CXIV. VARIATIONS IN THE QUALITY OF BUTTER, PARTICULARLY IN RELATION TO THE VITAMIN A, CAROTENE AND XANTHOPHYLL CONTENT AS INFLUENCED BY FEEDING ARTIFICIALLY DRIED GRASS TO STALL-FED CATTLE.

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THE natural diet of herbivora includes no vitamin A, but the carotene of green plants is transformed *in vivo* into the vitamin to an extent sufficient to provide for the needs of the animal. Milk from grazing cattle yields butter containing both vitamin A and carotene, the latter (with a small amount of xanthophyll) being responsible for the yellow colour. In the process of converting grass into hay for winter feeding, much of the carotene disappears, and in consequence winter butter is normally much paler than summer butter. A corresponding decrease in vitamin A content need not necessarily occur, since the lactating animal may utilise some of the vitamin A stored in the liver. It is important, therefore, that the vitamin A and carotene present in butter should be ascertained separately. Biological assay indicates the total growth-promoting effect, but this will be a summation of the contributions due to vitamin A and that fraction of the carotene which is transformed into the vitamin in the rat.

The spectroscopic method described by Morton and Heilbron [1930], whilst not differentiating between carotene and xanthophyll, provides a rapid means of measuring vitamin A content as well as total carotenoid content. By employing the phase separation between aqueous methyl alcohol and light petroleum [Kraus, 1872; Borodin, 1883; Willstätter and Stoll, 1913] the quantities of carotene, xanthophyll and vitamin A can now be determined separately, thus constituting a refinement of the earlier procedure. The method evaluates the carotene content irrespective of the efficiency with which the hydrocarbon can be utilised.

The scheme of investigation of which this work forms part involves a study of the variations in the quality of milk in relation to diet, which in turn involves the seasonal variations which occur in normal dairy practice. A general report on the organisation of the experiment and its broader implications will appear elsewhere [Watson, Drummond, Heilbron and Morton, 1933].

Four groups of cows were employed.

Group	Ration
1	Normal winter diet of hay, roots and purchased concentrates
2	Normal winter diet, hay replaced by medium quality grass silage
3	Normal winter diet, 75 % of concentrates replaced by artificially dried nitrogen-fertilised grass
4	As for 3 except that the grass had received no nitrogenous fertiliser dressing

Representative samples of milk were then taken at certain specified periods and butter made therefrom for the carotene-vitamin A analyses.

The treatment of the butters.

A 250 g. portion of each butter (the moisture content having been determined) was saponified for half an hour with a slight excess of 20 % alcoholic KOH. The product was poured into three times its volume of water and extracted with ether until the extract was colourless. The bulked extract was washed with a little water without shaking and then separated and washed with several portions of water, with shaking. The whole of the washed ether extract was reduced to small volume (about 10 cc.) in an atmosphere of nitrogen. The carotene and xanthophyll were then separated by the phase method as follows. 20 cc. of low-boiling light petroleum were added and the xanthophyll extracted from this solution by ten successive treatments with 85 % methyl alcohol, 300–350 cc. being used in all. The light petroleum solution, containing all the carotene, was washed with water, reduced to small bulk in an atmosphere of nitrogen and the concentrated solution transferred completely to an ampoule, where moisture and the remainder of the solvent were removed *in vacuo*, the residue being finally sealed off in nitrogen. The xanthophyll fraction in the combined methyl alcohol extracts was diluted with water and extracted with ether until free from yellow colour. The ether extracts were again mixed, washed and concentrated, transferred to an ampoule, evaporated and the residue sealed off in nitrogen as before. Thus two ampoules were available, the contents of which represented the whole of the carotene and xanthophyll respectively from 250 g. of fresh butter, the vitamin A being distributed between the two. By using ampoules of brown glass and sealing off in nitrogen, safe storage was ensured until the samples were required for analysis.

Before proceeding to report on the amounts of carotene, xanthophyll and vitamin A in the fractionated unsaponifiable matter of the butters it is necessary to refer to the standard spectroscopic data on the pure substances.

It has been established [Karrer, Morf and Schöpp, 1931; Heilbron *et al.*, 1932] that vitamin A is a colourless unsaturated alcohol characterised by a broad absorption band, free from fine structure, extending over the region 280–370 $m\mu$ approximately, with a maximum near 328 $m\mu$ in alcohol at an intensity $E_{1\text{ cm.}}^{1\%} = 1600$ [Carr and Jewell, 1933] (the molecular extinction coefficient calculated on $\text{C}_{20}\text{H}_{30}\text{O}/\sqrt{5}$ is probably close to 46,000 for alcoholic solutions). In any medium not exhibiting appreciable absorptive power at 328 $m\mu$, it is easy to determine quantitatively the proportion of vitamin A in solution by measuring the extinction at 328 $m\mu$ for a given solution and cell thickness. Vitamin A also gives rise to a blue solution with antimony trichloride in chloroform, characterised by a sharp absorption maximum at 617 $m\mu$ and a subsidiary band at 583 $m\mu$. For the apparently pure vitamin these bands have intensities which can be expressed as $E_{1\text{ cm.}}^{1\%} 617 m\mu = 5000$ and $E_{1\text{ cm.}}^{1\%} 583 m\mu = 2600$, the notation $E_{1\text{ cm.}}^{1\%}$ referring to $E = \log I_0/I$ (I_0 = intensity of incident light, I = intensity of

emergent light) for a solution containing 1 % of material in almost saturated antimony trichloride in chloroform and viewed through a 1 cm. cell.

Carotene in chloroform exhibits absorption maxima at 495, 463, 436, 348 and $280m\mu$. The intensity of absorption at the head of the main band in the visible $E_{1\text{ cm.}}^{1\%}$ $463m\mu = 1900$ ($e = 102,000$) [Dulière, Morton and Drummond, 1929]. With antimony trichloride, carotene gives rise to a blue solution with a clearly defined absorption band exhibiting its maximum at $585\text{--}590m\mu$ with an intensity of absorption $E_{1\text{ cm.}}^{1\%}$ $585m\mu = 420$ [Gillam *et al.*, 1932].

A specimen of xanthophyll (m.p. 193°), kindly supplied by Prof. Karrer, and probably consisting largely of "lutein" [Kuhn *et al.*, 1931], was found to give rise to the following absorption maxima: 485, 455, 425, 405, 340 and $272m\mu$ in chloroform. The intensity of absorption of this sample at the head of the main band in the visible $E_{1\text{ cm.}}^{1\%}$ $455m\mu$ is 1570 in chloroform solution. The nature of the solvent is important in this connection, as in alcohol-ether and in light petroleum the value is very much larger [McNicholas, 1931]. With the antimony trichloride reagent, xanthophyll gives a blue solution exhibiting absorption bands at 617 and $585m\mu$, superimposed on general absorption. The respective intensities can be expressed as $E_{1\text{ cm.}}^{1\%}$ $617m\mu = 250$ and $585m\mu = 192$ (for spectroscopic measurements on a number of carotenoids, *cf.* Euler *et al.* [1932]). The data on the pure substances are summarised in Table I.

Table I.

	Carotene	Xanthophyll	Vitamin A
Wave-length maxima (chloroform solutions)	495, 463, 436, 348, $280m\mu$	485, 455, 425, 405, 340, $272m\mu$	328–330 $m\mu$
Intensity of absorption $E_{1\text{ cm.}}^{1\%}$ (chloroform)	At $463m\mu = 1900$	At $455m\mu = 1570$	At $328m\mu = 1600$
Absorption bands with antimony trichloride	$585\text{--}590m\mu$	617 and $585m\mu$	617 and $583m\mu$
Intensity of absorption in the blue solutions with antimony chloride $E_{1\text{ cm.}}^{1\%}$	At $587m\mu = 420$	At $617m\mu = 250$ At $585m\mu = 192$	At $617m\mu = 5000$ At $583m\mu = 2600$

The absorption spectra measurements in the visible and ultra-violet have been carried out photographically on two Hilger E 3 quartz spectrographs, one being used with a short-focus rotating-sector photometer with a tungsten underwater spark as light source, and the other with an iron-nickel arc in air and a long-focus sector photometer. The absorption spectra determinations on the blue solution with antimony trichloride were all made by means of a Hilger-Nutting visual spectrophotometer, using the technique already described [Heilbron, Gillam and Morton, 1931].

The analytical problem therefore resolved itself into (a) the determination of carotene in a material substantially free from other coloured substances, (b) the determination of xanthophyll in a material similarly free from extraneous colouring matter, and (c) the determination of vitamin A in the presence of carotene in one fraction, and of xanthophyll in the other fraction. Direct determinations of the intensities of the absorption bands characteristic of carotene and xanthophyll respectively in comparison with standard data on the pure substances afford a satisfactory means of evaluation. Vitamin A can be detected in the presence of carotenoids by decolorising the solution with the minimum quantity of adsorbent carbon, after which the $328m\mu$ absorption band or the colour reaction with antimony trichloride serves to identify it. There is no certainty, however, that the vitamin will be wholly unadsorbed. If the

carotene or xanthophyll content of a given material is already known (from the direct absorption spectrum in the visible) it is easy to calculate the intensity of absorption at $328m\mu$ arising from a stated weight of the carotenoid by using the known absorption curves for the pure substances in the ultra-violet (Fig. 1).

Fig. 1.

— Carotene in chloroform, 0.00078 % 1 cm. cell.
 - - - Xanthophyll in chloroform, 0.0042 % 2 mm. cell.

By subtracting the calculated contribution of the carotenoid at $328m\mu$ from the observed intensity at this wave-length, a reasonably accurate figure for the absorption due to vitamin A is obtained. The total vitamin A content of the butter is then obtained by adding together the observed differences in the carotene and xanthophyll fractions. In actual practice the contribution of the carotenoid to the gross absorption about $328m\mu$ rarely exceeds 20 % in the carotene fractions, and in the xanthophyll fractions it is very much smaller.

The intensity of the blue colour developed on the addition of antimony trichloride to each of the fractions is measured at 617 and $585m\mu$, the value at the latter wave-length as shown below being particularly useful. The spectroscopic blue value (at $585m\mu$) due to carotene or xanthophyll can be calculated from the known carotenoid content as determined from the direct absorption spectrum, so that the difference between this calculated blue value and the observed one gives the blue intensity due to vitamin A. Two quantitative criteria for vitamin A, expressed in intensities of absorption, are thus obtained and it is necessary to calculate the amount of vitamin with which they correspond.

The ultra-violet method of assay is probably the most trustworthy for the determination of vitamin A, since the intensity of absorption is directly proportional to the amount of vitamin. On the basis of the spectroscopic data already given for pure vitamin A, the data have been calculated to percentages of vitamin. These are tabulated as the final values for the vitamin A content of the butters, but may need a slight constant correction throughout if and when vitamin A is isolated in a purer state.

The values obtained by the spectroscopic blue test provide approximate confirmation of the findings based on the ultra-violet method of measurement. When the antimony trichloride test is applied to liver oils (*i.e.* sources of

vitamin A free from carotene and xanthophyll) the ratios of blue to ultra-violet values are not constant over a reasonable range or among vitamin A-bearing oils from various sources. This lack of constancy is due mainly to the presence of substances which inhibit the formation of the blue colour. Norris and Church [1930] have shown that the blue colour is not strictly proportional to the vitamin A potency in liver oils, and in fact that the addition of certain substances (such as oleic acid) inhibits its formation quite appreciably. If inhibiting substances are present in liver oils they appear to be removed, at least partially, by saponification, since Smith and Hazley [1930] have shown that after saponification there is a considerable increase in the recorded blue value. That the ratio of blue colour value to ultra-violet value is not constant even after removal of the saponifiable matter is shown by the increase in this ratio as the vitamin A is concentrated in the unsaponifiable matter by various treatments [Heilbron *et al.*, 1932; Heilbron, Gillam and Morton, 1931; Gillam and Morton, 1931]. Both the 617 and the 583 $m\mu$ bands are inhibited but the latter band is affected much less than the former [Emmerie *et al.*, 1931; Eekelen *et al.*, 1931; Morton, 1932]. For this reason and also because vitamin A, carotene and xanthophyll all show absorption maxima near 585 $m\mu$, the intensity measurements at this wave-length are to be considered more trustworthy than those at 617 $m\mu$ in the case of the butter samples.

Table II.

Sample dated	The vitamin A value in terms of the ultra-violet absorption expressed as $E_{1\text{ cm.}}^{1\% \text{ fresh butter}}$ at 328 $m\mu$				Spectroscopic blue value due to vitamin A expressed as $E_{1\text{ cm.}}^{1\% \text{ fresh butter}}$ at 585 $m\mu$			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
9. xii. 31	0.0086	0.0098	—	—	0.0105	0.0113	—	—
16. xii. 31	0.00745	0.00504	0.00697	0.0063	0.0072	0.00579	0.00874	0.009
12. i. 32	0.00545	0.00838	0.0110	0.0103	0.00791	0.0125	0.0162	0.0167
26. i. 32	0.00554	0.00725	0.0119	0.0093	0.0071	0.0091	0.0202	0.0146
9. ii. 32	0.00522	0.00415	0.0090	0.00637	0.00683	0.00577	0.0168	0.0117
26. ii. 32	0.00373	0.00243	0.00823	0.00658	0.00613	0.00323	0.0177	0.0121
16. iii. 32	0.00263	0.00314	0.00668	0.0067	0.0043	0.00395	0.0118	0.01033
6. iv. 32	0.00339	0.00303	0.00559	0.0040	0.00434	0.00421	0.0080	0.00534
19. iv. 32	0.00338	0.00224	0.00402	0.0043	0.00568	0.00322	0.00573	0.0086
10. v. 32	0.0093	—	0.0115	—	0.0153	—	0.0187	—
23. v. 32	0.0105	—	0.0113	—	0.0162	—	0.0205	—

Table II summarises the observed values of $E_{1\text{ cm.}}^{1\% \text{ fresh butter}}$ (CHCl_3) for vitamin A by both the ultra-violet absorption and the 585 $m\mu$ band in the colour test. The data show a general parallelism between blue and ultra-violet values. In the complete absence of inhibitors the ratio of ultra-violet values to 585 $m\mu$ blue values should be 1600:2600 (*i.e.* the relative intensities of the two bands in the purest vitamin A preparations).

Tables III, IV, V, and VI show all the values calculated for carotene, xanthophyll and vitamin A for each of the butters, expressed as mg. of substance per 100 g. of dry butter. The difference between the butters of the various groups of cows will be better appreciated from the graphs (Figs. 2, 3, 4, and 5).

The statement of Carr and Jewell [1933] that carotene cures xerophthalmia and supports growth in rats at the level of 0.001 mg. per rat per day, whereas their richest vitamin A concentrates ($E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ = 1600) are 1.66¹ times as

¹ This is a conservative estimate of the superiority of vitamin A over carotene and has been used here pending agreement as to the relative growth-promoting effects of pure carotene and pure vitamin A.

Table III. *Group 1 butters.*

Cows receiving normal winter rations of roots, hay and concentrates.

Samples dated	Water %	Carotene in mg./100 g. on dry butter (1900 basis)	Xanthophyll in mg./100 g. on dry butter (1570 basis)	Vitamin A in mg./100 g. on dry butter (1600 basis)	Estimated growth-promoting activity in international units per g. (dry)
26. xi. 31	15.7	0.613	0.72	0.637	16.7
16. xii. 31	15.1	0.505	0.0180	0.549	14.2
13. i. 32	18.0	0.237	0.0124	0.416	9.3
26. i. 32	21.0	0.167	0.0105	0.438	9.0
9. ii. 32	15.0	0.137	0.0097	0.384	7.77
1. iii. 32	15.3	0.070	0.0127	0.276	5.3
16. iii. 32	24.7	0.0828	0.0106	0.218	4.46
6. iv. 32	18.14	0.0674	0.00824	0.259	5.0
19. iv. 32	17.5	0.0606	0.0069	0.256	4.88
10. v. 32	14.9	0.515	0.0360	0.683	16.5
23. v. 32	14.7	0.567	0.0469	0.770	18.5

Table IV. *Group 2 butters.*

Cows receiving normal winter rations of hay, roots and concentrates, except that the major part of the hay was replaced by grass silage.

Sample dated	Water %	Carotene in mg./100 g. on dry butter (1900 basis)	Xanthophyll in mg./100 g. on dry butter (1570 basis)	Vitamin A in mg./100 g. on dry butter (1600 basis)	Estimated growth-promoting activity in international units per g. (dry)
26. xi. 31	14.9	0.521	0.0747	0.720	17.2
16. xii. 31	14.0	0.344	0.0148	0.367	9.56
12. i. 32	17.6	0.327	0.0216	0.635	13.85
26. i. 32	13.75	0.244	0.0081	0.544	11.5
9. ii. 32	17.8	0.167	0.0085	0.315	6.92
1. iii. 32	14.0	0.0868	0.0103	0.176	3.80
16. iii. 32	17.1	0.0776	0.0107	0.238	4.75
6. iv. 32	19.2	0.0585	0.0077	0.234	4.48
19. iv. 32	16.8	0.0465	0.00685	0.168	3.26

Table V. *Group 3 butters.*

Cows receiving normal winter diet, except that the concentrates were largely replaced by artificially dried nitrogen-treated grass (30 % concentrates, 70 % dried grass).

Samples dated	Water %	Carotene in mg./100 g. on dry butter (1900 basis)	Xanthophyll in mg./100 g. on dry butter (1570 basis)	Vitamin A in mg./100 g. on dry butter (1600 basis)	Estimated growth-promoting activity in international units per g. (dry)
16. xii. 31	13.8	0.490	0.00963	0.506	13.3
12. i. 32	16.0	0.502	0.033	0.818	18.7
26. i. 32	16.6	0.538	0.0412	0.888	20.2
9. ii. 32	19.2	0.521	0.0371	0.696	16.8
1. iii. 32	12.2	0.464	0.0319	0.586	14.4
16. iii. 32	17.8	0.496	0.0319	0.508	13.4
6. iv. 32	13.5	0.289	0.0074	0.404	9.62
19. iv. 32	14.7	0.119	0.00375	0.294	6.09
10. v. 32	15.0	0.495	0.0360	0.846	19.0
23. v. 32	15.0	0.703	0.0629	0.829	20.8

effective, makes it possible to express the total growth-promoting activity of a butter due to carotene *plus* vitamin A in terms of the international carotene standard (1 unit = 0.001 mg. of carotene).

Fig. 2. Xanthophyll of the butters.

Group 1 + — +; 2 ● — — ●; 3 ○ — ○; 4 ○ — ○.

Fig. 3. Carotene of the butters.

Group 1 + — +; 2 ● — — ●; 3 ○ — ○; 4 ○ — ○.

The following example illustrates the method. A butter contains 0.703 mg./100g. carotene and 0.828 mg./100g. vitamin A on dry weight: 0.703 mg. carotene per 100 g. of dry butter fat equals 7.03 international units of carotene per g.;

Fig. 4. Vitamin A of the butters.

Group 1 +—+; 2 ●---●; 3 ○—○; 4 ○—○.

Fig. 5. Estimated total growth-promoting activity of the butters.

Group 1 x—x; 2 ●---●; 3 ○—○; 4 ○—○.

Table VI. *Group 4 butters.*

Cows receiving normal winter rations, except that artificially dried non-nitrogen-treated grass replaced 70 % of the concentrates in the diet.

Samples dated	Water %	Carotene in mg./100 g. on dry butter (1900 basis)	Xanthophyll in mg./100 g. on dry butter (1570 basis)	Vitamin A in mg./100 g. on dry butter (1600 basis)	Estimated growth-promoting activity in international units per g. (dry)
16. xii. 31	14.6	0.447	0.003	0.462	12.2
12. i. 32	17.8	0.385	0.028	0.780	16.8
26. i. 32	15.3	0.398	0.022	0.685	15.4
9. ii. 32	14.1	0.407	0.0205	0.463	11.8
1. iii. 32	13.6	0.367	0.0301	0.475	11.6
16. iii. 32	17.8	0.491	0.0263	0.508	13.3
6. iv. 32	17.1	0.193	0.0062	0.301	6.95
19. iv. 32	14.1	0.0882	0.0088	0.312	6.08

Table VII.

			Carotene			Xanthophyll			
			Mg./100 g.			Mg./100 g.			
			by spectroscopic colour test			by spectroscopic colour test			
			by yellow colour (Lovibond)			by yellow colour (Lovibond)			
			by direct absorption spectrum			by direct absorption spectrum			
			585 m μ			585 m μ			
			SbCl ₃			SbCl ₃			
			617 m μ			617 m μ			
			585 m μ			585 m μ			
Date of cutting grass									
1. vii. 31	Rye grass field	A	22	15.0	18.0	35.2	18.1	48.3	53.4
"	"	B	24.3	—	—	38.2	40.0	79.7	85.5
15. vii. 31	"	A	42.4	33.3	46.4	88.6	62.1	140	147
"	"	B	47.2	38.8	51.3	90.7	81.1	142	150
29. vii. 31	"	A	37.2	25.3	43.7	71.8	56.8	119	127
"	"	B	51.9	38.9	46.8	102.2	89.4	177	193
16. ix. 31	"	A	31.3	35.7	42.6	73.3	80.0	138	167
"	"	B	30.5	33.9	36.8	70.3	84.8	180	222
15. vii. 31	Clover grass field	A	33.01	25.1	38.4	48.5	43.2	71.3	69.0
"	"	B	33.08	28.5	36.0	48.9	52.8	90.0	94.7
29. vii. 31	"	A	37.44	24.8	31.8	74.95	58.0	111.2	120
"	"	B	40.0	33.9	36.0	75.9	76.6	122	130
16. ix. 31	"	A	51.5	46.1	53.8	106.1	87.3	210	212
"	"	B	51.7	42.4	56.3	107.1	101	189	218

0.829 mg. of vitamin A per 100 g. of fat equals 1.66×0.00829 mg. = 0.01381 mg. of carotene per g. Hence the total (estimated) growth-promoting activity of this particular butter = $13.81 + 7.03 = 20.84$ international units per g.

In the Tables will be found the growth-promoting value of all the butters calculated in this way. The results are also shown graphically. In connection with the Group 2 butters it should be noted that the tower silage was exhausted on January 20th and was replaced by stack silage. This appears to have affected the vitamin A content adversely (*cf.* Fig. 4).

Note on the unsaponifiable matter of grass.

In a study of the carotenoid content of grass it has been found that the apparent percentages of carotene, as measured by the spectroscopic antimony trichloride colour test, although in fair agreement with the values based on direct absorption spectra measurements, reveal, on closer examination, a dis-

crepancy in the sense that the colour test values are on the average 1.3 times those based on direct absorption spectra. A similar but greater discrepancy occurs in the xanthophyll determinations, the ratio in this case being approximately 2.2 (see Table VII).

The discrepancies are under detailed investigation, but it is already possible finally to rule out the plausible suggestion that they are due to the presence in grass of the vitamin A of liver oils. It seems much more likely that the carotenoids of grass include substances other than "carotene" and "xanthophyll," accepting the criteria of carotene and xanthophyll given earlier in this paper. The principal contaminant appears to accompany xanthophyll in the phase separation and may be the xanthophyll analogue of γ -carotene which contains only one ionone ring [Winterstein, 1933; Kuhn and Brockmann, 1933].

The presence of ergosterol in grass has not hitherto been fully established. We have obtained the characteristic absorption spectrum of ergosterol in the carefully purified total sterols isolated both from cocksfoot (kindly supplied by Prof. Chibnall) and perennial rye grass, the intensities corresponding with 1.0 and 1.4 % respectively. From this the ergosterol content of dried grass is of the order 0.002 %.

SUMMARY.

1. An experiment carried out in the winter of 1931-32 with four groups of cows was designed to measure the effects of two types of artificially dried grass, and of grass silage, on the quality of the resulting milk and butter in comparison with that produced on a normal winter ration. The values for the carotene, xanthophyll and vitamin A contents of the butters have been determined spectroscopically for the whole period of the experiment.

2. The results show that for cows fed on normal winter rations of hay and concentrates, the carotene, xanthophyll and vitamin A values fall steadily during the winter and remain at a very low level until the cows go out to grass in the spring. The consumption of fresh grass then results in a very rapid rise of the quantities of all three substances present in butter.

3. In the case of cows fed on artificially dried non-nitrogen-treated grass, the butters showed an increase in the content of the two carotenoids and of vitamin A very soon after the change over from the control diet. This higher level was then maintained until all the groups were again placed on the control diet, when the values dropped to the low control level. The results with the nitrogen-treated artificially dried grass were even better than with the non-nitrogen grass.

4. Grass silage of moderate quality was found to be little superior to the normal winter ration in its effect on the colour and vitamin A of the butter.

5. It may be concluded that the relatively high proportions of carotene, xanthophyll and vitamin A present in summer butter can be maintained during the winter period of stall feeding by the use of artificially dried grass.

6. In an examination of the unsaponifiable matter of grass it has been found that the spectroscopic blue test with antimony trichloride gives higher values for both carotene and xanthophyll than does the direct absorption spectrum method. The discrepancies are being investigated further.

7. The presence of ergosterol has been definitely established in the unsaponifiable matter of grass.

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