–60°) and acetone, and finally acetone alone. The results are calculated in mg./100 ml. for the carotene, and in i.u./100 ml. for the vitamin A.

RESULTS AND DISCUSSION

Butter-fat

The results (Table 1 and Fig. 1) represent vitamin A activity of butter samples examined between August 1941 and August 1943. During the first period of

Table 1. *Seasonal variation of vitamin A activity of butter-fat*

	I.u./g. of fat					
	1941-2			1942-3		
	Carotene	Vitamin A	Total	Carotene	Vitamin A	Total
August	7.7	27.2	34.9	8.6	23.7	32.3
September	7.5	22.4	29.9	7.8	22.4	30.2
October	6.7	24.2	30.9	8.2	23.3	31.5
November	—	—	—	9.2	24.1	33.3
December	6.5	24.3	30.8	6.2	15.6	21.8
Stall-feeding started						
January	5.5	16.4	21.9	3.7	11.4	15.1
February	3.8	13.4	17.2	2.8	8.7	11.5
March	2.5	11.8	14.3	2.4	12.0	14.4
April	2.7	11.7	14.4	2.7	10.7	13.4
Pasture-feeding started						
May	7.3	18.4	25.7	12.5	24.6	37.1
June	10.0	20.4	30.4	12.4	17.9	30.3
July	9.1	21.2	30.3	9.2	19.2	28.4

12 months, samples were examined at weekly intervals, and the average for each month is presented in the table; during the second period, samples were examined at intervals not exceeding 1 month.

Fig. 1. The seasonal variation of carotene, vitamin A and computed total vitamin A activity of butter-fat.

The results in Table 1 show clearly the effect of pasture on the vitamin A potency of butter-fat and emphasize the cyclic nature of the variations mentioned by Koehn (1941). The steady decrease found by him between August and December was not observed. It would appear that so long as the cattle had access to pasture, even if for only a few hours a day, the potency of the butter was maintained. As soon as the cows were placed on a diet low in carotene, there was a marked decrease in the vitamin A activity of the butter, the potency in winter being only about half that in summer. This relative potency agrees with the findings of Berl &

Peterson (1943), who obtained values of 22 and 40 i.u./g. of fat in winter and summer respectively. It is interesting to note that the carotene figures vary much more widely than do those for vitamin A *per se*. The carotene increases 4–5 times while the vitamin A is scarcely doubled. This observation agrees with those of Baumann & Steenbock (1933) and Sen & Sarkar (1943). It may be pointed out that the actual carotene figures, calculated as 94 % of the total yellow colour (Gillam, 1934) may be somewhat high. Koehn (1939) claims that only 91 % of the total colour is carotene, and other workers, for instance Zscheile, Nash, Henry & Green (1944) and Berl & Peterson (1943), have found only 75–80 % carotene in the total butter-fat carotenoids. Using the figures as they are, the contribution made by the carotene to the total vitamin A activity is 22–28 % during most of the year. This amount fell to 20 % or less during stall-feeding, and rose to the maximum of 35 % mentioned by Sen & Sarkar (1942) only during the first period of pasture-feeding in May and June.

Serum

The carotene and vitamin A content of samples of blood serum collected at approximately monthly intervals during 1944 and early 1945 are shown in Table 2. Carotene values are expressed as mg./100 ml. and vitamin A values as i.u./100 ml.

The maximum and minimum values both of carotene and of vitamin A agree well with those of Braun (1945), who obtained 1.35 and 0.3 mg./100 ml. for carotene and >100 and 40 i.u./100 ml. for vitamin A. The results show no decrease while the cows have access to pasture, and in this respect, they differ from those of Braun who found a steady decrease from March to October. It would appear, therefore, that access to pasture even during limited

Table 2. *Carotene and vitamin A values in blood serum*

	Carotene (mg./100 ml.)	Vitamin A (i.u./100 ml.)
August	1.12	117
September	1.68	235
October	1.14	159
November	1.20	106
December (beginning)	1.44	173
Stall-feeding started		
December (end)	0.38	60
February	0.26	25
March	0.14	None found
April (beginning)	0.13	40
Pasture-feeding started		
April (end)	0.84	62
May	0.78	65
June	1.52	120

hours is enough to ensure adequate vitamin A nutrition as measured by the carotene and vitamin A of both serum and butter-fat. It has been pointed out that the seasonal variation of carotene in butter-fat is more marked than that of vitamin A *per se*. Examination of the serum results reveals that this effect is by no means so marked as with butter-fat. The average carotene content of serum from August to December is 1.32 mg./100 ml., and the corresponding vitamin A value is 158 i.u./100 ml. The corresponding averages for the winter months January to April are 0.23 mg. and 31 i.u. respectively. (This last average includes the possibly anomalous vitamin A result found in March.) In each case there is a 5-6-fold decrease during the winter months. When the cows were again put on pasture at the end of April the serum carotene increased at a greater rate than the vitamin A. This might mean that the liver reserves of vitamin A, depleted during stall-feeding, are first built up before the serum vitamin A increases again. If this

is so, the results would seem to support the view of Boyer, Phillips, Lundquist, Jensen & Rupel (1942) who, from experiments on calves, suggested that measurement of serum vitamin A *per se* was a more sensitive criterion of vitamin A nutrition than was serum carotene. Finally, it may be mentioned that partly because of the small number of results available, and partly because they fall into two distinct groups instead of following a regular gradation, it is not possible to come to any conclusion about the use of the vitamin A/carotene ratio suggested by Braun as a means of assessing vitamin A nutrition.

SUMMARY

1. Data are presented showing the seasonal variation over a period of 2 years of the vitamin A activity of butter-fat of Ayrshire cows. The average values for pasture- and stall-feeding were respectively 31 i.u. and 15 i.u./g. of fat.

2. The contribution made by carotene to the vitamin A activity of butter-fat ranged from 28% during pasture-feeding to 20% or less while the animals were in stalls. Carotene made its maximum contribution during May and June, accounting for 34% of the total vitamin A activity.

3. Serum carotene and vitamin A values during pasture-feeding were respectively 1.35 mg. and 150 i.u./100 ml. The corresponding values for stall-feeding were 0.23 mg. and 31 i.u./100 ml.

4. The results indicate that pasture-feeding even for only a few hours a day maintains high levels of carotene and vitamin A in both butter-fat and serum.

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