A STUDY OF THE VITAMIN A CONTENT OF COLOSTRUM AND MILK OF DAIRY COWS

by

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INTRODUCTION

Milk is now recognized as an important source of vitamin A. Colostrum, the first milk secreted by a mammal after parturition, has long been considered important for the young of the species, but its value as a source of vitamin A, more potent than normal milk, has only recently commanded attention. It seemed of interest, therefore, to compare the vitamin A content of colostrum and of normal milk produced by individual cows with the total vitamin A yield per cow per day at different stages of lactation.

REVIEW OF LITERATURE

In 1921 Drummond and co-workers (5) found that rats fed colostrum in addition to a vitamin A-free diet made more rapid growth than those fed normal milk in the same amounts. They reported that colostrum was rich in vitamin A, and that there was a gradual fall in the amount of vitamin A in the secretion as the colostrum was replaced by the normal milk. These preliminary experiments also indicated that milk obtained from Jersey cows and closely related breeds tended to be richer in vitamin A than that of Shorthorn and Black Angus cows.

Later, in 1931, Davis and Hathaway (4) used the Sherman and Munsell rat growth method to determine the vitamin A content of pooled samples of milk from Holstein, Ayrshire, Jersey and Guernsey cows. No significant differences were found between the vitamin A content of the milks of these breeds. In 1933 Wilbur, Hilton and Hauge (16) tested biologically the vitamin A activity of samples of butter made from the milk of 4 Guernsey and 2 Ayrshire cows. These authors reported that the vitamin A potencies of these samples of butter were similar without regard to the breed of cow or the color of the butter. In the same year Hathaway and Davis (6) again studied the vitamin A content of milk from different breeds of cows and found that the vitamin A content is largely associated with the butterfat, that the standardization of the butterfat content of the milk by addition of skim milk reduces the vitamin A potency, and, that Holstein cream contains more vitamin A than Jersey cream.

In 1933 Baumann and Steenbock (1) reported on the carotene and vitamin A content of butter, using spectrographic methods of analysis. Carotene accounted for only

15 per cent of the total biological activity of the butter. Variations in carotene and vitamin A contents were seasonal and regular, the largest amounts appearing in the summer butter. Later, Baumann, Steenbock, Beeson, and Rupel (2) used much the same methods to study the carotene and vitamin A contents of butter made from pooled milk produced by Holstein, Ayrshire, Jersey, Guernsey and Brown Swiss cows, all on the same ration. They found that Jersey and Guernsey butters contained larger proportions of carotene and smaller amounts of vitamin A while the Holstein and Ayrshire butters contained little carotene and more vitamin A than did the other samples. There was little difference in the total activity of the butter produced from the milks of the different breeds. Semb, Baumann and Steenbock (10) used spectroscopic methods to study butterfat prepared from colostrum produced by cows on a uniform ration. The values in carotene and vitamin A fell rapidly during the first week of lactation, after which the decreases were slow. Breed tendencies were not definite, although the carotene content of colostrum from Guernseys was markedly higher than that from the other breeds. Carotene in the blood plasma and vitamin A storage were considered.. It is suggested that the high concentration of

carotene and vitamin A in the butterfat of colostrum might be attributed to an accumulation of these factors in the tissues during the later phases of pregnancy when the milk flow has ceased. In 1934 Dann (3) studied samples of colostrum of 14 individual dairy Shorthorns receiving the same ration, which contained a good supply of carotene. Carotene and vitamin A were determined with a Lovibond tintometer and the richest samples were also assayed biologically. For every animal but one the colostrum had a maximum vitamin A content immediately after birth of the calf or in the next 12 hours. Experiments while the cows were on winter feed indicated that the colostrum is enriched with vitamin A at the expense of the large store in the body. Heifers generally produced richer colostrum than cows, probably because the body reserves have not been depleted by previous lactation periods. Dann concluded that the vitamin A content of colostrum may be 10 to 100 times that of the later milk of the same cow, independent of season. He said that on the first day of life the calf receives supplies of vitamin A greater than the later milk could give in 20 to 50 days.

In 1932 Moore (7) concluded that carotene must be changed into vitamin A in the body of the cow, an herbivorous animal, because he found large amounts of vitamin A in the liver and in the milk fat, whereas he found only small amounts of carotene in the body fat. This is consistent with the finding that feeding large amounts of carrots to the cow results in an increase in the carotene and also in the vitamin A content of the milk fat.

PROCEDURE

Colostrum and milk for these experiments were obtained from cows of the dairy herd, Department of Dairy Husbandry, Kansas State College of Agriculture and Applied Science. These cows were fed the regular herd ration, which included a good quality alfalfa hay, Atlas sorgo or corn silage, and a grain mixture of corn, bran and cotton seed meal. All the cows used in these tests freshened in the spring of the year. They had access to limited rye pasture in April and early May.

Composite samples from the 24-hour yield of colostrum or milk from individual cows representing Guernsey, Ayrshire and Holstein breeds were saved by the Department of Dairy Husbandry through the courtesy of W. H. Riddell, and stored at 0° centigrade until needed for the experiment. According to Osborne and Mendel (8) butterfat may be stored at a temperature of $8^{\circ} - 18^{\circ}$ centigrade for at least as long as a year with no destructive effects on the vitamin A content. The total yield of milk, in pounds, the per cent of butterfat and the per cent of total solids were determined for the individual cow. The original plan called for taking samples on the first, second, fourth, seventh, fourteenth and twenty-eighth days of lactation. Unavoidable circumstances altered the time of the collection of the samples somewhat. Table 1 gives details concerning the cows used, the dates of collection of samples, the yields, and the percentages of butterfat and total solids, as well as calculated total yields of butterfat and total solids.

Biological assay is no longer necessary in estimating the vitamin A activity of a product. The yellow color of carotene and the blue color of antimony trichloride with vitamin A have been used to estimate the amounts of carotene and vitamin A present, in spite of the fact that the blue color is not specific for vitamin A. For example, Dann (3) used the Lovibond tintometer to determine the yellow units of carotene and the blue units of vitamin A in samples of colostrum and milk. Dann mentioned that this method measured carotene and xanthophyll together but that the error was small, with only about 7 per cent of the yellow color due to xanthophyll. He stated that this material need not be removed because the error in the colorimetric method used would be greater than that due to this amount of xanthophyll. The use of more painstaking spectroscopic methods marks recent advances in the technique of determinations of vitamin A activity. For example, Semb, Baumann and Steenbock (10) reported carotene readings made with a spectrophotometer, and spectrophotometric determinations of vitamin A made with a quartz spectrograph.

Biological methods of assay continue to be used for estimations of the total vitamin A value of a product, when it is desirable not to subject the sample to unusual processes which might change the vitamin A activity. The prolonged biological methods have lost popularity where quick results are necessary. On the other hand, animal experiments provide results not dependent upon the assumption that the total vitamin A activity of a material may be accounted for by the vitamin A plus the β-carotene present. Many laboratories have used the biological method of Sherman and Munsell (12). A modification of this, the single feeding method of Sherman and Todhunter (14), presents advantages which led to its selection for these experiments.

Rats from Wistar Institute stock were fed the diet described by Sherman and Crocker (11) which consisted of:

Dried whole milk - 1 part

Ground whole wheat - 2 parts

Sodium chloride 2 per cent of the weight of the wheat

This food and distilled water were supplied ad libitum. The young rats were weaned when 38 to 40 grams in weight and placed on the vitamin A-free diet recommended by Sherman and Munsell (12). The diet contains:

Vitamin A-free casein	- 18 per cent
Dried brewer's yeast	- 10 per cent
Osborne and Mendel salt mixture	- 4 per cent
Cornstarch	- 67 per cent
Sodium chloride	- 1 per cent

The Osborne and Mendel salt mixture was made by a short cut method, according to the suggestions of Wesson (15). As recommended by the U. S. P. and Druggists Committee (9) Squibbs' viosterol was used to furnish the vitamin D. The amount fed was 0.9 gram viosterol to 1,000 grams of food. The original recommendation was for "Viosterol in Oil

250 D", which is approximately equivalent to the viosterol in oil now on the market, containing 10,000 U. S. P. vitamin D units per gram.

During the first part of the depletion period, the animals were kept in groups of 5 or less in a cage with raised wire floors. The rats were weighed individually at frequent intervals and were placed in separate cages and weighed daily as the end of the depletion period approached. When the weight of the rat remained stationary for a week or the rat developed xerophthalmia, it was considered depleted and ready for the experiment. The depleted animals were divided into groups comparable as to weight, sex and litter. Weekly weighings of all rats were made during the experimental period, which lasted from the time of depletion until death of the animal.

Carotene (International Standard for vitamin A, held for the Health Organization of the League of Nations by the National Institute for Medical Research, Hampstead, London, N. W. 3) was secured through the Bureau of Chemistry and Soils, United States Department of Agriculture. The carotene was dissolved in cottonseed oil and stored in the dark below 0° centigrade, until needed. The β-carotene content of this solution was determined in the spectrophotometer, using the value for log I/I_0 of 0.44 as given by Baumann and Steenbock (1) for a solution containing 0.002 mg. of carotene per cc. of cottonseed oil measured at a depth of 1 cm. The carotene was given in single feedings to 2 groups of rats. The first group received 17 γ pure β-carotene or 28.3 International units, and the second group, 34 γ pure β-carotene or 56.6 International units. A γ is equal to 0.001 milligram or 0.000001 gram. The weight records of these animals furnished data for the carotene reference curves.

A group of negative control rats was maintained, matched as for the other series but receiving only water and the basal vitamin A-free diet.

Six groups of rats were used for testing the samples of colostrum and milk from each cow. Ten or more animals were started in each group, but a few were later dropped from consideration, as they were in some manner not suited to the experiment. In a few instances the rat refused to eat the weighed portion of sample presented to it, as, for example, some of those fed on the colostrum of Maggie, which showed presence of blood. In other instances the rats appeared to have been depleted too much or too little. Table 2 gives data concerning the size of portion presented for a single feeding and the number of rats supplying acceptable data in each group.

Weight records of the animals were used to compile data for composite growth curves. According to the suggestion of Sherman and Todhunter (14), the curves were plotted on cross section paper, 10 squares to the inch, (figure 1), and a planimeter used for measuring the areas. For each area the base line was the horizontal line representing the weight of the negative control rats at one week. The left boundary was the curve of the first week for the negative control animals and the remainder of the boundary was the composite curve of the group in question. Broken lines indicate figures for less than the entire group, as one or more animals died, and the end point of each curve represents the average weight and survival in days of all the members of the group.

Figure 1. Average gain curves for animals on single feedings of different materials.

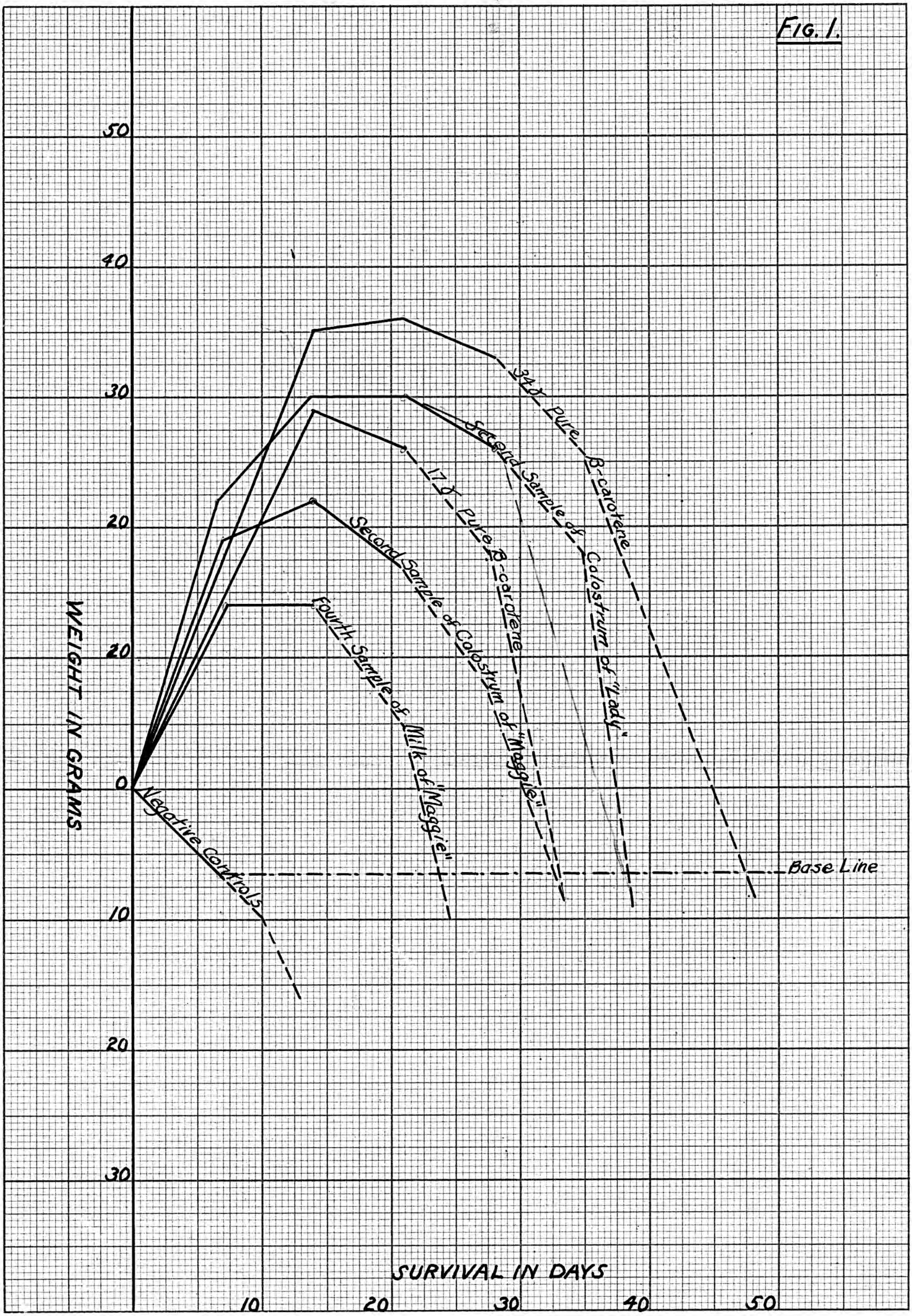


FIG. 1.
WEIGHT IN GRAMS
SURVIVAL IN DAYS
50
40
30
20
20
0
10
20
30
10
20
30
40
50
34 γ Pure β-carotene
Second Sample of Colostrum of "Lady"
17 γ Pure β-carotene
Second Sample of Colostrum of "Maggie"
Fourth Sample of Milk of "Maggie"
Negative Controls
Base Line

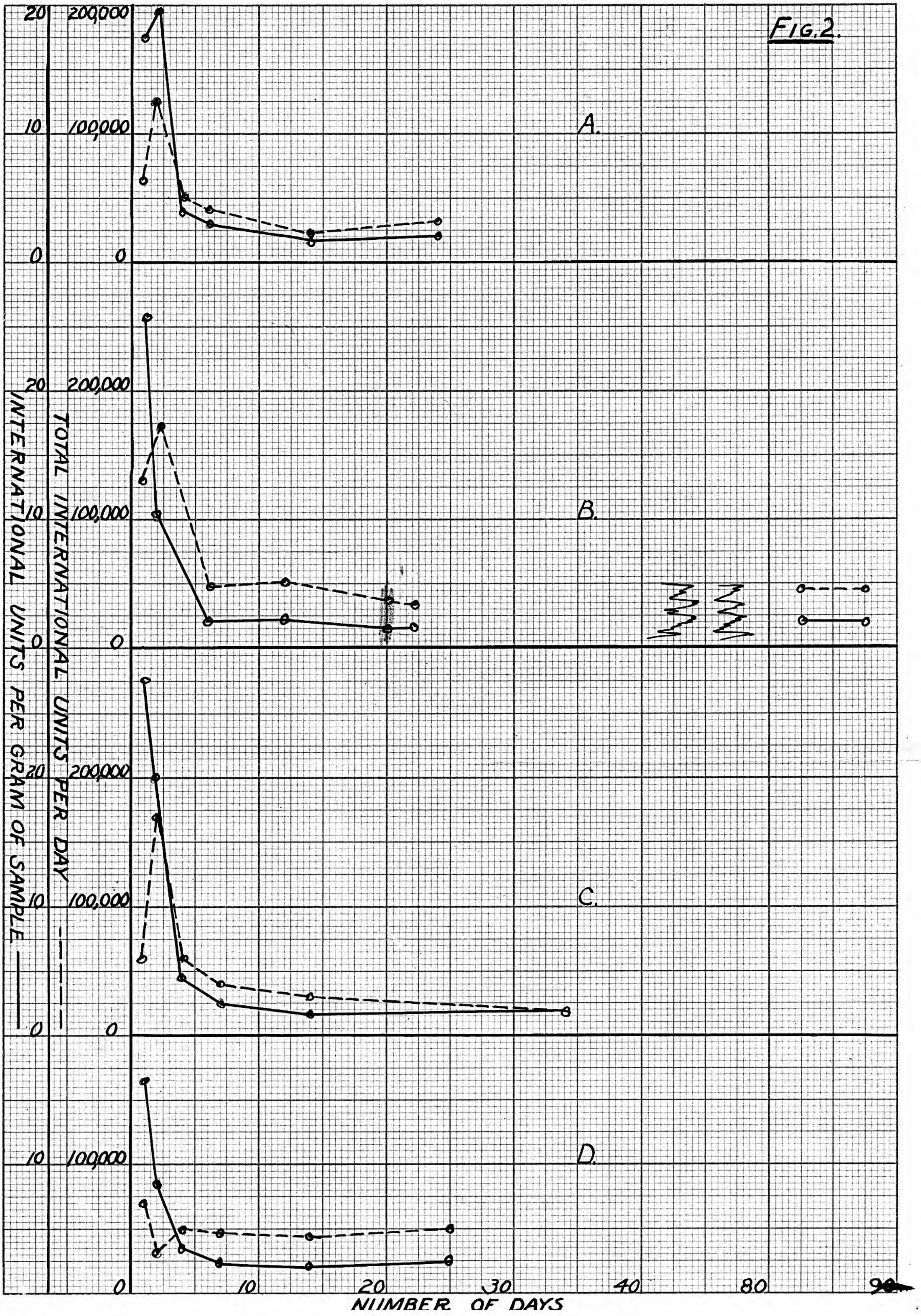
Figure 2. A comparison of vitamin A activity per gram of sample compared with the vitamin A activity of total yield per day.

A - Crystal

B - Maggie

C - Lady

D - Ella



INTERNATIONAL UNITS PER GRAM OF SAMPLE ——
TOTAL INTERNATIONAL UNITS PER DAY - - - -
20
200,000
10
100,000
0
A.
B.
C.
D.
10
20
30
40
80
90
NUMBER OF DAYS

Fig. 2.

Table 1. Records of cows used in experiment.

Cow	Date	Fat per cent	Total solids per cent	Total yield milk pounds	Total yield fat pounds	Total yield solids pounds
Elmbar 241	3- 4-35	5.1	22.76	8.0	0.41	1.82
Crystal	3- 5-35	9.0	21.92	13.9	1.24	3.02
3 yrs. old	3- 7-35	4.8	15.05	28.5	1.36	4.29
2nd calf	3-10-35	4.8	14.41	31.5	1.51	4.54
Ayrshire	3-17-35	4.3	13.28	32.5	1.40	4.32
	3-28-35	4.2	13.10	34.6	1.45	4.53
S. L.	5- 8-35	3.4	22.66	11.0	0.37	2.49
Maggie 220	5- 9-35	5.1	19.98	32.0	1.63	6.39
7 yrs. old	5-13-35	4.8	16.89	54.7	2.63	9.24
5th calf	5-19-35	5.0	14.19	55.8	2.79	7.92
Ayrshire	6- 1-35	4.8	12.77	59.2	2.84	7.56
	~~7-31-35~~	~~4.5~~	~~12.07~~	~~46.7~~	~~2.10~~	~~5.64~~
E. M. V.	5-26-35	5.2	20.11	5.2	0.27	1.05
Lady 471	5-27-35	5.1	15.37	18.5	0.94	2.84
6 yrs. old	5-29-35	4.5	13.69	31.6	1.42	4.33
4th calf	6- 3-35	5.3	14.90	32.8	1.74	4.89
Guernsey	6-10-35	4.7	13.65	33.4	1.57	4.56
	6-30-35	4.2	12.84	32.8	1.38	4.21
C. P. V.	2- 3-35	3.8	17.27	9.5	0.36	1.64
Ella 190	2- 4-35	3.9	12.76	7.4*	0.29*	0.94*
7 yrs. old	2- 6-35	4.9	13.91	37.6	1.84	5.23
5th calf	2- 9-35	5.7	14.54	42.2	2.41	6.14
Holstein	2-16-35	4.7	13.06	47.9	2.25	6.26
	2-28-35	4.6	12.23	44.0	2.02	5.38

* 7.0 pounds at morning milking and only 0.4 pounds at evening milking.

Table 2. Summary of data from rat feeding experiments.

Cows	Amt. fed: grams	Date produced	Rats used: No.	Rats used: Av. Wt. when depleted. grs.	Area in: sq. in.	Sq. in.: for each gram fed	Value per gram: β-carotene	Value per gram: I.U.	Yield for day: grams	Yield of vitamin A per day, computed: β-carotene	Yield of vitamin A per day, computed: I.U.
	2	3- 4-35	8	96	8.88	4.44	10.52	17.49	3629	38,177	63,471
	2	3- 5-35	9	92	9.94	4.97	11.78	19.58	6260	73,743	122,571
Elmbar	7	3- 7-35	10	93	7.03	1.00	2.73	3.84	12928	35,293	50,936
Crystal	15	3-10-35	7	94	10.63	0.71	1.68	2.80	14288	24,004	40,006
241	20	3-17-35	6	90	8.27	0.41	0.97	1.62	14742	14,300	23,882
	20	3-28-35	6	87	11.10	0.56	1.33	2.21	15695	20,874	34,686
	2	5- 8-35	9	96	12.95	6.48	15.36	25.53	4990	76,646	127,395
	2	5- 9-35	7	97	5.82	2.91	6.90	11.47	14515	100,154	166,487
S. L.	7	5-13-35	7	92	3.37	0.48	1.14	1.89	24812	28,286	46,895
Maggie	15	5-19-35	6	95	7.99	0.53	1.26	2.09	25311	31,892	52,900
220	20	6- 1-35	7	90	7.07	0.35	0.83	1.38	26853	22,288	37,057
	20	7-31-35	6	90	10.37	0.52	1.23	2.05	21183	26,055	43,525
	2	5-26-35	8	94	13.89	6.95	16.47	27.38	2359	38,853	64,589
	2	5-27-35	10	92	10.39	5.20	12.32	20.49	8392	103,389	171,952
	7	5-29-35	10	93	7.79	1.11	2.63	4.37	14334	37,698	62,640
E. M. V.	15	6- 3-35	8	91	9.55	0.64	1.52	2.52	14878	22,615	37,493
Lady	20	6-10-35	9	90	9.04	0.47	1.11	1.85	15150	16,817	28,028
471	20	6-30-35	5	90	8.28	0.41	0.97	1.62	14878	14,432	24,102
	2	2- 3-35	10	90	8.31	4.17	9.88	16.43	4309	42,573	70,797
	2	2- 4-35	7	90	4.47	2.24	5.31	8.83	3357	17,826	29,642
	7	2- 6-35	8	91	5.58	0.80	1.90	3.15	17055	32,405	53,723
E. P. V.	15	2- 9-35	6	93	9.41	0.63	1.49	2.48	19142	28,522	47,472
Ella	20	2-16-35	8	90	9.97	0.50	1.19	1.97	21729	25,855	42,802
190	20	2-28-35	8	91	11.58	0.58	1.37	2.29	19958	27,342	45,704

	Amount fed	No.	Av. Wt.	Area in sq. in.	Value of 1 sq. in.
Carotene	34 γ pure β-carotene or 56.6 I. U.	9	92	13.69	2.48 γ β-carotene or 4.13 I. U.
Carotene	17 γ pure β-carotene or 28.3 I. U.	11	91	7.55	2.25 γ β-carotene 3.75 I. U.

Average value for 1 sq. inch = 2.37 γ pure β-carotene or 3.94 I. U.

	No.	Av. Wt.
Negative controls A-free diet only	17	93

DISCUSSION

Table 1 presents the records for the cows used in the experiments. It will be noted that none of the cows from which the samples of colostrum or milk were taken was heifers. Dann (3) stated that colostrum from heifers tended to be richer in vitamin A than colostrum from cows. He accounted for this difference by the supposition that the vitamin A of the heifer had not been exhausted by previous lactations.

The dates on which samples of colostrum or milk were saved corresponded closely to the original plan. However, it will be observed that there was no fourth day milk for Maggie, and the last sample tested for Maggie was taken three months after freshening. Also the fourth sample for Lady was taken on the ninth day after freshening instead of the seventh day, as was planned.

The percentages of fat and the percentages of total solids were determined by the Department of Dairy Husbandry. Comparisons of fat percentages show that 3 cows had much the same per cent of fat in the colostrum as in the later milk. In one case the per cent of fat was much higher for colostrum than for later milk. Breed distinctions were not evident, the percentages of fat for the cows used being similar. The percentages of total solids were higher for colostrum than for later milk. Generally speaking, there was a gradual decrease in the total solids from the first day of lactation to the last day when samples were collected. In this regard, no noteworthy differences were found between the three breeds of cows.

The total yield in pounds was obtained by combining the weights of the morning and evening milkings on the day when a sample was saved. The collection of colostrum is beset by many difficulties. The total yield of colostrum in pounds is as accurate as is possible to obtain. However, there is little doubt that the total yields for some of the colostrum are low. For example, on the second day Ella gave 7.0 pounds of colostrum in the morning, and only 0.4 pound of colostrum in the afternoon. Beginning with the fourth day, the total yield of milk is considered reliable.

The calculated total yield of fat and of total solids per day presents further data. In each case, the total yield of fat for the first or second day is distinctly less than the yield per day for the same cow later in the first month of lactation.

The original records have been studied and used to compile data presented in table 2. Suitable amounts of the sample to give the rats for single feedings were determined by preliminary experiments. Two grams of colostrum proved satisfactory for a single feeding, but larger amounts of milk had to be used as lactation progressed, 20 grams of the last samples being necessary.

For each group more than 10 rats were started but because of unavoidable circumstances it was necessary to eliminate some of the rats from the records. The bloody colostrum of the first and second day of Maggie's lactation seemed very repugnant to the rats and many refused to eat their portions. Other rats seemed to have been depleted too much or too little. Slight amounts of mold in the first and second day's colostrum of Ella might have affected the results of these particular experiments. In other instances the rats also refused to consume the entire portion offered.

Depleted weights of the rats had a maximum range of 80 to 105 grams. The original plan was to use rats averaging about 92 grams for each group with sexes equally divided. Because some rats with poor records had to be dropped from the final summaries, the average weights for the groups ranged from 87 grams for the smallest to 97 grams for the largest. At the same time some groups became smaller in number than desired. The sex ratio remained well balanced in the groups.

The composite curves were plotted according to the method of Sherman and Todhunter (14). Each area was measured in square inches with a planimeter in triplicate, and the average recorded as indicated in table 2. The areas under the standard carotene curves were measured for reference. Dividing the area in square inches by the weight of the single feeding gave the square inches for each unit fed. It is evident that the vitamin A activities are high for the samples of the first 2 days of lactation of all 4 cows, because the areas produced by 1 gram of sample are much larger than those produced later. Per unit of weight, the product of the first or second day of lactation of the cow was from 8 to 19 times as rich as the less potent milk produced later in the month.

The results secured from the two carotene reference curves were considered reliable because they checked with one another. One group of rats received twice as much carotene as did the other group, and the area under the subsequent curve theoretically should have been twice as large as for the group receiving the smaller amount. This ideal condition was approached at least as well as in the experiments considered satisfactory by Sherman and Todhunter (14). In the present experiments, 34 γ pure β-carotene equivalent to 56.6 International units, produced an area of 14.40 square inches, using averages from the two reference curves.

The carotene reference curves were used to determine the value of 1 square inch of area in terms of β-carotene or International units. These values were used to calculate the vitamin A activity of 1 gram of each sample in terms of β-carotene and also International units. In terms of β-carotene or International units the secretions of the first and second days of lactation of the cow were richest in vitamin A per gram of weight. By the fourth day of lactation, there was a considerable drop in the vitamin A content per gram. For each of the 4 cows there was some further decrease in vitamin A content from the fourth to the seventh day of lactation. In general, the vitamin A content of the milk remained much the same from the seventh to the twenty-eighth day. These values for the vitamin A content of milk are slightly lower than those compiled by Sherman (13) who lists milk as containing 65 Sherman units of vitamin A per ounce. According to Baumann and Steenbock (1) 1 Sherman unit is equal to about 1.3 International units. On this basis several of the milk samples studied would contain 40 to 50 Sherman units per ounce.

The sudden decrease in the amount of International units per gram and the total International units per day is quite evident for all cows. The decline of vitamin A activity for the total yield of the second day of lactation for Ella is probably due to the difficulty in obtaining a complete yield of colostrum. After the fourth day of lactation the International units per gram remained on much the same level through the month tested. On the eighty-fifth day for Maggie, or at the end of the third month, the International units per gram and the total International units per day are still very much on the same level as for the seventh day. In these experiments the cows with the heaviest yield of milk per day had also the greatest amount of vitamin A activity per day. Figure 2 presents these relationships in graphic form. It is interesting to note the relatively high levels of vitamin A output maintained by both Maggie and Ella throughout the period of the experiment.

SUMMARY AND CONCLUSIONS

Samples of colostrum and normal milk from 1 Holstein, 1 Guernsey and 2 Ayrshire cows collected approximately on the first, second, fourth, seventh, fourteenth and twenty-eighth days were available for the experiments described. Each sample represented a composite from the 24 hour milking. Yield for the day, per cent of total solids and per cent of fat were determined in each case. All samples were stored in a frozen condition until needed.

Albino rats were depleted of their body reserve of vitamin A by feeding vitamin A-free diet. At the end of the depletion period, the experimental rats were divided into groups comparable as to weight, sex and litter; one group of rats served as negative controls and other groups received single feedings of colostrum or milk, following the method of Sherman and Todhunter (14). According to the single feeding method, the rats are given a single weighed portion of the food to be tested and then retained on the A-free diet. Weekly weighings of all rats were made during the experimental period which lasted from the time of the single feeding until death of the rat. Composite curves were plotted and a planimeter used for measuring the areas under the curves.

Computations were made to show the total vitamin A content per gram of sample expressed in two ways, as γ of β-carotene and as International units. The total yield of vitamin A per day for each cow was also calculated and expressed in the same terms.

When comparisons were made on the basis of the vitamin A activity per gram, the secretions of the first or second day of lactation of the cows were highest, from 8 to 19 times as rich as the less potent milk produced later in the month. By the fourth day of lactation, there was a considerable drop in the vitamin A activity per gram of sample. For each of the 4 cows there was some further decrease in vitamin A content from the fourth to the seventh day. In general, the vitamin A content of the milk remained much the same from the seventh day to the end of the experiment. Maggie and Lady produced the richest samples of colostrum tested. These two rich samples contained 25.53 and 27.38 International units per gram respectively. One sample of colostrum tested as low as 8.83 International units per gram. This was produced by Ella on the second day of lactation. Several of the samples of milk contained about 2 International units per gram. The least potent sample tested was produced by Maggie and contained 1.38 International units per gram. It so happens that the yield for Maggie on that particular day was the highest of the entire experiment. The number of animals used permits no statement regarding breed differences.

Comparisons may also be made on the basis of the vitamin A produced by the cow in a day. For each of the cows, the largest total output of vitamin A for one day was on the first or second day of lactation. The samples collected on approximately the fourth day of lactation gave results which showed total daily yields of vitamin A approaching one another. Total daily outputs of vitamin A for the remainder of the experiment were near the same level. The highest total output, that of Lady on the second day, was more than seven times as great as the lowest outputs, those of Crystal and Lady toward the end of the experiment.

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