8-1949

A study of carotene content of cow's milk and natural butter color changes during transition from winter to summer feeding

Ben B. Waggoner

To the Graduate Council:

I am submitting herewith a thesis written by Ben B. Waggoner entitled "A study of carotene content of cow's milk and natural butter color changes during transition from winter to summer feeding." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

R. H. Lush, Major Professor

We have read this thesis and recommend its acceptance:

Eric W. Swanson, T. B. Harrison

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 5, 1949

To the Committee on Graduate Study:

I am submitting to you a thesis written by Ben B. Waggoner entitled "A Study of Carotene Content of Cow's Milk and Natural Butter Color Changes During Transition from Winter to Summer Feeding." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

R. H. Lush
Major Professor

We have read this thesis
and recommend its acceptance:

Eric W. Swanson

Thos. B. Harrison

Accepted for the Committee

Dean of the Graduate School

A STUDY OF CAROTENE CONTENT OF COW'S MILK AND NATURAL BUTTER COLOR CHANGES DURING TRANSITION FROM WINTER TO SUMMER FEEDING

A THESIS

Submitted to

The Committee on Graduate Study

of

The University of Tennessee

in

Partial Fulfillment of the Requirements

for the degree of

Master of Science

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by

Ben B. Waggoner

August 5, 1949

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Professor C. E. Wylie, Head, Dairy Department for his valuable suggestions; to Professor R. H. Lush and Dr. T. W. Albrecht for their suggestions and criticisms in preparing the manuscript; to Professor Thomas B. Harrison who made arrangements for the collection of milk samples; and to Dr. Eric Swanson for his assistance and supervision on laboratory techniques.

I wish also to acknowledge the help of June Huston who typed the manuscript.

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CHAPTER I

INTRODUCTION

A study was made of the carotene content of milk and butter and its relationship to the natural yellow color, with observations concerning the color changes which occur during the transition from winter to summer feeding of cows.

Dairy products constitute a relatively large percentage of the average diet in this country, and it is important that they be maintained at the very highest level of quality possible. This responsibility belongs to the dairy industry as a whole, and calls for the full cooperation of the producer, as well as the distributor.

The vitamin A potency of milk and butter has been the subject of various investigations, and considerable data are available showing that these products may be good sources of vitamin A, depending upon the methods under which they are produced. The vitamin A potency of milk depends upon the carotene content of the feed of the cow. The carotene content of feeds is dependent on the plant species, stage of maturity, amount of rainfall and method of curing and storage.

The carotene content of milk and of butter is responsible for its yellow color. During periods when carotene is not available in sufficient amounts in the feed, the yellow color of butter is greatly diminished. This is of commercial significance. Furthermore, it indicates that the vitamin A potency has been reduced to a low level, particularly with the breeds producing a deep colored butterfat.

The above relationships should be understood by all producers and handlers of market milk. The consumer should demand a product of high vitamin A potency. Moreover, this demand should be exercised to the extent that all producers of milk for commercial distribution would be compelled to maintain a sufficiently high vitamin A potency, or suffer a price differential under appropriate trade considerations.

CHAPTER II

REVIEW OF THE LITERATURE

The characteristic yellow color of butterfat is due principally to the presence of carotene, and to a lesser extent to xanthophyll. These substances belong to a group of pigments referred to as carotenoids that are soluble in fats and most fat solvents. They are rather widespread in green plants, particularly the leafy ones, carrots and other yellow-colored vegetables, and to some extent in orange-colored vegetation. Carotene is known to occur in much greater concentration in plants than the other pigments, and is therefore largely responsible for the yellow color of butter and whole milk. This pigment has been isolated in crystalline form and the chemical formula representing its structure is known (25).

Foods having their origin in plants possess no vitamin A, but the vitamin A value is due almost entirely to the carotene content of the plant (50). Thus an animal consuming food of plant origin, converts the carotene to vitamin A for its own body requirements and to be secreted as such in milk. Some of the carotene remains unchanged upon ingestion and is secreted in the milk in like manner giving rise to the yellow color of milk and butter. The vitamin A potency of milk and butter is the combined biological performance of both carotene and vitamin A (35).

Besides the occurrence of carotene in plants and the milk fat of animals, the body fat of animals also is characterized by the presence of small amounts of carotene (48). The blood, liver, corpus luteum and other body tissue also are known to contain traces of carotene (60, 51).

Since it has been discovered that a cow can secrete carotene and vitamin A in the milk only when the carotene is present in the diet, it follows then that the cow must convert the carotene into vitamin A in the body. This conversion occurs chiefly in the liver, and when an animal ingests feed abundantly supplied with carotene the vitamin A is stored in the liver and to some extent in other tissues (50).

Moore (48) noted the conversion of carotene to vitamin A by the cow when, upon feeding a cow a large supply of carotene after a long period of winter-feed, she produced butterfat higher in both carotene and vitamin A. Dornbush, et al (16) found that the carotene of market milk dropped more abruptly in the fall than did the vitamin A content, suggesting the use of vitamin A stores in the animal body. Mitchell and Wise (46) noted that during the latter part of the grazing season, the decrease of carotene in milk followed the decrease of carotene in the herbage, and indicated that either the cows had no carotene reserve or did not utilize it sufficiently to maintain the carotene level in the milk for any appreciable length of time.

Theophilus, et al (62) observed that the carotene content of butter varied much more than vitamin A during the season and according to the type of feed fed. They are of the opinion that this is due to the body stores of these substances which permit a regulatory process by which the vitamin A content of milk can be maintained during certain periods of low carotene intake.

Koehn (41) conducted experiments in Alabama to determine the seasonal variation in the carotene and the vitamin A content of milk produced under general feeding practices in that State. His results led him to conclude that cows on permanent pasture failed to store sufficient carotene and vitamin A to prevent rapid decreases of these factors in the winter milk.

Of much importance from the standpoint of nutrition, is the contribution of carotene to the total vitamin A potency of milk and butter. A number of investigators have sought to determine the relationship of carotene to the vitamin A potency and color of milk and butter. Jenness and Palmer (38) recognized the distribution of carotene and vitamin A in the total potency of butter, and found that in winter-butter carotene furnished from 11 to 15 percent of the total potency, while 21 to 25 percent was furnished by summer-butter. Dornbush, et al (16) in studying the vitamin A potency of milk in Wisconsin, found that the contribution of carotene to the total potency varied considerably during the season. They report that the carotene is nearly tripled by the change to pasture, while the vitamin A content increased only 40 percent. During the period of maximum potency butter contained 9.9 micrograms of carotene per gram and 12.8 micrograms of vitamin A per gram.

Ashworth, et al (2) observed that carotene accounted for one-fourth to one-third of the total vitamin A activity. Their results indicate a much higher value for carotene. The results are tabulated in Table I.

A Technical Committee (61) reports that on the averave, 18 percent of the vitamin A potency of the Holstein milk and butter was due to carotene, whereas this figure for the Jersey milk and butter was 36 percent. Parrish, et al (52) found that carotene provided 14.9 percent of the total potency of butter during the months of December to April; 21.4 percent from May to November, and a mean annual percentage of 19.6.

Hodgson, and co-workers (36) conducted experiments to determine the effects of various roughages on the color,

TABLE I

THE AVERAGE VITAMIN A AND CAROTENE CONTENT PER POUND OF WASHINGTON CREAMERY BUTTER*

Month	Year	Weighted mean for state Carotene (I.U.)	Vit. A (I.U.)	Total (I.U.)
May-June	43	7,400	15,000	22,400
August	43	5,200	14,800	20,000
November	43	4,700	12,800	17,500
February	44	2,900	9,200	12,100
May-June	44	7,200	13,100	20,300
August	44	5,700	11,800	16,900
November	44	5,800	11,200	17,000
February	45	3,000	8,900	11,900
Average all seasons		5,200	12,000	17,000
Weighted mean for all seasons		5,500	12,400	17,900

*Converted into International Units which calculated on the basis of 1 microgram of carotene equals 1.67 I.U.; 1 microgram of vitamin A equals 4.00 I.U.

carotene content and vitamin A potency of butterfat when fed in the dairy ration. They concluded that carotene in the butterfat accounted for only a small percentage of the total vitamin A activity. These figures below represent the percentage for each type of roughage fed:

Hay ration (perennial grass and clover mixture). . 11.2
Hay and silage (grass and clover mixture). 12.7
Silage ration (oats and peas). 11.3
Pasture. 13.7

Theophilus, et al (62) reporting on the vitamin A potency of Idaho-produced butter states that in March the carotene supplied 22 percent of the vitamin A activity, while in July it accounted for 33 percent. Hathaway and Davis (27) report similar results. A number of other workers have determined values representing the contribution of carotene to total vitamin A potency, and in most cases the differences reported are not significant (18, 4, 63, 52).

As has been pointed out elsewhere, the carotene pigment is very largely responsible for the yellow color of butterfat. Numerous workers have shown that as the carotene intake of an animal varies, so does the color and vitamin A of the butterfat vary. Palmer and Eckles (51) of the Missouri station were probably the first investigators to show that the cow's milk derived its yellow color from carotene and to some degree from xanthophyll which accompany the chlorophyll in all green plants. It was originally thought that the color of milk was due to pigments synthesized in the body of the cow. Their classical experiments readily established the effects of the diet on the color of milk and milk fat, showing conclusively that the color increases in proportion to the amount of carotene fed, up to a certain level.

Fieger and Lewis (20) state that generally 75 to 90 percent of the natural color in butterfat is due to carotene derived directly from feed. They claim that as a result of the differences in the carotene content of the diet, some butterfats may be 15 times as highly colored as others, even though from the same breed. It is their belief that such differences in color may be a rough indication of the vitamin A potency of the milk. This point, however, is highly controversial (65, 15, 4, 63, 11).

Although Hodgson, et al (36) found no consistent relationship between the carotene content of the butterfat and its color, they concluded that on the average there was a good relationship between color and the carotene content of the butterfat.

Converse, Wiseman and Meigs (11) investigated the relation between color and vitamin A of butter and the feed of the cow. The experiments showed results as follows: (1) The butter color varies definitely with the green color and carotene of roughage fed; (2) the vitamin A content varies roughly with the changes of color, and (3) the color of milk and uncolored butter may be used as an index to the vitamin A content if the breed is known.

The intensity of the yellow color of the milk fat is positively correlated with the carotene content of the butter made from it, and also with its vitamin A content (65).

Moore (48) contends that the degree of pigmentation of butterfat when adequate carotene is available in the diet is determined by the breed. He found the Jersey breed gave a yellow value twice that obtained with the butterfat derived from Shorthorn cows under similar feeding conditions. Dornbush, et al (16) agrees that the Channel Island breeds, such as Guernsey and Jersey produce milk higher in color (carotene) than the other breeds. However, they state that the vitamin A constituent is less in these breeds as compared with other breeds producing a paler milk.

It seems that inheritance plays a part in determining how much yellow pigment cows are able to secrete in their milk. Some breeds, such as the Channel Island breeds, do not convert into the colorless vitamin A so large a proportion of carotene they assimilate as do the cows of the breeds which give a paler milk, such as Holsteins (16). Watson, et al (65) note a striking difference in the ability of Ayrshire and Shorthorn cows to produce a yellow colored butterfat. The Shorthorn group consistently secreted butterfat with a more intense yellow color, even though the same ration was fed to both groups. It appears, they conclude, that there is for each breed a "ceiling" value above which the depth of color of the butterfat does not rise, when fed unlimited amounts of carotene-rich feeds.

Baumann and Steenbock (4) studied the influence of breed on the carotene and vitamin A content of butter. The Ayrshire, Guernsey, Jersey, Holstein and Brown Swiss breeds were used in the trials. Two tests were made with all groups on a winter ration and one test with all groups on a green ration. The results are shown in Table II.

The data in Table II clearly shows that with the same feeding conditions, the milk fat of the Holstein and Ayrshire has more of its vitamin A activity present as true vitamin A and less as carotene. In the case of Jerseys and Guernseys the opposite is true. However, it should be understood that the total vitamin A potency of the different breeds, under the same dietary conditions, is about the same (4, 14, 68). Listed in the

TABLE II

CAROTENE AND VITAMIN A CONTENT OF BUTTERFAT
(in micrograms per gram of fat)

Conditions	Breed	No. of Cows	Carotene Range	Carotene Av.	Vitamin A Range	Vitamin A Av.
March	Ayrshire	4	4.0--5.9	4.8	6.1--11.5	8.4
Samples,	Guernsey	4	6.9-14.2	10.3	5.3-- 8.5	6.8
Winter	Holstein	6	3.8--7.2	5.2	5.9--13.0	10.2
Ration	Jersey	4	6.1--8.2	7.1	5.9-- 7.9	7.1
	Br. Swiss	4	3.9--8.8	6.0	6.75-10.0	7.8
June	Ayrshire	5	3.6--6.1	4.9	6.3-- 7.1	6.9
Samples,	Guernsey	6	5.9--9.4	7.8	2.1-- 6.6	5.1
Winter	Holstein	3	3.9--4.6	4.3	8.3--12.0	10.1
Ration	Jersey	6	4.1--9.2	5.5	4.2-- 6.0	5.3
	Br. Swiss	4	3.7--7.8	5.6	5.2-- 7.4	6.8
July	Ayshire	2	5.1--5.9	5.5	11.3--13.1	12.2
Samples,	Guernsey	2	15.7-18.2	17.0	7.5-- 9.4	8.5
Green	Holstein	2	6.1--7.2	6.6	13.4--16.9	15.1
Ration	Jersey	2	9.8-11.7	10.7	10.0--13.0	15.5
	Br. Swiss	2	7.4-12.3	9.8	12.0--15.6	13.8

order of the increasing ability to convert carotene to vitamin A, the breeds are Guernsey, Jersey, Ayrshire and Holstein (60).

Sutton and Krauss (59) have investigated the carotene content of butterfat from various breeds at different seasons. In the table below their data are tabulated:

TABLE III

CAROTENE CONTENT OF BUTTERFAT FROM VARIOUS BREEDS AT DIFFERENT SEASONS*

Breed	Winter-feeding	Summer-feeding	Composite
Ayrshire	1.45	4.70	1.75
Holstein	1.95	8.00	2.55
Jersey	2.05	12.10	3.20
Guernsey	3.45	20.50	9.80

*Expressed in milligrams of carotene per kilogram of fat.

In the above table the amount of carotene per unit of butterfat produced from each breed ranged in ascending order as shown. Although these figures indicate that these is considerable difference in carotene content among the breeds, Sutton and Krauss warn that on these results alone there is no basis for discrimination between milk of different breeds. Undoudtedly they have reference to other findings cited elsewhere in this paper (16, 4, 14, 68).

The effect of seasonal fluctuations of cartene and vitamin A on the welfare of human nutrition is very significant. To gain a better understanding of the effects of seasonal and regional feeding practices on the vitamin A potency of milk and butter produced in the United States, a recent nation-wide study was made (61). This study was begun in 1941 at the request of the Food and Nutrition Board of the National Research Council, and a Technical Committee which was appointed has recently reported the findings. The study gives a practical demonstration of the fact that the vitamin A potency of milk and butter depends upon the quantity of carotene in the cow's diet. The Technical Committee recommends the feeding of hays cured in such a way as to retain the green color and use of silages which have been properly preserved and stored. These roughages if harvested at the early stage of maturity are conducive to high carotene and vitamin A values in dairy products. That Committee stressed the necessity of improvement in pasture management and feeding methods.

Koehn (41) found that under Alabama conditions the total vitamin A activity of the milk decreased from 535 micrograms per quart in October to 200 micrograms in February. During the month of August it reached a peak of 1045 micrograms per quart and then fell rapidly to winter levels again.

Dornbush, et al (16) give the average vitamin A potency of market milk produced in winter as 372 micrograms per quart and 572 micrograms for that produced in summer.

According to Ashworth, et al (2) the carotene content of Washington creamery butter dropped from 7,400 International Units in May and June to 2,900 Units the following February. Idaho-produced butter varied from an average low of 12,499 I.U. in February to an average high of 19,281 I.U. in October (62). The results of work done in Arizona (19) show that butter produced in that State is quite uniform in vitamin A potency through the season. A range of from 15,000 to 20,000 I.U. per pound was found upon the examination of 25 samples. However, in Nebraska the seasonal fluctuation was very pronounced as shown by Hathaway and Davis (27). They observed that favorable climatic conditions can greatly increase the carotene content of milk and butter.

Ample experimental evidence has been presented concerning the effects of various diets on the carotene and vitamin A content of milk and butter. It has been shown conclusively that decreasing or increasing the carotene in the ration of the cow also causes like changes in the quantity of this substance in the milk produced by the cow. Whether other conditions besides the quantity of carotene in the feed affect the efficiency of its utilization by the cow has not been adequately investigated.

Green feeds, such as pastures, are known to be the best sources of carotene for the dairy ration. The problem seems to be in the providing of adequate year-round pastures.

Normally a cow gets all of her carotene from pasture and other roughages, which vary considerably in their vitamin A value. This variation may be due not only to difference in species of the plants, but also to the stage of maturity of the plants when pastured and harvested (32, 35, 10). Hodgson, et al (35) urge the making of hay before the plants reach maturity and while they are growing vigorously. They encourage the liberal use of nitrogen on forage crops, and state that the vigorous growth which is promoted is conducive to higher carotene content in the forage.

Guilbert (26) asserts that the degree of greeness of hays has serious limitations in indicating the relative carotene content. Hodgson, et al (36) also noted inconsistencies as to the relationship between these factors, but found that generally the greener the color of the hay the higher the carotene content. They found further, by comparison, that ensiling a forage crop was superior to haymaking practices in maintaining a higher level of carotene in the feed. These workers also studied the effectiveness of various roughages in supplying carotene to the butterfat when fed. Considering the effectiveness of home grown field-cured hay as 100, the results are as follows:

Roughage and its treatment	Relative carotene value of the B.F.
Hay, home grown, field cured	100
Hay, plus oat and pea silage (silo). . . .	175
Grass silage (silo).	188
Hay, plus grass silage (silo).	190
Oat and pea silage (silo).	199
Hay, plus grass silage (stack)	200
Grass silage (stack)	207
Pasture grass grazed	222

The manner in which hay crops are harvested and preserved greatly affect the quality. Carotene is lost or destroyed through bleaching, oxidation, leaching and by loss of leaves by shattering. Therefore, methods of hay-making and ensiling should be used that will reduce these losses (35).

Douglass, Tobiska and Vail (17) studied the changes in the vitamin A values of alfalfa hay. They summarize their work by stating that hay at the third cutting in the early bloom stage contains more carotene as a rule than that cut at other stages of growth, using similar curing methods. Hay cured in diffused light is superior to that cured in direct sunlight, according to their results. Snyder and Moore (58) made studies of the variation of carotene during the growing season of seven herbages which included alfalfa, bromegrass, corn leaves, oat plant, sudangrass and sweetclover. On the basis of their data they recommend the cutting of plants for hay at an early stage of maturity.

Moore (49) points out that green pastures may contain from 200 to 300 parts per million of carotene on the dry basis, whereas alfalfa hay which has undergone the curing process and has been stored, contains only about 15 to 50 parts per million. Russell (54) of the New Jersey station conducted an experiment investigating the contribution of alfalfa hay and corn silage to the vitamin A value of the dairy ration. According to that data, high grade alfalfa (field-cured), or machine-dried alfalfa and corn silage contributed 98 percent of the vitamin A value. Corn silage supplied from one-half to two-thirds of this value. In a previous experiment at this station by Russell, Taylor and Chichester (55) results revealed that machine-dried alfalfa has a higher vitamin A value than field-cured hay, often as much as 2 to 10 times as great. Under winter feeding timothy hay was not satisfactory in maintaining the vitamin A value of milk (33). Artifically cured hays (soybean and alfalfa) were superior to field-cured hays in their vitamin A values (32). Sudangrass was very satisfactory in increasing the carotene content of butterfat.

However, yellow corn cannot be fed in sufficient amounts to supply the necessary carotene (63).

Watson, Bishop, and Drummand (65) report that the inclusion of 50 percent of artificially dried grass in the concentrated ration of Shorthorn cows caused a distinct yellow color of the butterfat. The feeding of 40 pounds of A.I.V. fodder in the ration raised the color of the butterfat to a level comparable to that produced on the best pasture.

Koehn (41) in experiments designed to determine the effect of temporary pastures and certain roughages on the vitamin A content of milk, found that a pasture of Italian and abruzzi rye, crimson clover, and white Dutch clover, within two weeks increased the vitamin A content fourfold. Sorghum silage, permanent pasture and alfalfa hay (poor quality) had little effect on the vitamin A activity of the milk. Peanut and lespedeza hay caused only slight increases, whereas oat pasture doubled the vitamin A activity and rye pasture increased it threefold.

Several workers at Beltsville (47) determined that various kinds of roughages differ widely in their range of carotene content within grades. This is shown in the following tabulation, in which the carotene content is expressed in micrograms per gram as fed.

TABLE IV

THE AVERAGE CAROTENE CONTENT OF VARIOUS ROUGHAGES ACCORDING TO GRADE

Kind of feed	Average	Range
Alfalfa hay:		
Grade U. S. No. 1 in color.	43	19-121
Grade U. S. No. 2 in color.	15	12-20
Grade U. S. No. 3 in color.	4	1-11
Timothy hay:		
Grade U. S. No. 1 in color.	21	8-36
Grade U. S. No. 2 in color.	9	8-11
Grade U. S. No. 3 in color.	5	1-12
Corn stover (dry).	4	2-6
Corn silage.	14	1-40

The immediate affect of pasture in raising the vitamin A potency of Ohio milk was demonstrated by Krauss (42). He assayed butterfat from cows which had been on early spring pasture 16 days and found that the vitamin A potency was twice as potent as the butterfat obtained before the cows went on pasture. This rapid increase agrees favorably with the results of Baumann and co-workers (4), and with those of Booth and co-workers (7).

A comparison was made between the rotational and continual system of grazing to determine if either affected the carotene content of the pasture herbage or the milk produced therefrom. Neither of the systems caused any consistent differences in the carotene of the milk or the pasture plants (46).

Yapp (70) found that protein fed in concentrates from different sources has no effect on the color or carotene content of milk.

For normal calving, cows should receive during the last months of gestation 80 to 100 milligrams of carotene daily (12). These workers found that when U. S. No. 1 grade alfalfa hay was fed there was the usual number of normal calvings, while with the feeding of U. S. No. 3 grade alfalfa or timothy hay all calvings were abnormal where the cows had been on these rations for 5 months or more.

Very serious vitamin A deficiencies may be caused by long continued droughts (20, 49, 45). Not only does the growing plant show a rapid decrease in carotene during periods of little rainfall, but it has also been shown that hays in storage loose a large percentage during periods of high temperatures which accompany droughts. Thus Wiseman and others (35) at Beltsville present data based on tests of baled hay in storage at various temperatures, showing the loss of carotene in micrograms per gram of dry matter:

Storage temperatures	Loss of carotene
Above 67° F.	17.8
46° F. to 66° F.	6.6
Below 45° F.	2.6

The percentage of transfer of carotene in the diet to the vitamin A potency in the milk and butter has been investigated by various workers. All report that the percentage of transfer is very small regardless of the amount of carotene ingested. In all cases it was less than 5 percent, and generally ranged from less than 1 percent to 3.5 percent (54, 57, 1, 36, 3, 21).

Hodgson, et al (36) collected data as presented in the following table.

TABLE V

RELATIONSHIP BETWEEN THE AMOUNT OF CAROTENE IN ROUGHAGE AND THE AMOUNT SECRETED IN THE MILK

Kind of roughage	Average carotene content of roughage	Carotene content of butterfat
Hay ration	14.8 Mg	3.6 Units/gram
Hay and silage ration	57.0 Mg	6.5 " "
Grass silage ration	197.3 Mg	6.8 " "
Pasture ration	259.0 Mg	7.9 " "

The figures in the above table indicate that as carotene in the diet increases, no proportional increase of carotene occurs in the butterfat. The data would also suggest that there is an average minimum requirement of carotene for dairy cows, for the production of butterfat of maximum vitamin A value. Hilton, Wilbur, and Hauge (31) found that when the source of vitamin A in the ration was vitamin A per se (fish liver oil) the minimum requirement could be met with a daily intake of 200,000 Units, and that any amount above this caused no change in the level of vitamin A of the butterfat. Wilbur, Hilton, and Hauge (67) concluded from their results, that when artificially dried alfalfa was the source of carotene, approximately 550,000 Units were needed to restore the vitamin A potency of milk fat to its highest level.

Wiseman and Shepherd (69) show data indicating that a cow producing butter with an average potency of 10,000 I.U. per pound, requires 480 milligrams (actually 380 mg., according to their data) of carotene per day.

Stage of lactation has little or no effect on the vitamin A potency of milk or milk fat after the post-colostral period. However, the carotene content of colostrum is many times greater than in later milk, depending upon the diet of the cow.

The absorption of carotene from the intestinal tract may be considerably affected by the composition of the diet (1).

Carotene appears to be in some way related to the flavor of milk (9, 66, 24). The vitamin A activity of milk appears to not be effected by the treatment received in the manufacturing processes (37, 53).

A review of the literature relating to the effects of pasture management and feeding programs on the color and carotene of milk and butter, may be summarized as follows:

The yellow pigment carotene (pro-vitamin A) is mostly responsible for the yellow color of milk and milk fat; the seasonal flucuations which occur in the carotene of milk and butter is a result of carotene-deficient diets; the carotene of the plant varies with the species, stage of maturity, rainfall and possibly with the fertility level of the soil on which it is grown; cows consuming feeds low in carotene produce milk low in vitamin A potency, while the consumption of carotene-rich feeds results in the production of milk high in vitamin A values.

Carotene contributes from about 15 to 35 percent of the total vitamin A potency of milk, depending upon the diet and the breed; the Channel Island breeds secrete more carotene in their milk, but less vitamin A--while the other breeds secrete less carotene, but more vitamin A in their milk, resulting in very little difference in the total vitamin A potency of milk of the various breeds.

A cow normally derives her vitamin A from pasture and roughages in the form of carotene, and the feeding of other supplements has not been economically sound; methods of harvesting, curing and storing forages effect the carotene content of the resulting feed; the color of butter varies with the green color of feeds consumed.

There is evidence that the summer level of vitamin A potency in milk can be maintained during the winter by providing an abundant supply of carotene in the diet. This could be accomplished by the application of available knowledge concerning the improvement of pastures and the principles underlying the making of high quality hay.

CHAPTER III

EXPERIMENTAL

Selection of Herds

Studies were made on milk from ten local dairy herds supplying milk to the university creamery. Selection of herds was made with the idea of including in the study a number of herds representative of the average in the area. Each herd was managed under somewhat different feeding conditions. Although a number of breeds were represented, the Jersey breed predominated in all cases, except in the university herd. One-half the cows in this herd were Holstein. No effort was made to control any condition that might affect the results, but rather the study was conducted for the purpose of determining trends in natural color changes in butter and carotene content. The relation of these factors to various herd feeding practices was examined where possible.

Collection of Samples

About one quart of milk was collected from each herd from the daily milk delivery. Samples were taken in such manner that a composite for night and morning milkings was secured. Collections were made at two week intervals, beginning February 15 and ending June 30. Ten collections were made totaling 97 samples. When analyses were not made on the day of collection, the samples were refrigerated at 40° F. until the next day when they were made.

Method of Analysis

The method of analysis was essentially the same as that described by Boyer, et al (8). Modification of this method consisted of the following: Extension of the saponification time from 3 hours to 4 hours as previously described by Cannon (10). In order to bring the color readings within the sensitive range of the spectrophotometer, 15 ml. of petroleum ether was used instead of 10 ml., during the months when high carotenoid concentration occurred in the milk. In brief, the procedure was as follows:

Twenty milliliters of milk were mixed with 30 ml. of alcoholic potassium hydroxide in a separatory funnel and allowed to stand for 4 hours. The mixture was then extracted twice with ether. The ether extract was then washed with 75 ml. of distilled water, followed by a washing with 10 ml. of acidified alcoholic wash solution. Three milliliters of petroleum ether was added to reduce the water content. The ether extract was thereafter washed twice more with 10 ml. portions of acidified alcoholic wash solutions and allowed to stand for 15 to 20 minutes. Any water settling out was removed.

The extract was then transferred to a 75 ml. Pyrex tube and the solvents evaporated off under reduced pressure. The residue was then dissolved with 5.0 ml. of ether and then shaken with 5.0 ml. of saturated sodium chloride solution. Then 10.0 or 15.0 ml. of petroleum ether are added, depending on concentration desired (15 ml. of petroleum ether was used with all samples after April 28 analysis; two of the samples of the April 28 analysis required 15 ml. of petroleum ether in order to read accurately). The contents are shaken vigorously and allowed to stand several minutes until crystal clear. A 10 ml. aliquot was then transferred to a cuvette and the carotene concentration was measured by the Coleman spectrophotometer at 440 millimicrons wave length. The carotene content was determined from a standard curve based on readings with solutions of pure crystalline B-carotene.

Butterfat tests were made in duplicate on each composite sample by the Babcock method.

Color Determination

From each sample a portion of the cream was skimmed and the ten portions were combined and churned. The butter represented a composite for the ten herds for each period of carotene analysis. From these butter samples the determination of color changes during the experimental period was made. A total of eight butter samples were made, each corresponding to a period of analysis. The butter was stored as collected at -10°C. until the end of the experimental period. Two samples were disposed of by mistake which corresponded to the first and eighth periods (February 15 and April 28, respectively).

The determination of color changes in butter was made colorimetrically. This was accomplished in the following manner:

Adequate portions of each sample were melted in a hot water bath at 60° C. and transferred to a 50 ml. centrifuge tube. The water and curd was separated from the butter oil by centrifuging at 2000 R.P.M. for 5 minutes. One millileter of the oil was removed by a warm pipette and transferred to a cuvette containing 9 ml. of petroleum ether. The oil was thoroughly dissolved in the ether by shaking until the solution was crystal clear. From this solution color characteristics were determined with the Coleman spectrophotometer. A spectral transmission curve was plotted with the results shown in Figure 1. Maximum absorption occurred at 450 millimicrons. Therefore, this wave length was used for measuring the color of butter oil. Readings were made on all butter oil aliquots using the above concentration. Densities of each concentration were calculated using the following formula:

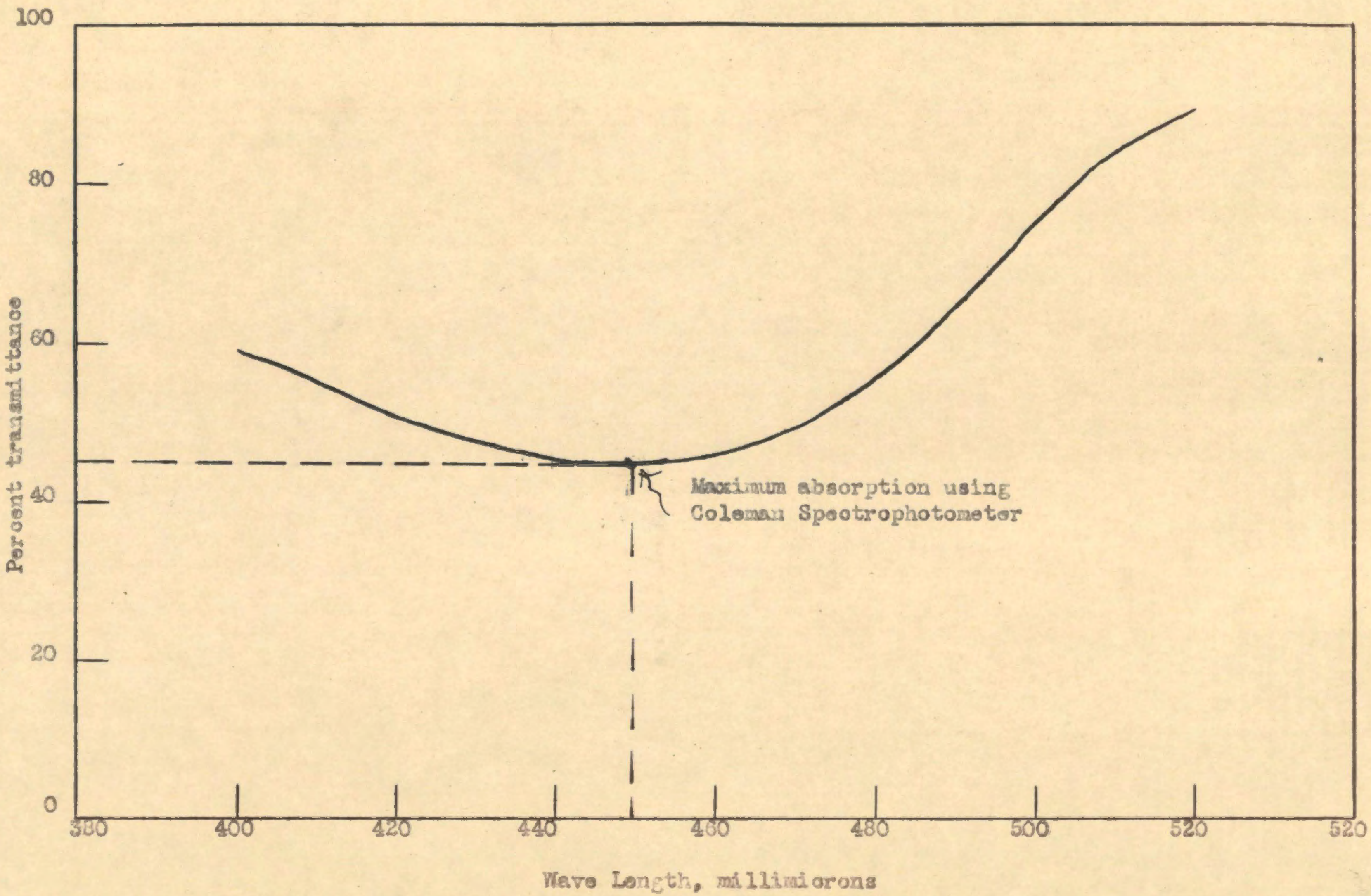

Figure 1. Curve showing wave length at which maximum absorption occurred using 1 ml. butterfat in 9 ml. of petroleum ether.

$$D = \text{Log } \frac{1}{T}$$

T equals the percentage of transmission as indicated by the galvanometer reading.

Pasture and Feeding Survey

A survey was made of the feeding conditions of each herd. Such contributing factors as breed, cows freshening and dried-off, were included.

CHAPTER IV

RESULTS

The color changes occurring in butterfat from ten herds from February 15 to June 30 are shown in Table VI. Color characteristics were determined by finding the density as explained in Chapter II. The density increased from .181 for March 1 sample to .431 for the April 28 sample, indicating an increase in carotenoid concentration in the butterfat during this period. After April 28 the concentration showed a gradual decrease to June 30. The increase in carotenoid concentration to a peak on April 28 was more rapid than the decrease thereafter.

The carotene content of milk from the ten herds for each 2 week interval is shown in Table VII. The results are expressed in micrograms of carotene per 100 ml. of milk. Differences in carotene content for the entire period with all herds considered ranged from 11 to 165 mcg. per 100 ml. Arithmetic averages for each herd varied from 53 to 78 mcg. per 100 ml. of milk, while a mean average of all herds was 64 mcg. Period averages of all herds showed considerable more variation, ranging from 36 to 103 mcg. per 100 ml. of milk. The March 16 period showed the lowest average and April 28 the highest, representing a 286 percent increase in carotene in one and a half months.

TABLE VI

COLOR CHARACTERISTICS OF BUTTERFAT PRODUCED
AT DIFFERENT PERIODS
FROM FEBRUARY 15 TO JUNE 30

Date	Sample No.	Percent Transmittance	Density*
3/ 1/49	2	66	.181
3/16/49	3	56	.252
3/31/49	4	51	.292
4/13/49	5	41	.387
4/28/49	6	37	.431
5/12/49	7	41	.387
6/16/49	9	47.5	.323
6/30/49	10	50.5	.297

* $D = \text{Log} \frac{1}{T}$; T = percent transmittance using Coleman spectrophotometer with wave length of 450 millimicrons. Note: Density of 1 percent transmittance equals 2.000, while the density of 100 percent equals zero.

TABLE VII

CAROTENE CONTENT OF MILK FROM TEN HERDS
(Micrograms per 100 ml. of milk)

Date	Herd Number										
	1	2	3	4	5	6	7	8	9	10	Average
2/15/49	37	42	48	51	65	44	61	34	--	--	48
3/ 1/49	33	28	37	36	60	34	26	28	68	57	41
3/16/49	18	17	38	11	63	48	--	20	66	45	36
3/31/49	35	49	39	34	72	48	68	28	78	60	51
4/13/49	63	70	94	70	103	82	105	54	100	72	81
4/28/49	79	73	118	85	165	116	118	71	110	97	103
5/12/49	85	82	40	99	74	80	69	90	65	80	75
5/27/49	70	79	61	65	71	97	77	71	75	70	74
6/16/49	59	71	64	89	59	63	85	56	74	68	69
6/30/49	49	45	52	52	51	80	75	75	70	74	62
Average	53	55	59	59	78	69	76	53	78	62	64

TABLE VIII

CAROTENE CONTENT OF BUTTERFAT FROM TEN HERDS
(Micrograms per gram butterfat)

Date	Herd number										
	1	2	3	4	5	6	7	8	9	10	Average
2/15/49	9.2	11.3	9.7	12.7	15.0	9.1	12.8	8.0	----	----	11.0
3/ 1/49	6.9	7.0	7.8	7.1	13.8	7.0	5.1	4.5	12.0	11.5	8.3
3/16/49	4.2	4.2	8.0	2.5	14.2	9.9	----	5.9	11.8	10.1	7.9
3/31/49	8.1	11.3	8.4	6.7	18.3	9.7	11.0	7.8	13.0	13.5	10.8
4/13/49	15.3	15.8	19.8	18.3	23.7	18.0	22.6	13.1	17.3	18.8	18.3
4/28/49	18.2	19.7	25.9	19.6	36.3	22.9	23.8	16.4	19.4	21.3	22.3
5/12/49	15.5	17.0	9.4	20.0	16.3	18.4	17.1	21.8	17.5	22.8	17.6
5/27/49	16.1	18.6	13.4	16.5	15.3	21.8	16.2	15.3	14.0	15.8	16.3
6/16/49	15.1	18.1	14.0	21.0	13.3	12.7	15.2	14.2	13.8	16.9	15.4
6/30/49	12.5	12.4	12.6	12.6	13.4	19.8	16.4	16.9	13.6	14.9	14.5
Average	12.1	13.5	12.9	13.7	18.0	14.9	15.6	12.4	14.7	16.2	14.2

TABLE IX

RECORD OF BUTTERFAT TESTS

Date	Herd Number									
	1	2	3	4	5	6	7	8	9	10
2/15/49	3.9	4.6	4.1	4.2	4.0	4.2	5.3	4.2	3.8	3.8
3/ 1/49	3.6	3.9	3.9	4.2	4.3	3.6	4.1	4.1	3.8	3.5
3/16/49	4.8	4.6	4.6	4.5	4.6	4.4	4.1	4.4	4.4	4.0
3/31/49	3.9	4.9	4.2	4.9	3.7	4.2	4.8	3.8	4.1	4.0
4/13/49	4.2	4.2	4.3	3.8	4.2	4.4	4.4	4.5	4.3	3.7
4/28/49	4.7	4.7	4.7	4.8	4.4	4.9	4.2	4.3	5.8	4.5
5/12/49	4.6	4.9	---	5.1	4.5	4.8	3.9	4.6	5.4	4.4
5/28/49	4.1	4.2	3.3	3.5	4.0	4.0	4.0	4.6	3.8	4.3
6/16/49	---	5.5	5.4	5.8	5.6	5.5	3.6	5.2	5.2	5.0
6/30/49	---	4.8	4.3	4.3	3.7	4.4	3.4	4.3	3.9	4.8

TABLE X

DESCRIPTION OF HERDS FROM FEBRUARY 15 TO APRIL 12

Herd	Number Cows	Predominating Breeds	Number of Cows Freshened	Number of Cows Dried off
1	15	Jersey	3	0
2	42	Holstein	4	1
		Jersey	9	1
3	12	Jersey	0	1
4	22	Jersey		
		Guernsey		
		Holstein	6	4
5	24	Jersey	6	4
6	28	Jersey	7	3
7	10	Jersey	8	1
8	11	Jersey	2	0
9	15	Jersey	2	0
10	10	Jersey	3	2

TABLE XI

RECORD OF PASTURE FEEDING *

Herd Number	Kind of temporary ** Pasture	Condition	Date Started
1	Oats, crimson clover, and ryegrass	excellent	4/49
2	Oats, crimson clover, and ryegrass	excellent	2/49
3	Oats	excellent	2/49
4	Crimson clover and ryegrass	good	3/49
5	Oats, barley, and vetch	good	4/49
6	Oats and crimson clover	good	2/49
7	Crimson clover	fair	2/49
8	None	-----	----
9	Crimson clover and rye	excellent	2/49
10	Crimson clover and rye	poor	2/49

* All herds except 2, 9, 10 had access to permanent pasture of bluegrass and white clover.

** Hours on pasture ranged from 3 to 8 hours per day, and averaged 6 hours.

TABLE XII

RECORD OF TYPE, QUALITY, AND AMOUNT OF ROUGHAGE FED PER DAY PER HEAD

Herd Number	Type	Quality	Estimated Pounds	Date On Or Off
1	Lesp. and oat hay	good	25	Off 4/10
2	Lesp. hay and	good	5	----
	corn-sorghum silage	very good	15	Off 4/1
3	Alfalfa hay	good	20	----
4	Alfalfa hay	good	5	Off 4/10
	Corn silage	good	15	Off 4/1
5	Lesp. hay	good	25	Off 4/15
	Alfalfa hay	good	25	On 4/15
6	Lesp. hay	good	5	Off 4/1
	Oat straw	good	5	Off 4/1
	Corn silage	fair	25	Off 5/2
7	Lesp. hay	fair	25	----
8	Lesp. hay	good	25	Off 4/15
9	Oat hay	good	4	On 2/1
10	Alfalfa hay	fair	10	On 4/1
	Lesp. hay	fair	10	Off 4/1

TABLE XIII

RECORD OF KIND AND AMOUNT OF PROTEIN SUPPLEMENT
PER DAY PER HEAD

Herd Number	Kind	Amount	Months Fed
1	20% dairy mix	4-6 lbs.	All winter
2	Corn, oats, wheat bran, cottonseed meal	12 lbs.	February-May
3	Crushed corn, 20% dairy mix	9 lbs.	All year
4	Cottonseed meal	3 lbs.	All year
5	Oats, barley, citrus pulk, vetch, 32% dairy mix, soybean oil meal, cottonseed meal	9-10 lbs.	All year
6	Cottonseed meal, crushed corn bran	5-6 lbs.	All winter
7	18% dairy mix	6 lbs.	All year
8	18% dairy mix	10 lbs.	All year
9	32% dairy mix, corn, wheat, bran cottonseed meal, soybean oil meal, ground alfalfa	7 lbs.	Since December
10	Cornmeal, wheat meal	---	All winter

The carotene content per gram of butterfat for each sample is given in Table VIII. Variations are quite consistent with those of milk. Herd 4 for the March 16 period showed the lowest amount of carotene, while herd 5 for the April 28 period showed the highest. The content was 2.5 mcg. and 36.3 mcg. of carotene per gram of butterfat respectively. Arithmetic averages for each herd ranged from 12.1 mcg. for herd 1 to 18.0 mcg. for herd 5, with a mean average for all herds of 14.2 mcg. per gram butterfat. Period averages increased from 7.9 mcg. on March 16 to 23.3 mcg. on April 28, representing a 282 percent increase during this period.

Table IX shows a record of butterfat percentages for each sample analyzed, which varied from period to period.

A description of the ten herds studied is presented in Table X.

A survey of the feeding conditions of each herd was made April 12. The findings represent estimates and may offer the explanation of possible variations caused by feeding practices. Presented in Table XI is a record of the pasture conditions for each herd. All herds had access to winter pasture except herd 8. Due to favorable climatic conditions during the winter most pastures were grazed much earlier than under usual conditions. The quality of roughage fed ranged from fair to good. Alfalfa and lespedeza were the principal hays fed. Three herds were fed silage in addition to hay. A record of roughage feeding is shown in Table XII. The grain and protein supplement feeding is shown in Table XIII, indicating most herds were fed an 18020 percent protein ration in proportion to daily milk production.

CHAPTER V

DISCUSSION

Although the conditions of this study were not controlled, the results of the experiment indicate very definite trends in the effects of transition from winter to summer feeding on the color and carotene content of milk and butter. The data, when analyzed, demonstrate very clearly the correlation of carotene content to the depth or intensity of color in butterfat. Figure 2 shows graphically a comparison of color changes and variation in carotene content of butterfat occurring during the experiment. It can readily be seen that a positive correlation exists, suggesting that the depth of color occurring in butter during the season may be useful in indicating its vitamin A potency. Fieger and Lewis (20), Watson, et al (65), and Hodgson and co-workers (36) have suggested that the color of butter may be used to indicate roughly its vitamin A potency. During the period of carotene increase the color of the butterfat was paralleled closely by an increase in depth of color, but after the peak was reached the carotene content decreased more rapidly during the following two weeks. Thereafter the color intensity decreased more rapidly.

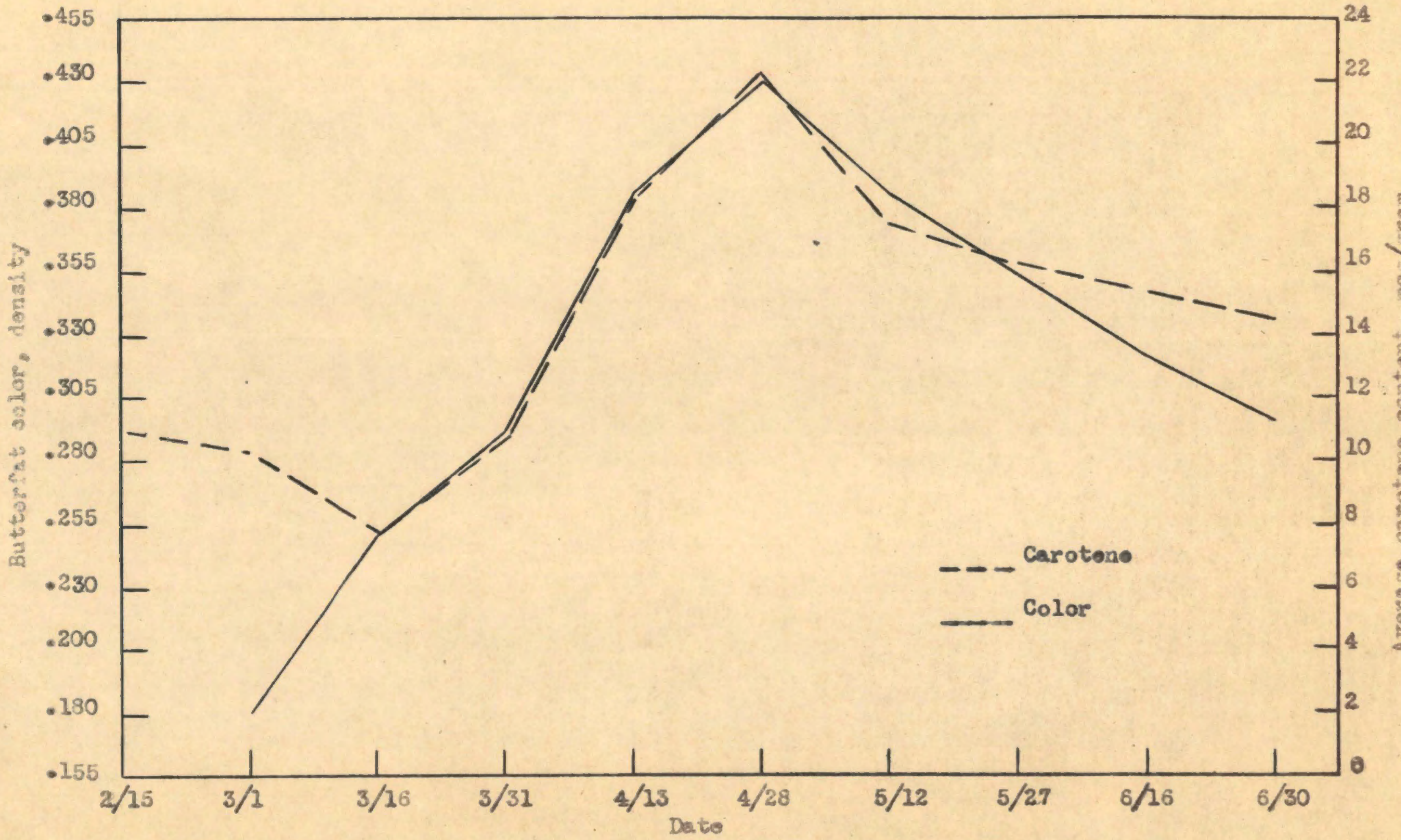

Figure 2. Comparison of color changes in butterfat and its carotene content.

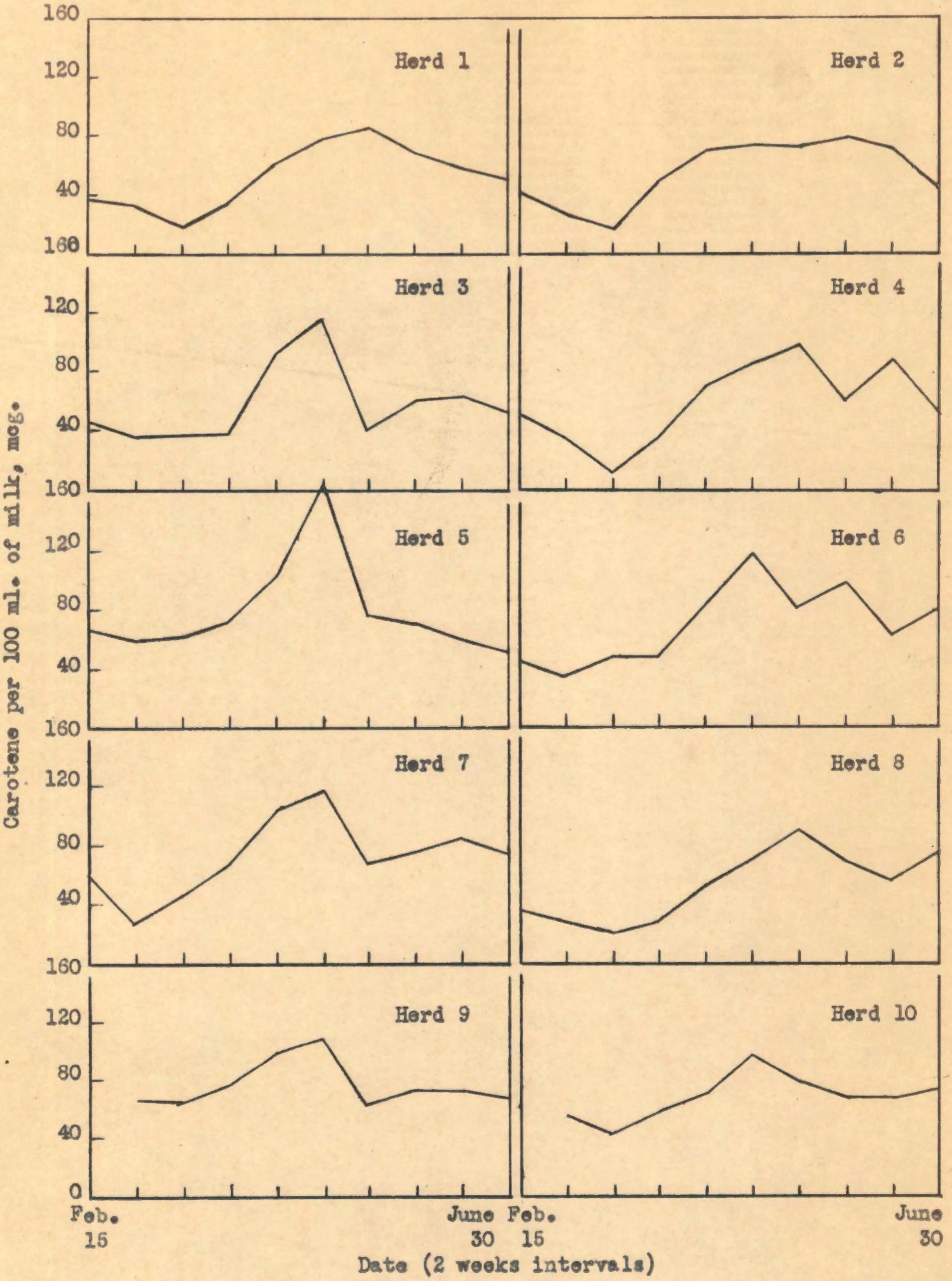

Figure 3. Variation in carotene content per 100 ml. of milk from February 15 to June 30.

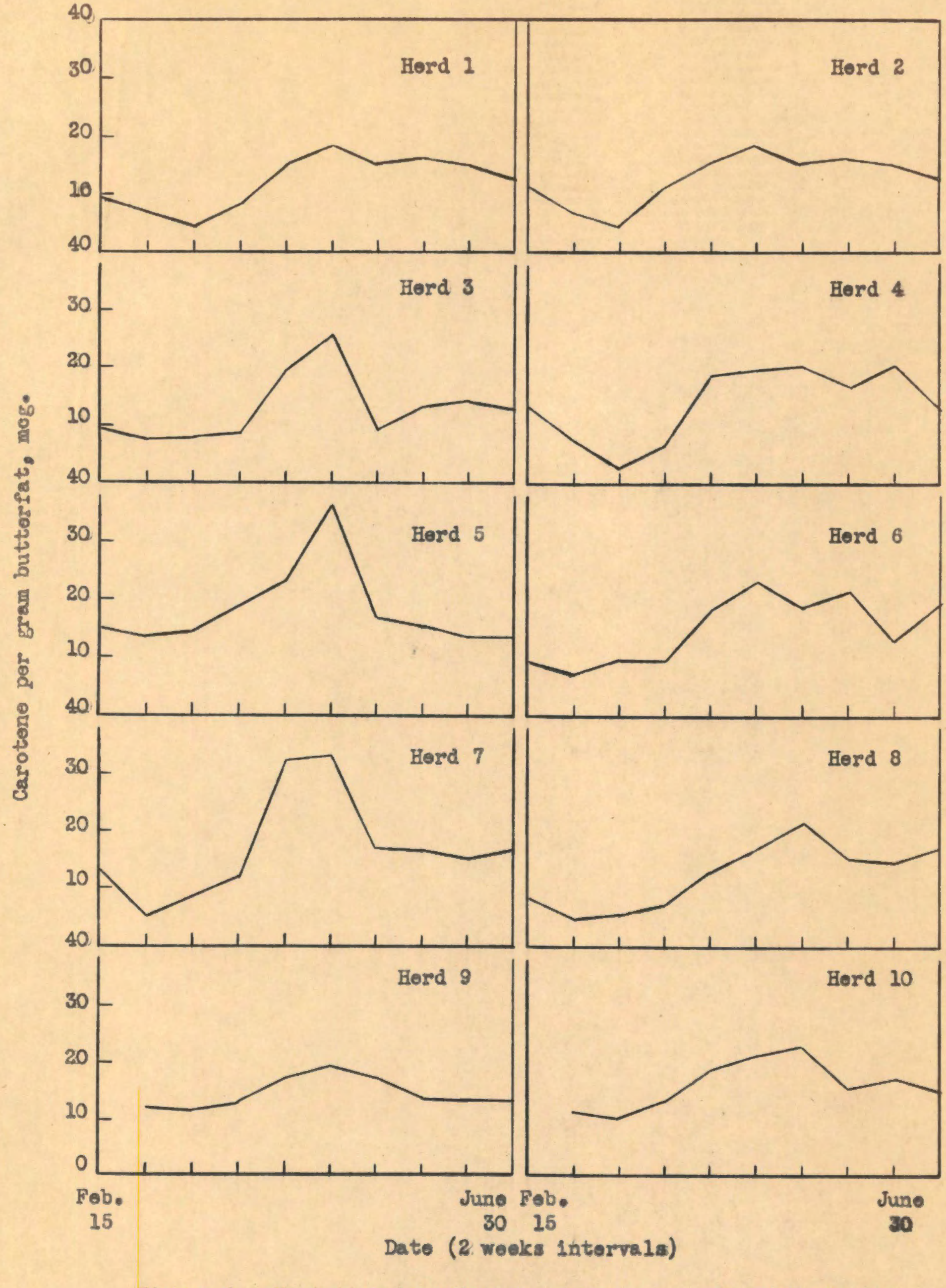

Figure 4. Variation in carotene content per gram of butterfat from February to June 30.

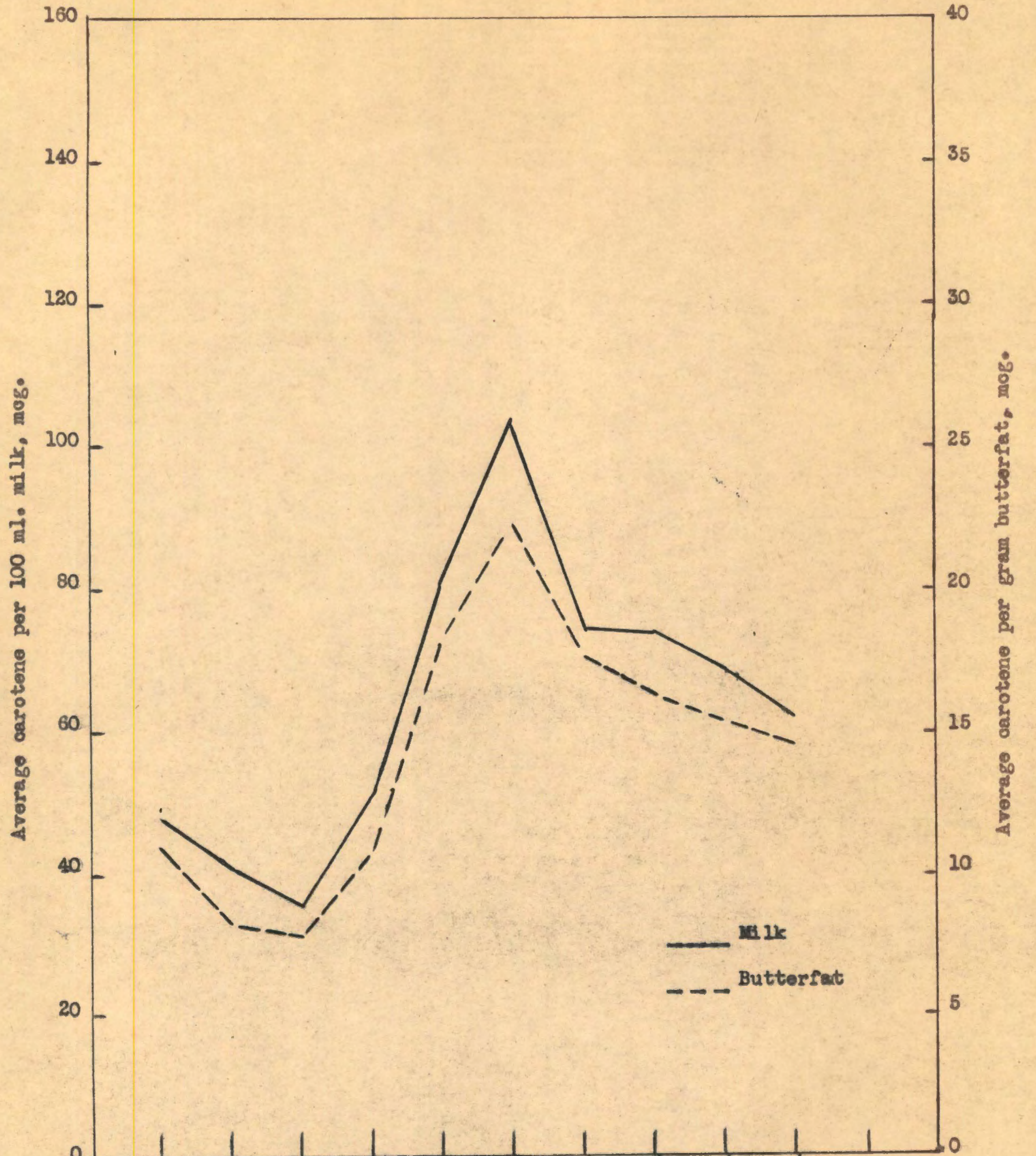

Figure 5. Comparison of the average carotene content of milk and butterfat from February 15 to June 30.

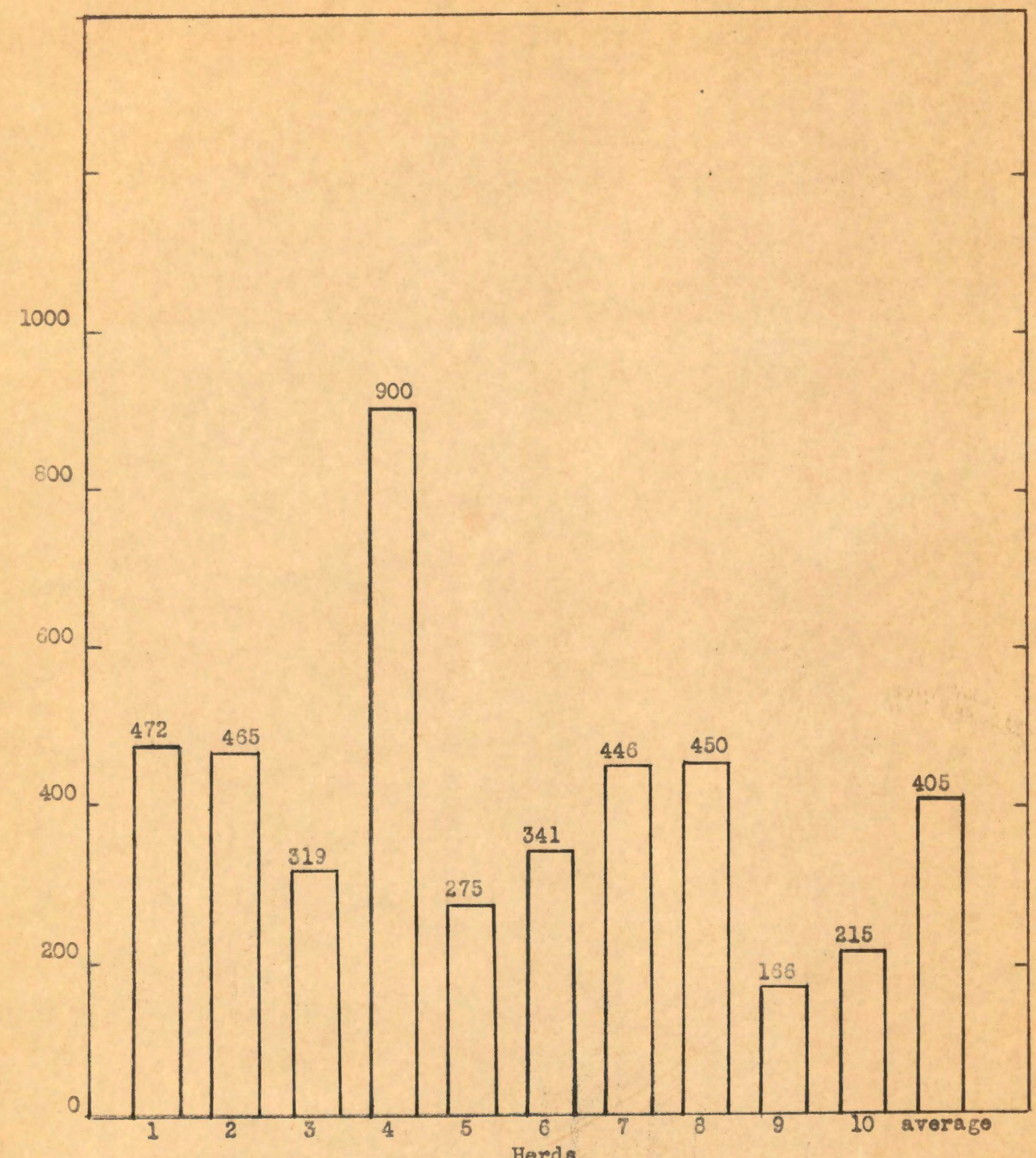

Figure 6. The percentage of increase in carotene content of milk during the period of transition from winter-feeding to summer-feeding of cows.

The increases in both color intensity and carotene content of butterfat occurred coincident to the turning of cows on pasture. There is no evidence that a maximum level of carotene in the butterfat was reached during this experimental period. This is in agreement with the results reported by Koehn (41).

The carotene values in milk clearly reflect the changes in feeding conditions that occur when cows are changed from winter to summer feeding. A study of the variations for each herd as shown in Figure 3 typify these changes. The milk of most herds show a slight decline in carotene from February 15 to March 16, during which time most herds were on winter feeding. This decline is probably due to the decreasing quality caused by storage of the roughage being fed. During this time lespedeza hay was the principal roughage fed.

Table XI shows that most herds were on pasture by March or before and at this time an increase in carotene was apparent as shown in Figure 3. For most herds the carotene increase started about the middle of March, but the rapid increases occurred during April when pastures were at their best. Milk from some herds showed minor fluctuations in carotene content during the months of May and June. During these months intermittent periods of rainfall tended to revive pastures and consequently the carotene was increased.

Herd 5 and 9 were found to have the highest average values for carotene per 100 ml. of milk. Both herds averaged 78 mcg. per 100 ml. The pasture conditions for these herds was superior to all others, except possibly that of herd 2. Herd 2 averaged 55 mcg. per 100 ml. of milk, which was comparatively low. This is largely due to the fact that half the cows in this herd were Holsteins. This breed normally converts more of the carotene into the colorless vitamin A. Dornbush, et al (16) and Baumann and Steenbock (4) and others have shown conclusively that the Holstein milk has more of its vitamin A activity present as true vitamin A and less as carotene.

Herds 1 and 8 produced milk of the lowest average carotene value as compared with the other herds. Both herds had an average value of 53 mcg. per 100 ml. milk. The study made showed that the feeding and pasture conditions of these herds were inadequate, although herd 1 had access to an excellent pasture in April, the acreage was too small to carry the herd for any length of time. Herd 8 had no temporary pasture and good permanent pasture was available only after April.

Figure 4 shows the variations of carotene per gram of butterfat for each herd. It can be seen that the changes are similar to those in Figure 3. However, herd

10 shows a higher carotene content per gram of butterfat than herd 9, which showed a higher content per 100 ml. of milk. This difference is due to the higher fat tests of herd 9, and would indicate that butter made from milk of this herd would contain less carotene than that made from herd 10.

Figure 5 shows for each 2 week period a comparison of the average carotene content of milk and of butterfat for all herds. This comparison demonstrates the variation of carotene in butterfat as influenced by the fat percentage in an aliquot of milk. The higher the fat test the lower the carotene concentration, assuming the carotene content per volume of milk to be the same.

Maintaining a uniform level of carotene and vitamin A during the year appears to be the problem needing most attention as shown by the results of this study. On the average carotene content of milk from the ten herds increased about fourfold. Some herds show a greater percentage of increase than others. Figure 6 shows the percent increase in carotene content per 100 ml. milk for each herd calculated from lowest to highest carotene values obtained during the experiment. Herd 4 milk increased in carotene content 900 percent while herd 9 increased only 166 percent. These great increases resulting from summer feeding emphasize the inadequacy of winter feeding practices in maintaining a high level of vitamin A potency in milk.

CHAPTER VI

SUMMARY

A study was made of the color and carotene changes occurring in milk and butter during the period of transition from winter to summer pastures.

The milk of ten herds supplying milk to the university creamery was analyzed for carotene at 2 week intervals, beginning February 15 and ending June 30. Color determinations were made on butter composites representing all herds for each of the two week intervals during the experiment.

The color changes were in correlation with the carotene changes during the experimental period, indicating that the color of butter or milk may be a means of roughly estimating vitamin A potency.

Color intensity and carotene content of butterfat increased coincident to the turning of cows on productive spring pastures.

The carotene content of the milk from the various herds varied considerably, ranging from 11 to 165 mcg. per 100 ml. of milk during the experiment. A mean average for all herds was 64 mcg. per 100 ml., and period averages ranged from 36 mcg. to 103 mcg. per 100 ml. of milk.

The carotene content of the butterfat per gram was calculated for each sample of milk. Variations were similar to those occurring in the original milk. The average carotene content for all herds for the entire period was 14.2 mcg. per gram of butterfat. Herd averages ranged from 12.1 to 18.0 mcg. per gram butterfat. Period averages showed a greater variation, ranging from 7.9 mcg. for March 16 to 23.3 mcg. on April 28.

The carotene changes in milk and butterfat clearly reflect the feeding conditions of each herd. The milk of most herds showed a decrease in carotene during the latter part of the winter feeding period, but a marked increase resulted when cows had access to better pastures during March and April. The percentage increase in carotene content of milk for each herd varied from 900 percent for herd 4 to only 166 percent for herd 9. These great fluctuations in the carotene content of milk during the season suggest the desirability of the improving of seasonal feeding conditions of herds producing milk for human consumption.

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