SEASONAL VARIATIONS IN THE VITAMIN A CONTENT OF VICTORIAN BUTTERFAT

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Summary

The total vitamin A potency of butterfat (derived from cheese or butter) has been determined on samples from three different Victorian districts and from King Island in Bass Strait in 1945 and 1947-48. Carotene was determined colorimetrically and the Carr-Price test was used for vitamin A. The glycerol dichlorhydrin reagent was too insensitive.

Sufficient samples were obtained from west Gippsland and the Western District (Victoria) to show the seasonal fluctuation over a whole year. There is a minimum value for total vitamin A potency in the late summer or early autumn when the pastures have dried off and a maximum in the late winter or early spring following the appearance of new growth.

Values obtained in 1945 were higher than those in the 1947-48 series. It is suggested that this is a concentration effect in that in 1945 normal quantities of carotene were ingested by the cows and secreted in a much smaller volume of milk than in the same period in 1947. It is apparent that there can be wide variations between values obtained each year at the same time.

Comparison of samples of February (1949) butterfats from the areas studied, with one from Orbost in east Gippsland where pastures remain green and plentiful in late summer, underlined the importance of carotene in the feed and suggested that seasonal fluctuations in this area would be very much smaller than in the rest of Victoria.

Comparison with figures in the literature shows that Victorian butterfats compare favourably with those in other parts of the world so far as vitamin A potency is concerned, the normal values probably being of the order of 18,000 I.U./lb. Minimum values of the order of 11,000 I.U./lb. are likely in late summer.

I. Introduction

It has long been recognized that the vitamin A content of butterfat is subject to seasonal fluctuations which are dependent largely on the variations in the feed available to the cow (Price 1931; Fraps and Treichler 1932; Gillam *et al.* 1933; Converse, Wiseman, and Meigs 1934; Jenness and Palmer 1945; Kon 1945; Parrish *et al.* 1946). Thus in the northern parts of the United States and in Europe, winter stall feeding of cattle with low carotene feeds is linked with substantial decreases in vitamin A potency in the butterfat (Morgan and Pritchard 1937; Dornbush, Peterson, and Olson 1940; Hvidsten 1943; Lord 1945). At the same time, Kunerth and Riddell (1938) have shown that cattle living under drought conditions produced butterfat with a very much reduced vitamin A potency, the carotene content being particularly low.

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It seemed likely, therefore, that a study of seasonal fluctuations of vitamin A potency of Australian butterfat which is obtained from cows which are at pasture the year round would show minima in the late summer when the pastures, having dried off, would be low in carotene. Furthermore, it was reasonable to expect that the overall average values for vitamin A potency would be higher than those obtained in countries with greater seasonal differences.

The work reported here was begun in 1945, but had to be laid aside for a time because of circumstances beyond the control of the authors. In the meantime, Parrish *et al.* (1946) reported the deleterious effect of hot summer weather on vitamin A values of Kansas butter and Barnicoat (1947) showed conclusively that (for the season 1935-36) the minimum values for vitamin A potency in New Zealand (North I.) butter were, in fact, found in late summer (February) at the time when the pasture normally tends to dry up.

It would appear that the results reported in this paper are the first referring to Australian butterfat and that the conditions existing, in Victoria at least, closely parallel those obtaining in the North Island of New Zealand.

II. Experimental

(*a*) *The Determination of Vitamin A*

The Carr-Price (1926) method was used. Carotene also reacts with antimony trichloride to give a blue colour, but the rates of reaction are quite different according to Oser, Melnick, and Pader (1943), as carotene does not give its maximum blue colour until some two hours have elapsed. The error introduced by reading the colour formed by a mixture of vitamin A and carotene in the first four seconds after addition of the antimony trichloride, and converting this to units of preformed vitamin A is extremely small in such products as butter where the carotene contributes only a small proportion of the total vitamin A potency. This error has been ignored in this work.

(*b*) *The Determination of Carotene*

In butterfat, by far the greater part of the yellow colour normally encountered is β-carotene and some workers, e.g. Booth *et al.* (1933), ignore the small amount of xanthophyll and accept the intensity of the yellow pigmentation as a measure of the carotene present. That this is virtually correct has been demonstrated by Gillam (1934) who showed that the ratio of carotene to xanthophyll in English butter was 14:1 by weight. This is so constant that one is quite justified in accepting 94 per cent. of the absorption at 455-460 mμ as a measure of the carotene present.

In the early part of this work (1945) the determinations were carried out according to the method of Fraps, Kemmerer, and Greenberg (1940) in which the xanthophyll was removed from the solution of the unsaponifiable fraction in petroleum ether by shaking with "light $MgCO_3$" before the solution was

placed in the colorimeter. The results so confirmed those of Gillam that in the later studies 94 per cent. of the total was accepted as carotene.

(*c*) *Reagents*

Chloroform.—Pure chloroform was washed two or three times with water, dried over Na_2SO_4, refluxed and finally distilled. It was stored in a dark brown bottle away from the light.

Carr-Price Reagent.—Antimony trichloride (C.P.) was distilled directly from a retort into chloroform (250 ml.) until 75 g. had been added. The solution was set aside to come to saturation equilibrium in the presence of excess crystalline $SbCl_3$. It was stored in the dark at 20°C.

Peroxide-free Ether.—Ethyl ether (B.P.) was shaken with a saturated solution of ferrous sulphate, separated, dried over Na_2SO_4 and distilled in an all glass still painted black on the outside.

Alcohol.—Absolute alcohol (C.P.) was distilled once.

Petroleum Ether.—The fraction of an industrial solvent (Shell X222) boiling below 80°C. was distilled and dried over Na_2SO_4.

Magnesium Carbonate.—Light, B.D.H.

Acetic Anhydride.—C.P.

Potassium Hydroxide 60%.—This was prepared from the C.P. reagent and distilled water.

(*d*) *Preparation of Samples*

Four dairying districts have been studied. They are Allansford-Garvoc in the Western District of Victoria, Drouin in Gippsland, Leitchville in the Murray Valley irrigation area, and King Island in Bass Strait. Both butter and cheese have been used as the source of fat, the latter when it was inconvenient or impossible to obtain butter from the factories under consideration. It was thought safe to do this as the work of Dearden *et al.* (1946) points to the complete conservation of vitamin A /g. of fat during the manufacture and maturing of cheese and the unpublished work of one of the authors (W.M.B.) has shown that there is no loss of vitamin A potency even in the processing of cheese.

Samples were drawn at random from production, an 8 oz. pat, if butter, and for cheese, production samples covering 2-3 days. The butter samples were melted and filtered through a coarse, dry filter paper which removed water and curd. Cheese samples were taken with the conventional cheese trier, minced, and held at 50-60°C. for 1-2 hours, whereupon the fat separated. It, too, was decanted through a coarse, dry filter paper.

(*e*) *Saponification*

Twenty g. of the filtered fat was weighed into a 250 ml. conical flask painted black on the outside and saponified on a hot plate with 12.5 ml. of 60 per cent. KOH and 25 ml. of absolute alcohol. One hundred ml. of cold water was then added and the flask cooled under running water. The solution

was then extracted first with 100 ml. and then with three lots of 50 ml. of peroxide-free ether. The ethereal extracts were combined, washed with four 50 ml. lots of water, dried overnight over anhydrous sodium sulphate, and then adjusted to some convenient dilution and divided into two parts. The ether was boiled off each part and the flasks blown out with nitrogen gas. The dry unsaponifiable matter left in the flasks was dissolved, in one case in 25 ml. of dry chloroform, and in the other, in 25 ml. of petroleum ether. At all stages the solutions were shielded from strong light.

(*f*) *Determination of Vitamin A and Carotene with the Lange Photo-Electric Colorimeter* (1945)

The Lange photo-electric colorimeter consists of two selenium photo-electric cells activated by a common light source and balanced by suitable adjustments of iris diaphragms and resistances. It is graduated to read directly in extinction or percentage absorption. For the Carr-Price test, a yellow Wratten filter was used and the instrument was standardized from day to day with a copper sulphate solution (34.64 g. $CuSO_4.5H_2O$/500 ml.).

A diluted fish liver oil kindly supplied by Nicholas Pty. Ltd. was used as a standard. It contained 3025 I.U. of vitamin A/g. The tubes containing the chloroform solutions of unsaponifiable matter were placed in the instrument and 9 ml. of Carr-Price reagent added rapidly from an automatic pipette. The speed of addition was sufficient to mix the solution and the galvanometer deflection was read at the point of temporary stability. From the results obtained a standard curve was constructed.

In assaying unknown samples, 20 g. of butterfat was saponified and 1 ml. of the final chloroform solution of the unsaponifiable matter (equivalent to 0.4 g. of fat) was taken for the final determination. The vitamin A present was read off from the standard curve.

Carotene was determined colorimetrically using the deep blue filter supplied with the instrument, petroleum ether to set the zero point, and a solution of potassium dichromate (0.018 g./100 ml.) to standardize the instrument.

A standard curve was obtained by diluting with petroleum ether a sample of peanut oil containing 0.0364 g. of pure β-carotene/250 ml. and plotting deflection against μg. of β-carotene.

The butterfat samples were saponified and the unsaponifiable material dissolved in petroleum ether (100 ml.) which was then shaken with 5 g. of "light magnesium carbonate" to remove xanthophyll. After centrifuging the solution was read in the colorimeter and the β-carotene present determined by reference to the standard curve.

(*g*) *Determination of Vitamin A and Carotene with the Spekker Absorptiometer*

It has already been pointed out by Innes and Birch (1945) that the Spekker absorptiometer used normally as a null point instrument is not satisfactory for the measurement of the evanescent antimony trichloride colour.

However, these authors describe a modification of the use of this instrument by which it is possible to obtain reliable measurements of the Carr-Price colour. This method has been followed with further slight modification to permit the use of the galvanometer actually supplied with the absorptiometer.

A diluted fish liver oil, standardized by the Carr-Price procedure on the whole oil, was made available by Nicholas Pty. Ltd. It contained 2,000 I.U. of vitamin A/g. and was used to prepare a standard curve. For each determination, 6 ml. of antimony trichloride solution and 2 drops of acetic anhydride (to prevent any precipitation of antimony oxychloride) were placed in the cell. With this in place the setting of the instrument was checked and 1 ml. of vitamin A solution rapidly blown in from a wide bore pipette. The mercury switch button was kept depressed and the maximum rest point of the galvanometer noted.

In assaying unknown samples, 1 ml. of the chloroform solution of the unsaponifiable matter was used and the vitamin A content calculated from the graph.

The determination of carotene is a normal colorimetric procedure and a graph was constructed for β-carotene (the pure crystals) in concentrations of from 0.64 to 16 µg./ml. of petroleum ether against Spekker drum readings using the violet filters supplied with the instrument, and a cell containing water as the blank. Unknown samples were assayed by comparing the drum reading of the petroleum ether solution of the unsaponifiable matter with the standard graph. As already indicated, 94 per cent. of this value represents the β-carotene content.

(*h*) *The G.D.H. Reagent for Vitamin A Determination*

The obvious disadvantages of the Carr-Price reagent have led to a search for another reagent. One of the latest suggestions is that of Sobel and Werbin (1945, 1946, 1947) who used the reaction of glycerol dichlorhydrin (1, 3-dichloro-2-hydroxypropane), referred to as G.D.H., with vitamin A to measure the potency of the latter. Several attempts were made to use this reagent but all failed as it was far too insensitive even when "activated" by distillation over $SbCl_3$.

III. Results and Discussion

Vitamin A values were determined directly as International Units by comparison with an oil so standardized. Carotene was determined as µg. of β-carotene and was converted to vitamin A potency on the basis of 1 I.U. = 0.6 µg. The results are concerned only with butterfat from bulk milk as supplied to dairy factories and no account could be taken of variations due to breed differences.

Results obtained from Gippsland and Western District areas are shown in the figures (Figs. 1, 2, 3) where total vitamin A and carotene potencies are graphed with time. The curve for the year 1947-48 shows a definite seasonal fluctuation in total vitamin A potency with a minimum in the late summer or

early autumn when the pastures in all three districts have dried off, and a peak in the spring following the appearance of new lush growth.

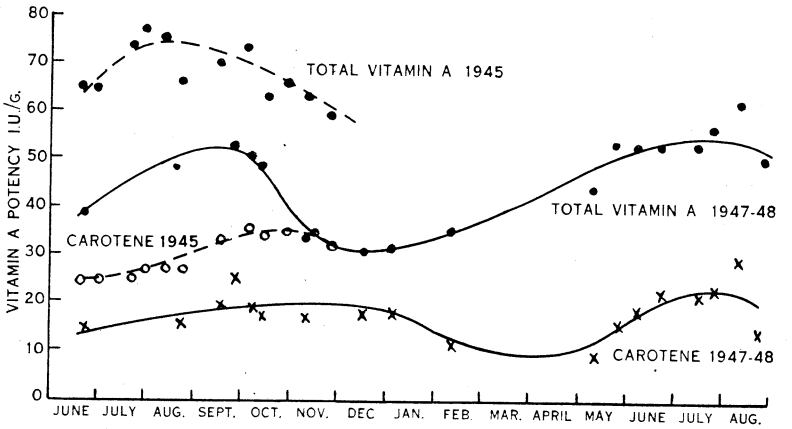

Fig. 1.—Seasonal fluctuations in vitamin A and carotene at Drouin (Gippsland).

As the Allansford and Garvoc factories are only some thirteen miles apart and draw milk from virtually the same area, a much smaller number of samples was taken from the latter factory which was used merely to confirm the trend shown by the Allansford results.

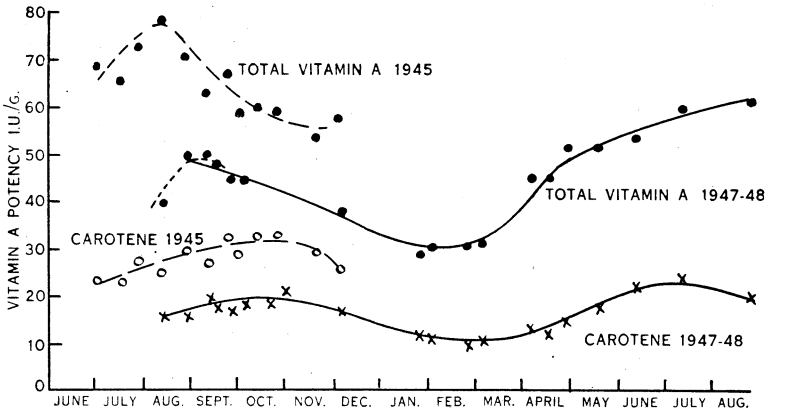

Fig. 2.—Seasonal fluctuations in vitamin A and carotene at Allansford (Western District).

Values for Leitchville and King Island are shown in Figures 4 and 5 respectively. It is unfortunate that these two areas could not be studied for a whole year, as one would expect to find smaller fluctuations in these districts than in the others: Leitchville is an irrigation district and King Island has abundant rain and cool summer temperatures. February butters from these areas are compared with Gippsland and Western District butterfat later (see Table 4).

A statistical analysis* has been made of the effect of rainfall on vitamin A and carotene in butterfat from Allansford and Drouin where complete yearly cycles were available. The total rainfall for the fortnight before the date of

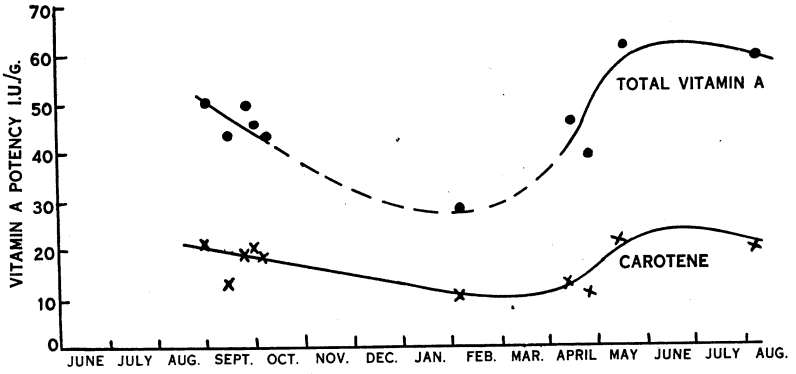

Fig. 3.—Seasonal fluctuations in vitamin A and carotene in butterfat at Garvoc (Western District), 1947-48.

test and for the fortnight before that were worked out and correlated with these two factors. For both Allansford and Drouin the effect on vitamin A was very much more pronounced than that on carotene. Moreover, it was found that the effect was much greater at Allansford than at Drouin. For the Allansford data the regression of vitamin A on the total rainfall for the preceding fortnight was significant at the 1 per cent. level, and that of carotene at the 5 per cent. level.

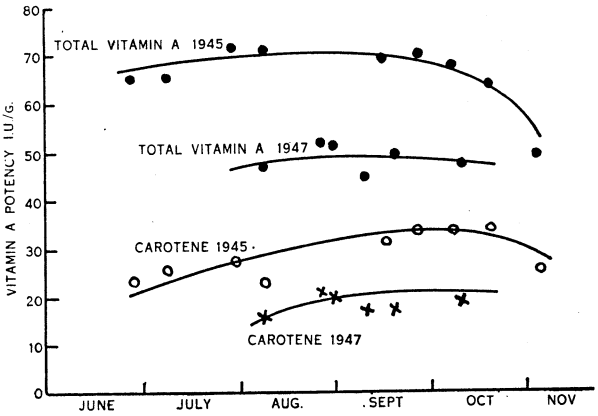

Fig. 4.—Seasonal fluctuations in vitamin A and carotene at Leitchville (northern irrigation area).

At Drouin neither of the regressions was significant. In no case was there any significant effect of the rainfall between 4 weeks and 2 weeks prior to test. This may be due to the greater total rainfall in this district which

* The authors are indebted to Mr. E. J. Williams, Section of Mathematical Statistics, C.S.I.R.O., for this treatment of their results.

averages about 34 per cent. more per annum than Allansford. While the relationships found are not very consistent or very impressive it may be taken as established that rainfall, through its effect on the chemical composition of the grass, does affect the vitamin A and carotene content of the butter produced. There are, of course, other factors such as temperature and soil which are concerned with pasture production.

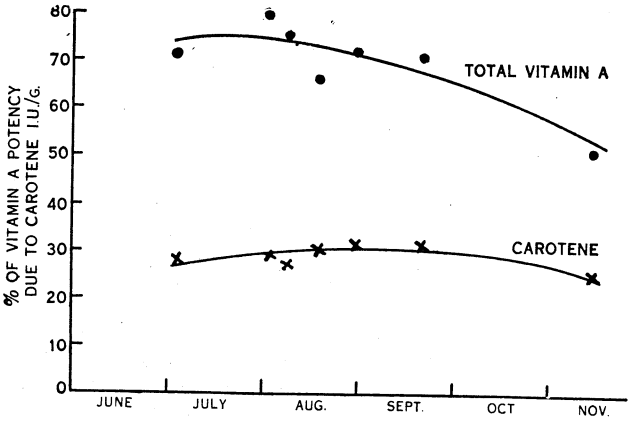

Fig. 5.—Seasonal fluctuations in vitamin A and carotene on King I. (Bass Strait), 1945.

From the vitamin A aspect the year may be subdivided into three periods, August-December, January-March, and April-July. The average vitamin A potency/g. of butterfat for all assays done in these periods is shown in Table 1.

TABLE 1
AVERAGE VITAMIN A POTENCY I.U./G. OF BUTTERFAT

District	Year	Aug.-Dec.	Jan.-Mar.	Apr.-July
Drouin	1945	68.0		
Drouin	1947-48	46.0	33.1	51.7
Western District*	1945	64.7		
Western District†	1947-48	45.7	29.8	52.1
Leitchville	1945	63.9		
Leitchville	1947-48	50.0		
King Island	1945	69.0		

* Allansford; † Allansford and Garvoc.

The results obtained in 1947-48 are generally comparable with those of Barnicoat (1947) obtained 12 years earlier in New Zealand, where similar dairying conditions exist. They also confirm those of Kunerth and Riddell (1938) who found very low vitamin A potency in fat produced by cattle on drought-stricken pasture. It is usual, of course, in northern countries to associate low vitamin A values with winter butterfat. Our results shown the vitamin A

potency in winter butterfat to be well up and approaching the maximum because of the availability of green, though slow growing, pastures after the autumn rains. Das Gupta (1937) has recorded a similar state of affairs in Bengal, though in this case the cattle are denied pasture in the summer because of wet season flooding.

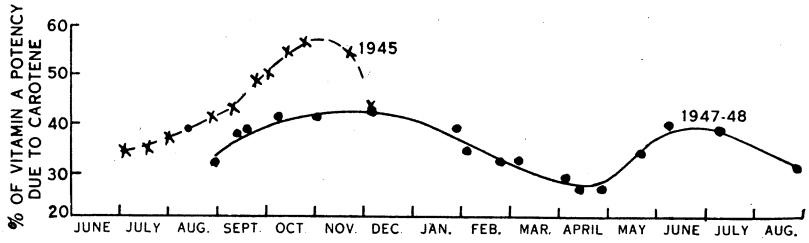

Fig. 6.—Seasonal fluctuations in the percentage of vitamin A potency due to carotene in Allansford butterfat.

It is noteworthy that the carotene figures are more constant than the vitamin A throughout the year. The percentage of total vitamin A potency due to the carotene has been graphed for Allansford (Fig. 6) and Drouin (Fig. 7). Two peaks are apparent; one in summer and one in winter. The former corresponds roughly with falling total vitamin A potency and the latter with rising total potency. The minima occur in late winter or early spring and in the autumn.

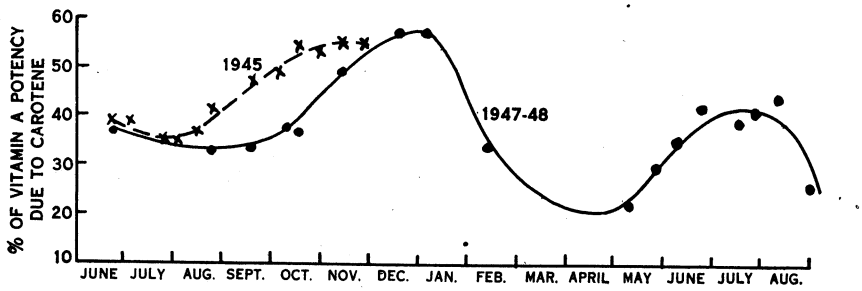

Fig. 7.—Seasonal fluctuations in the percentage of vitamin A potency due to carotene in Drouin butterfat.

The proportion of total potency due to carotene rises above 50 per cent., is usually 35 to 40 per cent., and sometimes below 35 per cent. This is a much higher proportion than is reported by Ronnenberg (1945) for Danish butterfat (about 20 per cent.), by Jenness and Palmer (1945) for Minnesota butter (11 to 15 per cent. in winter, 21 to 25 per cent. in summer), or by Parrish *et al.* (1946) in Kansas butter (14.9 per cent. in winter, 21.4 per cent. in summer). However, Barnicoat (1947) shows comparable figures for New Zealand butterfat; 37-42½ per cent. for Manawatu and 34 to 52 for Waikato. This phenomenon can probably be related to the carotene-rich diet of continuous pasture.

It will have been noticed that the 1945 values are much higher than those for 1947-48. These figures were supplied to Dr. F. W. Clements of the Institute of Anatomy, Canberra, in 1946, and formed the basis of the vitamin A figures in Australian butter and cheese published by Osmond (1946). A critical study of the methods and standards, etc. used in the two phases of the work failed to reveal any anomalies and an explanation of the phenomenon was sought in the climatic conditions obtaining in the two periods and in the statistics of milk production.

Table 2 records for Victoria the total dairy cattle for the year and the milk produced in the period June to December for the past four years as set down in the "Summary of the Dairy Industry in Australia," an annual publication of the Commonwealth Statistician.

TABLE 2
TOTAL DAIRY CATTLE AND MILK PRODUCED IN VICTORIA

Year	Total Dairy Cattle (thousands)	Milk Produced from June-December (millions of lb.)
1945	1364	2013
1946	1299	2777
1947	1411	2728
1948	1476	2758

In 1945 a slightly smaller number of cattle produced in the crucial months little more than two-thirds as much milk as in 1947 and 1948. The 1945 season followed a comparatively dry autumn.

Table 3 shows the percentage of the mean average rainfall received in each period in the three Victorian districts in the years under discussion and the pasture notes of the Commonwealth Meteorological Bureau, Melbourne, refer to unsatisfactory pastures in June 1945, "slow," "retarded," and "fair" pastures in all areas (including King Island) in July and August and good growth but accompanied by frosts in September in all three Victorian areas. This slow start would account for the lower milk production for the June-December period in 1945 compared with the same periods in 1947 and 1948 when rainfall was more nearly normal, and the pasture notes show the satisfactory development of pastures with no abnormalities. Actually milk production for the whole 1945-46 season was almost normal as the season "held on," the number of cows in milk on March 31, 1946 being abnormally high.

It is submitted that the higher vitamin A figure for the 1945 "flush" can be attributed to the lower volume of milk in that the cattle on the slow but green pastures ingested normal quantities of carotene which then appeared in a smaller volume of milk, leading to a high vitamin A potency in the fat actually produced. This is in fact a concentration effect.

It is well known that when the rest of Victoria is dry and parched in late summer the Snowy River flats at Orbost in the far east of the State retain an

abundance of green feed. On King Island, too, with a more even summer temperature and absence of drying north winds, the pastures usually last better. It seemed, therefore, that a comparison of vitamin A potency of February butterfat (corresponding with the minima in the curves in Figs. 1, 2, 3) from the different areas mentioned would reveal higher values for Orbost and, possibly, King Island. A detailed study was not possible, and isolated samples have been assayed.

TABLE 3

RAINFALL IN YEARS AND DISTRICTS STUDIED EXPRESSED AS PERCENTAGE OF AVERAGE MEAN RAINFALL FOR THE PERIODS SHOWN

	Jan.-Mar.	Apr.-July	Aug.-Dec.
1945			
Allansford	124	41	85
Drouin	80	78	88
Cohuna (Leitchville)	45	106	101
King Island	90	80	123
1947			
Allansford	145	101	134
Drouin	127	112	126
Cohuna (Lietchville)	138	112	167
King Island	148	160	138
1948			
Allansford	54	111	105
Drouin	52	124	101
Cohuna (Leitchville)	30	175	100
King Island	64	138	115

Table 4 compares the vitamin A potency of February butterfats from six dairy products factories in five areas. Leitchville, Drouin, Allansford, and Garvoc samples were from cheese manufactured during the first week in February; King Island and Orbost from butter manufactured in the third week.

These results point to the superiority of the Orbost butterfat over that from the other areas so far as vitamin A potency is concerned and strikingly confirm the influence of feed on this property. Although there is only one result from the Orbost district, it seems likely from what is known of seasonal variation and the influence of fresh pasture on vitamin A potency of butter that there is no serious seasonal fluctuation in this area. While so few results cannot be regarded as conclusive, they are in line with the recorded facts.

It is noteworthy that the values for Gippsland and Western District butterfats are higher than those obtained at the corresponding period in 1948; 37 as against 30 I.U./g. for Allansford and 40 as against 33-34 for Drouin. This is probably due to the abnormally low rainfall in the first quarter of 1948 (see Table 3) and further serves to underline what has already been noted — that the vitamin A potency of butterfats from the same area at the same period

each year may be subject to significant fluctuations. This must follow from the prosperity of the season, but may be a much greater fluctuation than hitherto imagined.

TABLE 4

VITAMIN A POTENCY OF LATE SUMMER BUTTERFAT, FEBRUARY 1949

Area and Factory	Vitamin A Potency, I.U./g.		
	Vitamin A	Carotene	Total
King I. (Loorana)	23.4	11.0	34.4
King I. (Loorana)	28.0	11.0	39.0
Murray Irrigation (Leitchville)	21.0	18.3	39.3
East Gippsland (Orbost)	34.3	19.8	54.1
West Gippsland (Drouin)	24.0	15.6	39.6
Western District (Allansford)	24.0	13.1	37.1
Western District (Garvoc)	22.0	12.5	34.5

In Table 5, average values for vitamin A in Victorian butter are compared with values recorded in the literature.

TABLE 5

COMPARISON OF VITAMIN A POTENCY OF BUTTER OF DIFFERENT COUNTRIES

Location	I.U./lb.	Authority
U.S.A.; Washington	8,700 to 25,900	Ashworth *et al.* 1945
U.S.A.; Kansas	11,050 to 17,700	Parrish *et al.* 1946
U.S.A.; Minnesota	9,000 to 17,000	Jenness and Palmer 1945
U.S.A.; Wisconsin	9,500 to 18,000	Berl and Peterson 1944
U.S.A.; Texas	17,000	Kemmerer and Fraps 1943
Denmark	20,700	Ronnenberg 1945
Denmark	7,000 to 17,100	Wilkinson 1939
Scotland	5,700 to 12,180	Wilkinson 1939
England	4,600 to 18,200	Morgan and Pritchard 1937
Norway	9,000 to 17,000*	Hvidsten 1943
New Zealand	13,200 to 18,600	Barnicoat 1947
Australia; Victoria 1945	Up to 29,000*	—
Australia; Victoria 1947-48	11,000 to 21,000*	—

* Calculated as 80 per cent. of the values obtained for dry butterfat.

IV. ACKNOWLEDGMENTS

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V. References

Ashworth, U. S., McGregor, M., and Bendixen, H. A. (1945).—Wash. St. Agric. Exp. Sta. Bull. No. 466.

Barnicoat, C. R. (1947).—*J. Dairy Res.* **15**: 80.

Berl, S., and Peterson, W. H. (1944).—*J. Nutrit.* **26**: 527.

Booth, R. G., Kon, S. K., Dann, W. J., and Moore, T. (1933).—*Biochem. J.* **27**: 1189.

Converse, H. T., Wiseman, H. G., and Meigs, E. B. (1934).—Amer. Soc. Animal Prod. Rec. Proc. 27th Ann. Mtg. p. 50.

Das Gupta, S. M. (1937).—*Science and Culture* **3**: 244.

Dearden, D. V., Henry, K. M., Houston, J., Kon, S. K., and Thompson, S. Y. (1946).—*J. Dairy Res.* **14**: 100.

Dornbush, A. C., Peterson, W. H., and Olson, F. R. (1940).—*J. Amer. Med. Ass.* **114**: 1748.

Fraps, G. S., Kemmerer, A. R., and Greenberg, S. M. (1940).—*Industr. Engng. Chem.* (Anal. Ed.) **12**: 16.

Fraps, G. S., and Treichler, R. (1932).—*Industr. Engng. Chem.* **24**: 1079.

Gillam, A. E. (1934).—*Biochem. J.* **28**: 19.

Gillam, A. E., Heilbron, I. M., Morton, R. A., Bishop, G., and Drummond, J. C. (1933).—*Biochem. J.* **27**: 878.

Hvidsten, H. (1943).—*Meld. Norg. LandbrHöisk.* **23**: 169.

Innes, R. F., and Birch, H. F. (1945).—*Analyst* **70**: 304.

Jenness, R., and Palmer, L. S. (1945).—*J. Dairy Sci.* **28**: 491.

Kemmerer, A. R., and Fraps, G. S. (1943).—Texas Agric. Exp. Sta. Bull. No. 629.

Kon, S. K., (1945).—*J. R. Soc. Arts* **93**: 124.

Kunerth, B. L., and Riddell, W. H. (1938).—*J. Dairy. Sci.* **21**: 41.

Lord, J. W. (1945).—*Biochem. J.* **39**: 372.

Morgan, R. S., and Pritchard, H. (1937).—*Analyst* **62**: 354.

Oser, B. L., Melnick, D., and Pader, M. (1943).—*Industr. Engng. Chem.* (Anal. Ed.) **15**: 724.

Osmond, A. (1946).—"Tables of Compositions of Australian Foods." (Australian Inst. Anatomy: Canberra.)

Parrish, D. B., Martin, W. H., Atkeson, F. W., and Hughes, J. S. (1946).—*J. Dairy Sci.* **29**: 91.

Price, W. A. (1931).—*Cream. Milk Pl. Mon.* **20**: 26-37, 46-50.

Ronnenberg, P. (1945).—*K. VetHöjsk. Aarsskr.* 47-58.

Sobel, A. E., and Werbin, H. (1945).—*J. Biol. Chem.* **159**: 681.

Sobel, A. E., and Werbin, H. (1946).—*Industr. Engng. Chem.* (Anal. Ed.) **18**: 570.

Sobel, A. E., and Werbin, H. (1947).—*Anal. Chem.* **19**: 107.

Wilkinson, H. (1939).—*Analyst* **64**: 17.