BLOOD CHEMICAL DATA IN OKLAHOMA DAIRY CALVES

BLOOD CAROTENE LEVELS IN OKLAHOMA DAIRY CALVES

By

EVERETT B. HANSON

Bachelor of Science

University of Wisconsin

1938

Submitted to the Department of Dairying

Oklahoma Agricultural and Mechanical College

In partial fulfillment of the requirements

for the Degree of

MASTER OF SCIENCE

1939

Approved:

G. N. Kuhlman
In charge of Thesis

G. N. Kuhlman
Head of Department of Dairy

D. C. McIntosh
Dean of Graduate School

ACKNOWLEDGMENTS

The author wishes to take this opportunity to sincerely thank the many persons who were of service in conducting this study. Especially appreciated were the valuable suggestions offered by Dr. A. H. Kuhlman and Dr. W. D. Gallup. Indispenable was the assistance of Dr. L. H. Moe who collected all blood samples.

TABLE OF CONTENTS

INTRODUCTION

The following study was undertaken in order to attempt to find a normal blood carotene level for dairy calves of different ages fed under practical Oklahoma conditions. Obviously what is termed "practical Oklahoma conditions" may vary from herd to herd, season to season, or year to year; yet , there should be enough similarity in composition of feeds and feeding practices throughout the state so that blood carotene levels established in one herd should at least serve as a guide in others.

Interest in carotene arises from the fact that it is the precurser of vitamin A which is necessary for the proper growth and well-being of dairy calves.

REVIEW OF LITERATURE

General

In 1920 Steenbock and Boutwell (20) made the important correlation between yellow color in foods and good sources of vitamin A. They even went so far as to suggest that the pigment carotene is the source of vitamin A. This view was not accepted by other workers and the relationship remained unsolved until a decade later. In 1928 von Euler, of Sweden, as cited by Maynard, (14) furnished evidence that highly purified carotene would cure rats exhibiting typical vitamin A deficiency. Moore (16) in 1930 working with rats proved that carotene was transformed into vitamin A in vivo.

In 1932 Karrer in Switzerland and Drummond in England, cited by Maynard (14), isolated a very active vitamin A fraction from halibut liver oil which was identified as being an unsaturated alcohol with the formula $C_{20}H_{30}O$. This alcohol is now regarded as vitamin A. Soon after Karrer, Kuhn, and others, cited by Maynard (14) established the formula of carotene as $C_{40}H_{56}$. It is now recognized that there are four different carotenes which have vitamin A activity, namely: α-, B-, γ-, and hydroxy-B-carotene or krytoxanthine. On hydrolysis the B-carotene yields two molecules of vitamin A while the rest yield only one.

Although having been suspected for several years previously, it was not until 1926 that Jones, Eckles, and Palmer (11) demonstrated the needs of calves for vitamin A. With a skim milk, white corn ration the calves showed typical

symptoms of an A deficiency; namely, failure to grow, xeropthalmia, respiratory trouble, diarrhea, and death. Cod liver oil corrected these symptoms. Beohdell, Eckles, and Palmer (2) in the same year reported that calves developed symptoms of vitamin A deficiency when cod liver oil was not included in their experimental ration of corn gluten feed, casein, polished rice, and butterfat. Since then experimental vitamin A deficiency has been produced by many investigators.

Of importance to the Oklahoma farmer is the relationship of vitamin A to the so-called "cottonseed meal" poisoning. In 1930 Halverson and Sherwood (9) reported a long time investigation from which it was concluded that failures in cattle attributed to cottonseed meal injury were primarily due to a deficiency of vitamin A. Through extensive work at the Oklahoma, Pennsylvania, and Michigan experiment stations this belief has been substantiated.

Although most vitamin A deficiencies reported have been experimentally produced, Guilbert and Hart (7) observed a condition in range cattle in California which was suspected to be vitamin A deficiency. A number of types of disturbances occurred: (a) failure of the female to come in estrus; (b) more or less frequent occurrences of estrus, but with failure in fertilization; (c) mating and fertilization followed by death of the fetus and abortion or resorption at various stages of gestation; and (d) failure in lactation. The conditions could be corrected by the addition of cod

liver oil or green alfalfa hay to the ration.

Guilbert and Hart (8) in 1935 reported on a continuation of their studies and stated that the daily minimum carotene requirement of the bovine is 26 to 38 micrograms per kilogram of body weight.

Comparatively little work has been done involving actual plasma carotene levels in calves. Palmer (17) in 1914 made the observation that the blood carotene of a newborn calf was very low. He extended his studies to blood carotene levels in mature cattle and found a definite correlation between the type of ration fed and carotene levels. Moore (15) in 1938 reported on plasma carotene levels in calves which were experimentally depleted of vitamin A reserves. Plasma carotene fell to low levels which were quickly raised by either supplements of carotene in cottonseed oil or by alfalfa hay. He concluded that as a rule plasma carotene remained below about 0.13 micrograms per c.c. for a considerable period before the development of nyctalopia and ocular changes.

Whitnah, Peterson, Atkeson, and Cave (21) made daily carotene determinations in studying carotene balances in heifers and dairy cows. They gave evidence of a large daily variation. Madsen and Davis (13) concluded from blood plasma samples taken from several beef heifers that the degree of carotenemia depends upon the exogenous supply of carotene. The vitamin A of the blood content reached a less variable physiological level which is maintained as long as the carotene supply is adequate, but which declines during the state of deficiency.

They found high values in pasture fed animals. Phillips, Rupel, Oleson, and Bohstedt (18) found no carotene in the blood of a calf 46 days old at the onset of vitamin A deficiency.

Semb, Baumann, and Steenbock (19) reported that twelve cows receiving 140 mg. of carotene daily averaged 5.2 micrograms carotene per c.c. of blood plasma. By feeding A.I.V. silage, the daily carotene intake was increased to 500 mg. per cow resulting in an average blood carotene level of 6.5 micrograms per c.c. in two weeks, and 8.8 micrograms in six weeks. They reported a decrease in blood carotene immediately after parturition.

Gillman and El Ridi (6) reported that five cows on a winter ration averaged 0.40 mg. of carotene per 100 ml. of serum and four bulls on a winter ration averaged 0.08 mg. On a summer ration six cows averaged 1.11 mg. per 100 ml. of serum and six bulls on a summer ration averaged 0.42 mg.

Methods of Analysis

Definite identification of the lipochrome of cattle serum as carotene was made by Palmer and Eckles (17) in 1914. Previously Van den Bergh and Snapper, as cited by Palmer (17), had distinguished the lipochrome of cattle serum from bilirubin.

Early workers were puzzled by the fact that carotene which was readily soluble in fat solvents could not be extracted directly from the blood serum. Palmer (17) showed that the pigment was closely bound to the protein of the blood and could be separated from this union by the addition of 95% alcohol to the plasma. He took advantage of this fact and evolved a

method of measuring blood carotene by treating the serum with 95% alcohol, mixing with plaster of Paris and extracting the yellow color with petroleum ether. Van den Bergh, Muller, and Broekmeyer, cited by Conner (4) modified this method by ommitting the plaster of Paris, and comparing the yellow color to a 1/24 per cent solution of potassium dichromate as a standard. Conner (4) modified the method further and compared the yellow color to a 0.02% or 0.04% solution of potassium dichromate as was done in this study.

It should be realized that the preceding methods of analysis do not differentiate between the biologically active pigments, the carotenes, and the other ether soluble pigments such as xanthophylls and xanthophyll esters. Such separations can be made by chromatographic determinations, but these procedures are very slow and not suited to routine determinations. Willslatter and Stoll, cited by Palmer (17), in 1913 described in detail a colormetric method for the quantative estimation of carotene and xanthophyll in green plant tissues. They took advantage of the fact that xanthophylls could be removed from a petroleum ether solution by washing with successive portions of 85 and 90 per cent methyl alcohol until no more color could be removed. The carotene would remain in the petroleum ether solution. This fact has been used in separating the carotene from xanthophylls in blood.

Clauson and McCoord (3) in 1935 proposed a method of separating the carotinoid pigments in blood with diacetone. Hegsted, Porter, and Peterson (10) adopted a modification of

this method for silages and stated that it was superior to the old method of extracting the Skellysolve with methyl alcohol.

PROCEDURE OF ANALYSIS

Blood samples were drawn from the jugular vein into flasks containing lithium citrate as the anti-coagulent.

Carotene content of the plasma was determined by slight modifications of the procedure of Conner.

The blood samples were centrifuged to separate the plasma from the cells. Ten ml.[1] of plasma were then pipetted into a small-necked centrifuge tube. Ten ml. of 95% ethyl alcohol were added, the tube tightly corked, and shaken for one minute. Then ten ml. of Skellysolve (petroleum ether B. P. 90° - 94° C.) were added and the tube again shaken for one minute. Ten ml. of water[2] were then added and the tube shaken for one minute. Since several samples were treated in this manner simultaneously a short time elapsed between the addition of each reagent. All reagents added were cold and the small-necked centrifuge tubes were kept in ice water during the above procedure except when being shaken.

The samples were then placed in the refrigerator for approximately one half hour until the ether layer had clearly risen to the top. Samples were then centrifuged for a short time and a portion of the ether layer drawn off and transferred into a tightly stoppered vial.

---

[1] Conner originally proposed three c.c. of plasma. Ten c.c. were used at the suggestion of W. D. Gallup of the Agricultural Chemistry Department.

[2] Water used on the recommendation of W. D. Gallup.

The yellow color of the plasma was compared with permanent standards of a 0.02 or 0.04% potassium dichromate solution in a Klett colorimeter with artificial illumination. Since the colorimeter used in this study was located in the Agricultural Chemistry Department, the Skellysolve carotene solution had to be carried from the Dairy building to Whitehurst Hall, a distance of about two blocks. In warm weather the vials were surrounded by ice to help prevent evaporation.

The determinations in most all cases were made immediately after the samples were collected. If any time did elapse between the drawing of the blood and analysis the samples were kept in the refrigerator. Colorimeter readings were made as rapidly as possible in order to prevent ether evaporation.

Calculations of carotene values were derived from the following equation: $x = \frac{F}{R} x\ S$ in which, x = concentration of carotene in micrograms per 100 ml. of plasma, F = scale reading of standard in mm., R = scale reading of unknown in mm., and S = concentration of the standard in standard terms. With the 0.02% potassium dichromate solution S = 100 and with 0.04% solution S = 200. These values for S were adopted at the suggestion of Dr. Gallup who had previously checked the potassium dichromate standards against carotene extracts from fresh green alfalfa using a spectrophotometer.

EXPERIMENTAL PROCEDURE

Purebred calves in the Oklahoma A. & M. College dairy herd including Jerseys, Guernseys, Holsteins, and Ayrshires were used in this study. Blood carotene determinations were started in February and continued into July. It is obvious that a continuous record of blood carotene could not be kept on any animal for over four months. Therefore, in order to secure data on older calves determinations were started immediately on all available calves in the herd. All calves born during the experimental period were included in the study.

It would have been desirable to have had accurate records of feed consumption for each individual animal as well as carotene contents of feeds used, but due to the large amount of labor involved and lack of facilities this was not done. Instead, an attempt was made to group animals in accordance to breed, age, and type of ration.

These groups are briefly summarized as follows:

Group I. Fifty calves were fed regular college calf ration consisting of praire hay ad libitum, and milk and grain in accordance to age and weight.

Group II. Seven calves used in a feeding experiment received the same general type of ration as Group I but different levels of milk and 30 c.c. of cod liver oil daily.

Group III. Five calves changed from regular calf ration to pasture.

Group IV. Four calves fed definite quantities of fresh green oats in addition to the regular calf ration.

Group V. Four calves fed definite quantities of a commercial carotene preparation in addition to the regular calf ration.

Group VI. Four mature cows changed to pasture from a ration containing praire hay as the roughage.

Group VII. Six mature cows changed from a mungbean silage ration to pasture.

Group VIII. Eleven calves which could not be included in Group I because of the possibility of eating fresh grass. This group includes most of the calves under one month of age and the calves that died during the study.

As previously indicated in this paper, all yellow color extracted from the blood plasma by the Skellysolve was reported as carotene. Attempts to ascertain the amounts of xanthophyll pigments in the Skellysolve carotene solutions were made only in a tentative sort of a way.

Attempts were made several times during the course of the study to extract composite Skellysolve carotene solutions obtained from routine carotene determinations of animals on the regular calf ration with 85 and 95 per cent methyl alcohol. In all attempts color removed by the methyl alcohol was so small that it was not quantitatively estimated and it was assumed that the major portion of the yellow color was due to carotene. These results were not unexpected since Palmer (17) in his early work reported that xanthophylls appear to play very little part in coloring the tissues or fluids of dairy cattle. In 1935 Gillman and El Ridi (6) stated that in

the metabolism of the cow carotene is absorbed preferentially in contrast to the xanthophyll, lutein.

A similar attempt was made to separate the carotene from xanthophyll pigments in the blood of grass fed calves. Again, little if any pigment was removed by the methyl alcohol.

A separation was attempted for the mungbean silage cows by extracting a composite Skellysolve solution from the routine carotene determinations with diacetone. The diacetone removed over 50% of the pigment. This may be in line with the work of Hegsted, Porter, and Peterson (10) who have reported that silages, especially high acid silages, contain pseudocarotenes, that is, biologically inactive pigments derived from xanthophylls which are very similar to carotene in their solubility in petroleum ether. Since these acid derived pigments were also detected in butterfat, it would be only logical to expect that they would also be found in the blood of animals on a silage ration.

In making the routine carotene determinations on very young calves a yellow precipitate was sometimes apparent in the plasma alcohol mixture which could not be directly extracted with Skellysolve. Upon acidifying with trichloroacetic acid, extraction of all the color was complete. For example, a Jersey calf at three days of age showed a blood carotene value of 35.3 micrograms per 100 c.c. of plasma. Upon acidifying a duplicate plasma sample with 5 c.c. of trichloroacetic acid a value of 208.3 micrograms was obtained.

The identity of the yellow precipitate was not ascertained.

In the routine carotene determinations trichloroacetic acid was not added. A notation is made in the presentation of the data where such a precipitate occurred.

Again it should be emphasized that the values reported as carotene may not all be true carotene. Likewise, no differentiation is made between the different carotene isomers.

EXPERIMENTAL RESULTS

Group I

Animals in this group consisted of 5 Guernseys, 12 Holsteins, 13 Ayrshires, and 13 Jerseys, ranging in age from 1 to 273 days. Management and feeding practices were typical of those found on many Oklahoma farms.

The general practice in the college herd is to maintain dry cows in an outside paddock until a few days after parturition. In the early portion of the experimental period, these animals were therefore essentially on dry lot feeds. However, in the latter months of the experimental period cows consumed considerable pasture. Care was taken in the selection of Group I to include only calves, which did not, or whose dams did not have access to green feed. Since the greater part of the experimental period occurred after pasture was available the number of young calves in Group I was limited.

Newborn calves were kept with their dams for about four days and then moved into the barn where they received whole milk from Holstein cows until about six weeks of age when a gradual change was made to skim milk. Milk was fed in accordance with the general rule of 1 pound of milk for every 10 pounds of body weight until a total of 18 pounds daily was reached (9 pounds per feeding). This allowance was maintained until the calves were weaned from milk. Most heifers were fed milk until about six months of age and the bulls to 4½ to 5 months of age.

The grain mixture was offered at an early age and the

amount was gradually increased until a maximum of 2½ pounds per day was fed until the calves went to pasture. The grain mixture until March 1 consisted of corn meal--4 parts, wheat bran--3 parts, oats--2 parts, and cottonseed meal--2 parts. From about March 1 to May 25 the cottonseed meal in the above mixture was cut down to 1 part. From about May 25 on, the grain mixture consisted of 4 parts wheat bran, 3 parts barley, 2 parts oats, 1 part corn, and 1 part cottonseed meal.

Since records of the feed intake of individual calves were not kept, and since carotene analysis of the feeds were not made, no attempt was made to calculate the carotene intake of individual animals. While the calves were with their dams and receiving colostrum the carotene intake probably was greater than during the period immediately following when animals received whole milk. According to Dann (5) carotene content of colostrum may be ten to one hundred times greater than later milk from the same cow. After being changed to skim milk the calves were dependent largely on praire hay for their carotene intake. Although carotene content of the hay fed was not known, carotene determinations were made every month on similar hay by Dr. Gallup. These determinations for hay fed in February, March, April, May and June were respectively: 23.0, 10.2, 8.6, 9.0, and 15.5 milligrams per kilogram. From these results it was assumed that the hay fed during the period covered by this study was relatively low in carotene.

Blood samples were collected as often as possible. For the majority of animals this resulted in a determination every ten days and in many cases more often.

As previous workers had reported a considerable daily variation in blood carotene, six animals were chosen from Group I and II and daily determinations made for a short period. Results are shown in Table 1. Animals 116 and 124 are from Group II.

Table 1: Daily Variation in Blood Carotene

Animals	Breed	Age in days on 5-10-39	Micrograms of Carotene per 100 ml. plasma					
			5-10-39	5-11-39	5-12-39	5-13-39	5-14-39	5-15-39
			10 AM	1:30 PM	1 PM	8:30 AM	8:30 AM	7:30 AM
3	G	205	307.7	279.7		266.7	231.2	
116	J	176	47.5	50.0	50.0	47.5		
124	J	207	91.3	86.2	91.3	90.9		
1	H	214	107.0	111.1	111.1	105.3	108.1	108.1
16	G	210	367.0		307.7	320.0	307.7	303.0
12	J	270		133.3	131.6	126.6	122.7	122.0
35	J	249	142.9	125.0	129.0	128.2	133.3	134.2

It is evident that there was considerable daily variation, yet, not as much as was suspected. Whether or not these variations could be attributed to experimental error was not determined. Evidence that the daily variation may have been greater than indicated in Table 1 will be presented later in this paper.

Although no determination at intervals within the same

day were made the data in Table 1 indicate that such a variation was not large. The time of day when samples were taken was recorded in most cases but no account was taken of this in analyzing the data.

Since the calves in Group I ranged in age from 1 to 273 days and since in no case were samples collected from one individual for even half that long, it becomes evident that in order to establish a continous record of blood carotene for the full range of 272 days the determinations of several animals at different ages must be combined.

An example of how this combination was arranged is shown in Table 2 for Holstein calves varying in age from 155 to 180 days. The data were first summarized by establishing age groups with 10 day intervals from 0 to 280 days. In most cases each individual animal had only one determination for each 10 day period. An average for the period was taken if there were two or more determinations. An average was then obtained of all animals of the same breed in each 10 day age group. The 10 day averages for all individuals of the same breed were then combined to make a 30 day average.

The 10 day averages for the four breeds are shown in Table 3. It is apparent that there is a definite rise in blood carotene as calves increase in age. When it is considered that the average for one 10 day period may be based on an entirely different group of animals than another 10 day period, the increase in all breeds shows a surprising uniformity. Although only a few Guernseys were available their

Table 2 Example of Method Used in Summarizing Data

No. of Animal	Date	Age in Days	Carotene*	Results for 10 day Period			Results for 30 day Period
				151-60	161-70	171-80	
59	4-11-39	155	77.5				60.6
	4-19-39	163	60.7				77.5
	4-30-39	174	74.6	77.5	60.6	74.6	74.6
18	3-3-39	155	90.0				90.0
	3-18-39	170	120.0	90.0	120.0		120.0
17	2-22-39	160	50.0				50.0
	3-4-39	170	80.0				80.0
	3-10-39	176	105.0				
	3-14-39	180	95.0	50.0	80.0	100.0	100.0
60	4-8-39	169	115.0		115.0		115.0
5	2-21-39	169	125.0				125.0
	2-28-39	176	80.0		125.0	80.0	80.0
1	3-18-39	161	85.0				85.0
	4-4-39	178	66.6		85.0	66.6	66.6
107	2-22-39	171	57.5				
	3-3-39	180	65.0			61.3	61.3
			Total	217.5	585.6	382.5	1185.6
			Average	72.5	97.6	76.5	84.7

*Carotene in Micrograms per 100 c.c. Plasma

Table 3
Blood Carotene Levels in Calves on Regular Calf Ration

Periods in Days	Jerseys 1		Holsteins		Ayrshires		Guernseys		Jerseys 2	
	Carotene*	No. of animals	Carotene	No. of animals	Carotene	No. of animals	Carotene	No. of animals	Carotene	No. of animals
0-10	23.8	2	10.7	1						
11-20	13.8	2	10.0	1	36.3	1				
21-30	18.8	2	10.0	1	22.4	3				
31-40	17.9	2	27.5	1	24.9	3				
41-50	31.3	3	40.0	1	38.7	2				
51-60	29.1	5	37.7	2	32.0	2	105.0	1		
61-70	24.2	5	40.8	2	55.0	3	80.0	1		
71-80	45.0	1	48.8	2	53.9	2	70.0	1		
81-90	52.7	5	40.0	1	50.9	2				
91-100	52.9	6	58.0	2	53.1	2	129.9	1	25.0	1
101-110	55.5	6	50.9	4	65.1	1	100.2	2	23.8	3
111-120	66.0	3	55.9	4	76.4	1	113.4	2	22.5	3
121-130	78.7	3	63.5	4			141.7	3	27.4	3
131-140	62.5	4	65.3	5	56.3	2	162.0	2	39.1	4
141-150	63.9	2	69.0	4	95.0	3	135.7	3	43.3	5
151-160	73.4	3	72.5	3	108.3	3	142.9	1	47.8	4
161-170	77.1	3	97.6	6	93.2	6	161.7	2	43.8	4
171-180	66.7	2	76.5	5	99.8	4	140.9	3	44.1	5
181-190	111.6	2	101.0	5	118.4	4	173.6	3	65.1	4
191-200	81.7	3	93.9	5	137.4	6	231.0	3	82.2	4
201-210	78.7	1	103.0	2	139.0	5	259.4	3	119.7	2
211-220	85.2	2	95.3	6	148.9	6	227.0	1		
221-230	139.8	4	83.0	2	149.6	3				
231-240	131.4	4			159.5	2				
241-250	119.7	2	101.7	2						
251-260	157.8	4	111.1	1						
261-270	158.8	2								
271-280	130.5	1								

Jerseys 1--Group I
Jerseys 2--Group II

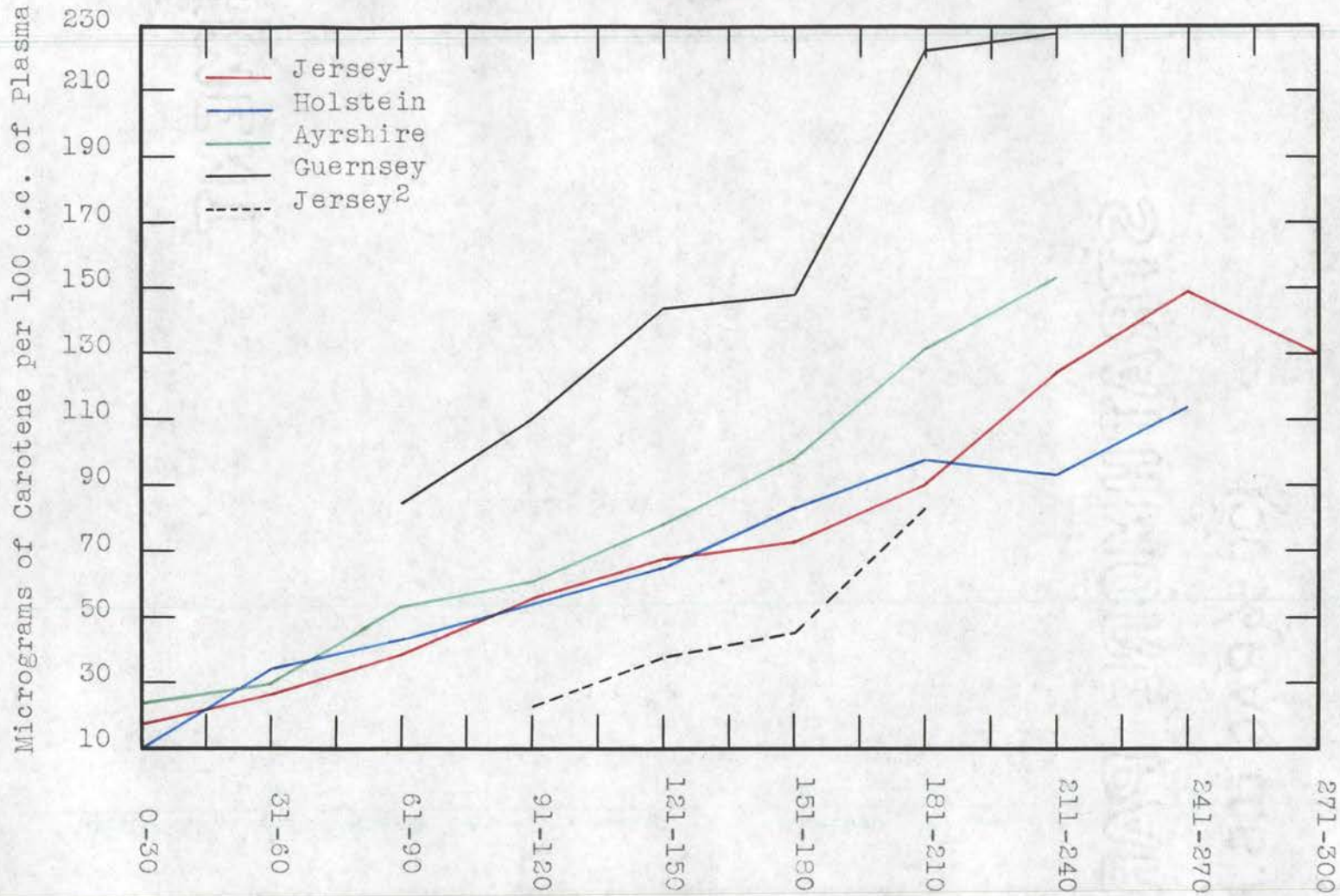
*Carotene in Micrograms per 100 c.c. Plasma

Table 4 Blood Carotene of Calves on Regular Calf Ration

Age Group in Days	Jerseys[1]		Holsteins		Ayrshires		Guernseys		Jerseys[2]	
	Carotene*	σ	Carotene*	σ	Carotene*	σ	Carotene*	σ	Carotene*	σ
0-30	18.8	7.5	10.4		25.9					
31-60	27.5	9.3	35.7		30.3	8.9				
61-90	39.0	18.0	43.8		53.5	8.9	85.0			
91-120	56.6	11.5	54.3	10.8	61.9		110.5		23.4	2.6
121-150	68.9	19.2	65.7	8.6	79.5		144.5	30.3	37.9	14.0
151-180	73.1	17.6	84.7	23.1	98.7	38.4	148.2	38.3	45.1	12.5
181-210	91.1	20.7	98.4	14.2	132.9	27.9	221.4	79.4	83.4	30.2
211-240	125.5	51.5	93.2	14.1	153.9	40.9	227.0			
241-270	148.5	39.6	114.4							
271-300	130.5									

σ Calculated when six or more records were available for that period.
1. Jerseys on regular herd ratio.
2. Jerseys on milk feeding experiment.
*Micrograms per 100 c.c. Plasma.

Figure 1 BLOOD CAROTENE OF CALVES ON REGULAR CALF RATION



blood carotene levels were about twice as high as in the other three breeds which showed a marked similarity.

Table 4 and Figure 1 show the same data summarized for 30 day intervals. The standard deviation (σ) was calculated if six or more records were available for a given 30 day period. Since the averages for the 30 day periods were calculated from all the 10 day averages it is apparent that the 30 day averages are weighted averages. For example, a given 30 day average may be based on eight 10 day averages, three of them from one animal, two from another, and one from each of two more. Therefore, the animal with three records would influence the 30 day average more than any other single animal. The σ was also calculated from all 10 day averages.

Again the calves showed a definite increase in blood carotene with advancing age. In only one case was the value of one 30 day interval lower than the preceding interval in all cases in which sufficient records were available to calculate the σ.

Group II

Group II consisted of seven Jersey calves fed different levels of skim milk. Two of the calves received one pound of milk daily for every ten pounds of body weight, three of them received one pound of milk for every eight pounds body weight, and the other two received one pound of milk for every six pounds body weight. In no case were more than eighteen pounds fed daily.

The group was very similar to Group I in that the same praire hay was fed ad libitum and both groups received the

same grain mixture. In addition, each calf in Group II received about 30 c.c. of cod liver oil daily.

In analyzing the results no significant difference was noted between calves on the different milk levels. However, when records of all the calves were combined and compared with the Jerseys in Group I a very surprising difference was apparent.

The animals in Group II, as shown in Tables 3 and 4 and Figure 1, were only about half as high in blood carotene as Jerseys of similar age in Group I. This difference is difficult to explain but must be due to the cod liver oil supplement for otherwise the rations were practically the same. It will be noted in Table 3 that as the calves passed the age of 180 days (when they were discontinued from the milk experiment) the carotene values rose to a level comparable to the carotene levels of Jerseys fed the regular calf ration in Group I. No explanation can be given as to why cod liver oil should have this effect.

Group III

The calves used to show the influence of pasture on blood carotene were formerly in Group I on the general herd ration. They were turned to pasture on the morning of May 15, and daily determinations were made until May 20. A record of the daily blood carotene prior to May 15 is shown in Table 1 for four of the calves. Determinations on the fifth calf, number 55, was started on May 15. Results are shown in Table 5.

Table 5 Effect of Pasture on Blood Carotene Level

Animal	Breed	Age on 5-15-39	Micrograms of carotene per 100 c.c. plasma					
			5-15-39	5-16-39	5-17-39	5-18-39	5-19-39	5-20-39
			7:30 AM	3 PM	10 AM	8 AM	1 PM	10 AM
1	H	219	108.3	108.7	129.0	142.9	181.8	200.0
16	G	215	303.0	333.3	380.9	400.0	493.8	533.3
12	J	275	122.0	125.0	133.3	153.8	150.9	166.7
35	J	254	134.2	137.9	142.9	173.9	216.2	242.4
55	A	250	205.1	219.8	210.5	199.0	209.4	235.2

The samples on May 15 were taken before the calves were turned out to pasture so that May 16 was the first day on which any rise could be expected. It so happened that a rise occurred in every case. However, all increases, with the exception of the Guernsey calf, number 16, were so small that they probably were not significant. All calves showed an increase on May 17 over the previous day with exception of the Ayrshire, number 55. On the following day all calves showed further increase over the previous day, with the exception of the Ayrshire. On May 19 all animals increased except the Jersey, number 12. All animals showed additional increase on the last day.

The Guernsey, even though much higher in blood carotene at the start, rose at a greater rate than any of the other animals. The Ayrshire showed the least response. It cannot be stated definitely that these results show breed difference in that the animals never had access to pasture before. It may have taken some of the calves a much longer time to learn to eat grass than others. It would be expected that if the

animals had been accustomed to eating grass, the rise would have been more apparent. The calves also had access to hay which probably cut down the pasture consumption more for some animals than others.

Group IV

An attempt was made to feed four calves definite quantities of fresh green oats, most of which had not begun to bloom, in addition to the regular calf ration. The grass supplement was fed about 6:30 A.M. before the calves received their morning quota of hay. As none of the animals were accustomed to green feed some difficulty was encountered in getting the calves to consume the desired amount of grass. The daily blood carotene levels and quantities of grass fed are shown in Table 6 and Figure 2.

It had been hoped that by the second day the two Ayrshires would consume four pounds of grass. Actually four pounds were not consumed until the fifth day. The two Jersey calves were fed one pound of grass the first day and two pounds each day thereafter. Although care was taken to prevent waste some did occur.

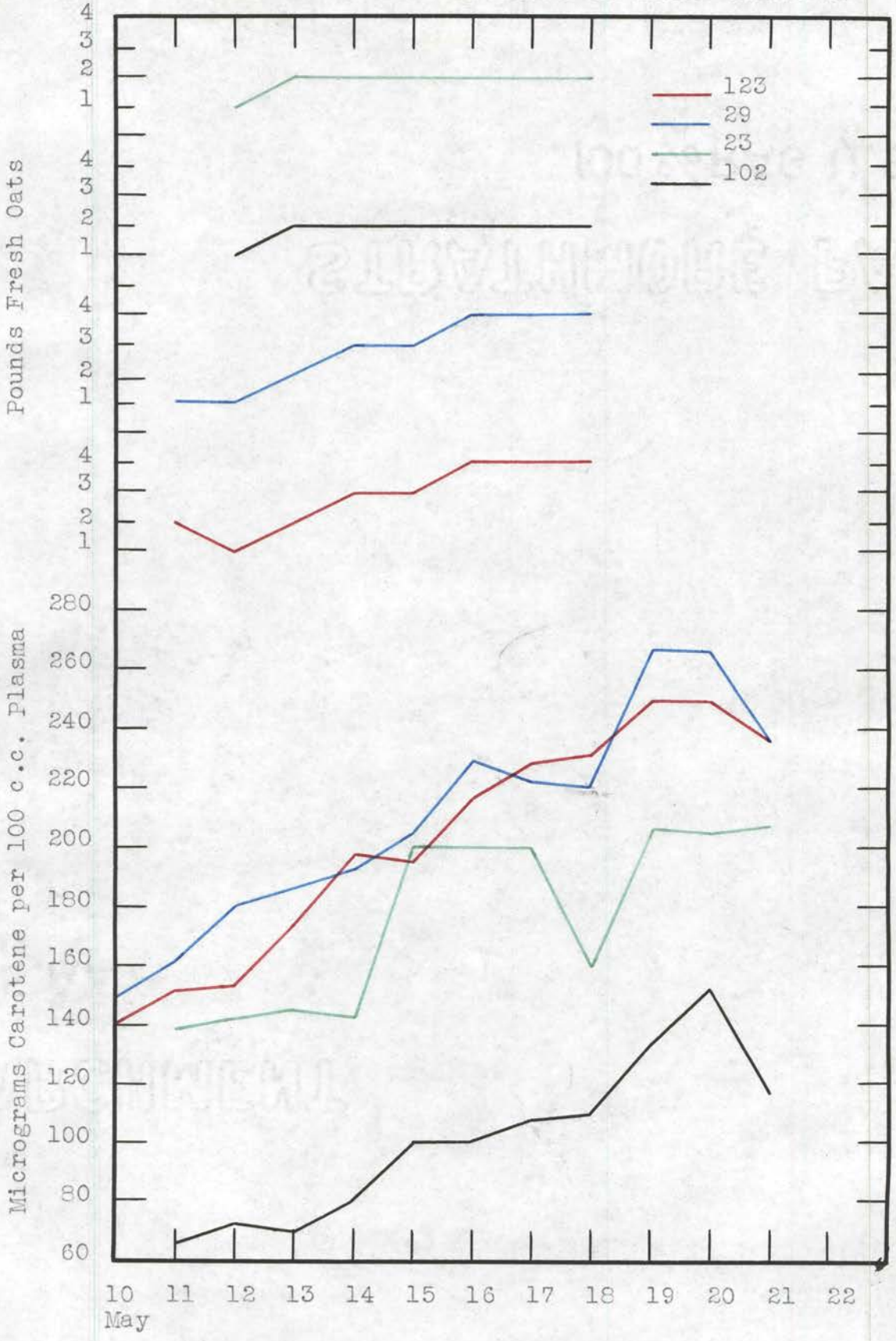
All animals showed a slight increase in blood carotene the day following initiation of grass feeding. The general trend continued upward as long as grass was fed. Three of the calves were highest in blood carotene on May 19, the day following the termination of grass feeding, while the fourth calf, number 102, was highest on May 20. All calves with the

Table 6 Effect of Grass Supplement on Blood Carotene

Animal	Ayrshires				Jerseys			
	123		29		102		23	
Age in days on 5-11-39	231		210		195		210	
Date and time sample taken	Carotene*	lbs. grass fed	Carotene	lbs. grass fed	Carotene	lbs. grass fed	Carotene	lbs. grass fed
5-10-39 10 A.M.	141.8		150.4					
5-11-39 1:30 P.M.	151.5	2	160.0	1	66.4		138.9	
5-12-39 1 P.M.	153.8	1	180.2	1	73.0	1	141.8	1
5-13-39 8:30 A.M.	173.9	2	186.0	2	70.2	2	146.0	2
5-14-39 8:30 A.M.	198.0	3	192.3	3	80.0	2	143.9	2
5-15-39 7:30 A.M.	195.1	3	205.1	3	100.0	2	200.0	2
5-16-39 3 P.M.	217.4	4	229.9	4	100.0	2	200.0	2
5-17-39 10 A.M.	228.6	4	222.2	4	108.1	2	200.0	2
5-18-39 8 A.M.	231.2	4	221.0	4	109.3	2	160.0	2
5-19-39 1 P.M.	250.0		266.7		133.3		208.3	
5-20-39 10 A.M.	250.0		266.6		152.7		205.1	
5-21-39 9 A.M.	236.7		235.3		117.6		207.2	

*Carotene in Micrograms per 100 c.c. Plasma

Figure 2
EFFECT OF GRASS SUPPLEMENT ON BLOOD CAROTENE



exception of number 23 showed a slight drop on May 21 when the determinations were discontinued.

Table 7 shows the difference between blood carotene values for each calf at the beginning and end of the grass feeding period. An average of the first two determinations was used for the beginning level while the end level is an average for the two days immediately following termination of grass feeding.

Table 7 Summary of Grass Feeding Experiment

Animal	Total pounds grass fed	Micrograms Carotene per 100 c.c. Plasma			Per Cent Increase
		Beginning	End	Increase	
123	23	146.7	250.0	103.3	70.4
29	22	155.2	266.7	111.5	71.8
102	13	69.7	143.0	73.3	105.2
23	13	140.4	206.7	66.3	47.2

The two Ayrshires show a marked similarity in actual and percentage increase in blood carotene. Although the two Jerseys were very similar in actual increase the percentage of increase for calf number 102 was more than twice as great as for the other Jersey. Perhaps, this was due to the fact that the initial level of carotene in calf number 102, was much lower. A comparison of the Ayrshires with the Jerseys indicates that the actual blood carotene increase is roughly proportioned to the total amount of grass fed.

Although no reference to the carotene content of green oats was found in the literature Atkeson, et al (1) reported

that green rye averaged 25.1 milligrams of carotene per pound. If it is assumed that the oats fed in this study was similar to rye, then additions of 1, 2, 3, and 4 pounds of grass supplement, respectively, increased the carotene content of the regular calf ration by 25.1, 50.2, 75.3, and 100.4 milligrams.

Group V

Definite amounts of "Puratene", a commercial carotene preparation, were given orally in capsule form to five calves in Group V in addition to the regular calf ration. The Puratene was guaranteed to contain a minimum of 1,500,000 vitamin A units per pound. One unit of vitamin A is equivalent to 0.6 micrograms of B-carotene so that each gram of Puratene would contain about 1984.1 micrograms of carotene. The 10 c.c. capsules used contained approximately nine grams of Puratene which would be equivalent to 17,856.9 micrograms or 17.8 milligrams of carotene. Results are shown in Table 8 and Figure 3.

The carotene supplements were always given in the morning at about 9 A.M. and the blood samples were taken at about 8 A.M. Daily blood carotene determinations were made until June 5, after which determinations were made every other day.

From June 1 to June 8, inclusive, all calves received 10 c.c. of Puratene daily. On June 9 Jersey number 54 and Ayrshire number 108 were raised to 30 and 20 c.c. respectively. The others were left on the original level until June 21 when all supplemental carotene feeding was discontinued.

Table 8 Effect of Puratene on Blood Carotene

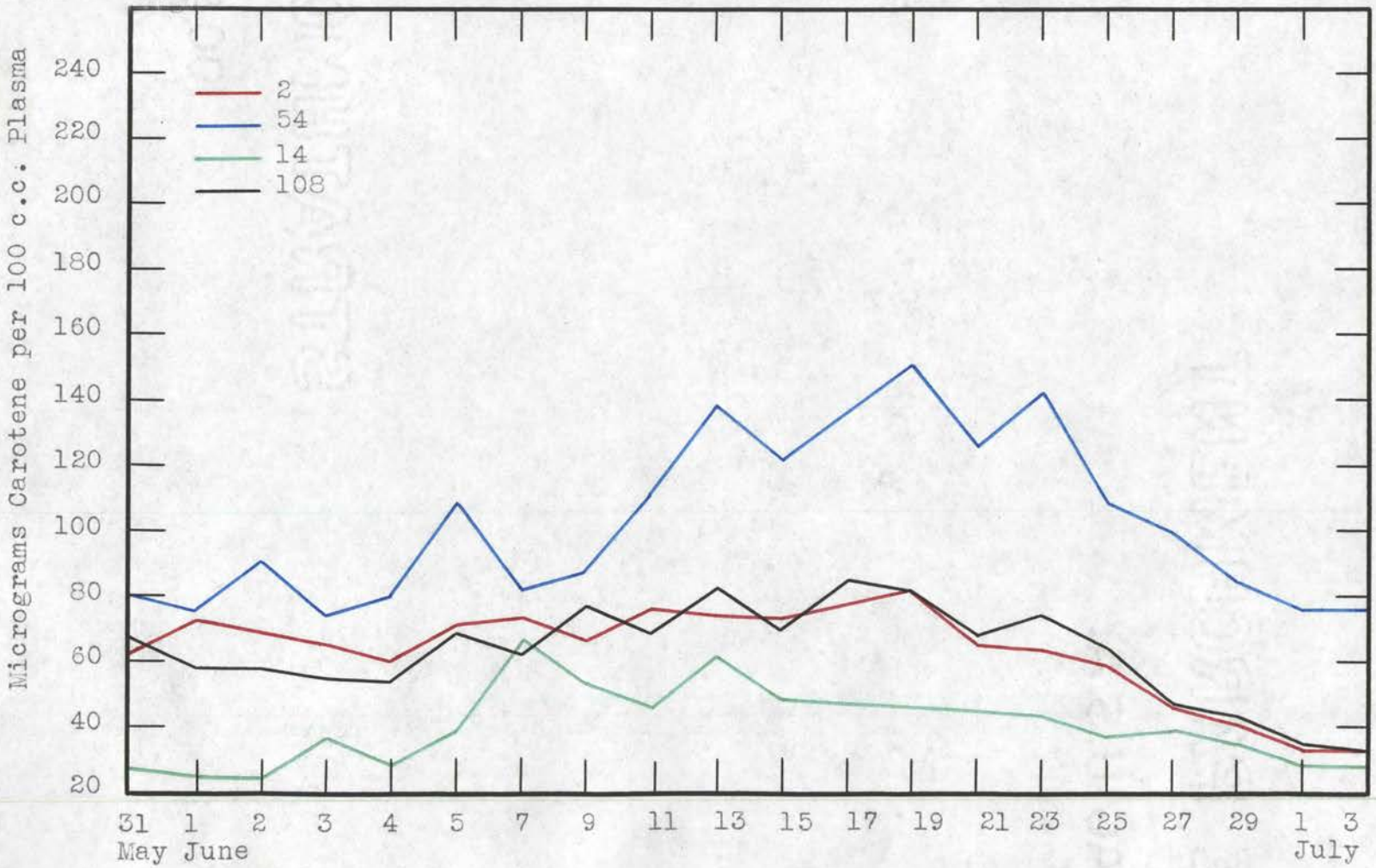
Breed	Jersey		Ayrshire		Jersey		Holstein		Guernsey	
Animal	54		108		2		14		110	
Age*	127		68		108		42		151	
Date	Carotene**	Puratene c.c.	Carotene**	Puratene c.c.	Carotene**	Puratene c.c.	Carotene**	Puratene c.c.	Carotene**	Puratene c.c.
5-30-39	80.3		67.8		63.5		27.5		169.5	
6-1-39	76.9	10	58.8	10	73.3	10	25.8	10	177.8	
6-2-39	90.9	10	58.8	10	69.0	10	24.5	10	130.7	
6-3-39	74.5	10	55.5	10	66.3	10	37.5	10	142.9	10
6-4-39	80.0	10	54.4	10	60.1	10	29.3	10	140.3	10
6-5-39	109.3	10	69.4	10	71.7	10	39.8	10	186.9	10
6-7-39	82.6	10	62.5	10	74.6	10	66.5	10	186.0	10
6-9-39	88.5	30***	77.8	20***	67.8	10***	53.8	10***		
6-11-39	111.7	30	69.0	20	76.9	10	46.3	10		
6-13-39	138.9	30	83.0	20	74.1	10	61.8	10		
6-15-39	121.2	30	70.9	20	73.3	10	49.5	10		
6-17-39	137.9	30	85.8	20	78.4	10		10		
6-19-39	151.5	30	81.3	20	82.6	10	46.3	10		
6-21-39	126.6	30	68.7	20	66.4	10	45.8	10		
6-23-39	143.9		74.3		64.9		43.8			
6-25-39	109.9		64.5		59.7		38.8			
6-27-39	100.0		46.5		46.0		39.8			
6-29-39	85.8		43.0		41.3		35.3			
7-1-39	76.6		35.5		33.5		28.8			
7-3-39	76.9		33.8		33.8		28.0			

*Age in days on 6-1-39.

**Carotene in Micrograms per 100 c.c. Plasma.

***Same amount of Puratene given on June 10, 12, 14, 16, 18, and 20.

Figure 3 EFFECT OF PURATENE ON BLOOD CAROTENE



The Ayrshire calf, number 108, and the Jersey, number 2, showed very little rise in blood carotene throughout the whole supplemental feeding period. The Jersey, number 54, showed some response especially after being raised to 30 c.c. of Puratene daily. A significant response was also apparent in the Holstein. The Guernsey was sold June 8.

Blood carotene in all cases dropped off rapidly after Puratene feeding was discontinued. Animals 54 and 14 reached their initial level about five days after the termination of Puratene feeding. At this time the other two animals dropped to about half of their original blood carotene level. This seems to indicate that the carotene content of the regular calf ration was lower at the end of the experimental period than at the beginning.

Table 9 shows difference between blood carotene values for each calf at the beginning and end of the Puratene feeding period. The beginning level is an average of the two determinations just prior to feeding the carotene supplement and the end level is an average of the last two determinations before Puratene feeding was discontinued.

Table 9 Summary of Puratene Feeding Experiment

Animal	Total c.c. Puratene fed	Micrograms Carotene per 100 c.c. Plasma			Per cent Increase
		Beginning	End	Increase	
54	480	78.6	139.1	60.5	77.0
108	330	63.3	75.0	11.7	18.5
2	200	68.4	74.5	6.1	8.9
14	200	26.7	46.1	19.4	72.7

In comparing Group V to Group IV it is evident in general that calves fed Puratene did not show as great an increase in blood carotene as the grass fed animals. The lower additional daily carotene intake of all calves receiving Puratene, excepting the Jersey number 54, may partially explain this condition. However, this explanation would not hold true for the Holstein which made substantial gains in blood carotene on 10 c.c. of Puratene daily. The low initial blood carotene level in the Holstein may be a factor, since in both Group IV and Group V the highest percentage of gain in blood carotene occurred in the calves with the lowest initial level.

Group VI

The daily blood carotene trend was studied for four cows in Group VI consisting of two Ayrshires and two Holsteins which had been fed dry lot rations since the preceding fall.

During the ten days previous to May 25 all cows were on a full alfalfa hay ration getting at least two pounds of hay per 100 pounds of live weight. In addition to the hay the general herd concentrate mixture was fed to provide ample nutrients for milk production and body maintainance.

Blood samples were collected between 5:30 A.M. and 6:30 A.M. The animals were first turned to pasture the evening of May 25. From then on the cows were on pasture both night and day with the exception of the time spent in the barn for milking and feeding of grain. Results are shown in Table 10 and Figure 4.

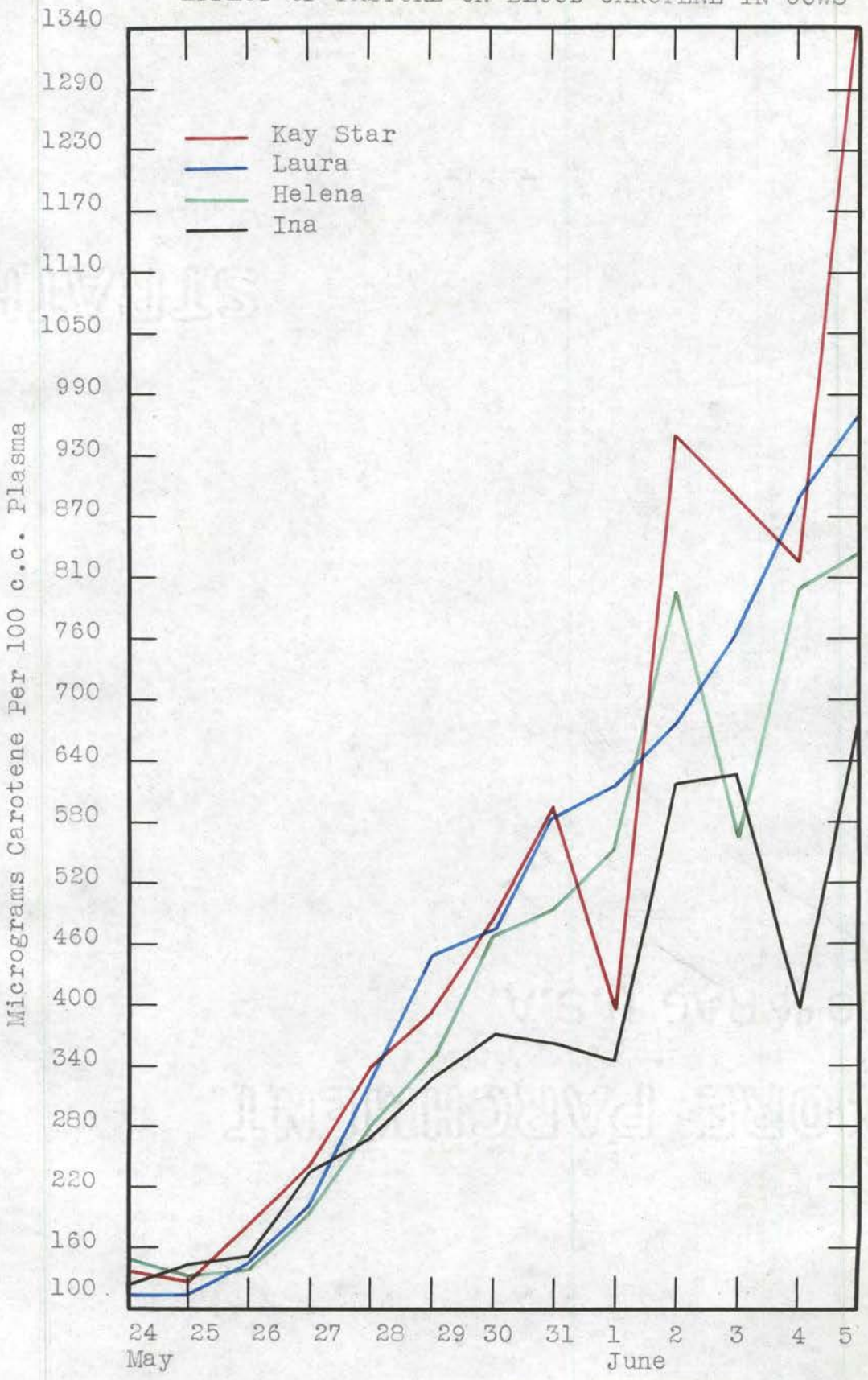
The four animals were at a comparatively low level of

Table 10 Effect of Pasture on Blood Carotene in Cows

	Group VI				Group VII				
	Micrograms Carotene per 100 c.c. Plasma								
Breed	Ayrshire	Ayrshire	Holstein	Holstein	Ayrshire	Guernsey	Jersey	Jersey	Jersey
Animal	Kay Stor	Laura	Ina	Helena	Minerva	Viola	Nannette	Nancy	Fontaine
5-24-39	137.0	114.3	129.9	155.2	517.4	800.0	550.8	495.8	495.8
5-25-39	129.0	114.3	145.9	139.9	540.5	833.3	339.0	500.0	571.4
5-26-39	135.2	142.9	153.8	142.8					
5-27-39	240.9	304.0	238.9	139.0					
5-28-39	338.9	325.2	263.1	283.7					
5-29-39	392.1	454.5	320.0	347.8					
5-30-39	431.9	476.2	370.3	476.2					
5-31-39	597.0	588.2	387.0	500.0					
6-1-39	400.0	615.4	444.4	355.5	666.7	727.3	563.4	606.1	540.5
6-2-39	952.4	889.6	615.4	800.0	916.3	1000.0	571.4	571.4	545.4
6-3-39	806.9	769.2	525.0	366.0	833.3	952.4	303.0	625.0	600.0
6-4-39	824.7	850.3	400.0	800.0	919.5	1212.1	500.0	606.0	781.9
6-5-39	1332.3	975.5	606.4	833.3	1200.0	1304.3	600.0	761.9	816.3

Figure 4

EFFECT OF PASTURE ON BLOOD CAROTENE IN COWS



blood carotene at the initiation of the study. An increase in blood carotene was evident after the cows had been on pasture for one night. This increase was greater for the two Ayrshires than the two Holsteins. After May 26 all animals showed a very rapid increase until the study was discontinued. The Ayrshires increased at a greater rate and to a higher level than the Holsteins.

A considerable daily variation was evident. This might have been due to experimental error, since the color in the carotene-Skellysolve solutions was so much more intense than the standard used that any error in reading the colorimeter would account for a relatively large error in the calculated carotene.

It is interesting to note that the cows on pasture increased much more rapidly in blood carotene than did the calves in Group III. The explanation would logically lie in the fact that the mature animals were accustomed to eating grass. The mature animals also reached a higher maximum level than did the calves. This may not have significance since the study was continued for a longer length of time for the cows. The calves also had access to hay while the cows did not.

Group VII

In Group VII the daily blood carotene is followed on five cows changed from a mungbean silage ration to pasture. Carotene determinations were made on May 24 and May 25, and started again on June 1 when the cows were discontinued from mungbean silage and changed to pasture. Blood samples were taken at 6 A.M.

From May 8 to May 17 the cows were changed from a level of about four pounds of hay to no hay. By May 17 all animals were getting mungbean silage as the sole roughage. In addition about five pounds of oats was fed daily. This ration was continued until the cows were turned to pasture. Results are shown in Table 10.

Even though the initial blood carotene was very high, a substantial rise occurred for all animals the first day after being on pasture. From then on until the end of the study the trend in blood carotene was upward for all the cows except Nannette. The Guernsey and Ayrshire were higher than the three Jerseys both at the beginning and end of the study.

In comparing Group V with Group IV it is of interest to note that the initial blood carotene of cows on the mungbean silage ration was several times higher than of the cows on the alfalfa hay ration.

Group VIII

Group VIII for the most part consisted of animals that could not be included in Group I because of the possibility of a higher carotene intake due to pasture. In addition the blood carotene levels of four calves that died during the study are also included. Data for these four calves is shown in Table 11.

Table 11 — Calves That Died During the Study

Animal	Breed	Date	Age in Days	Blood Carotene	Remark
Hadie Sybil	Jersey	2-28-39	5	22.5	Included in Group I
		3-7-39	12	12.5	Died 5-1-39
		3-14-39	19	7.5	Sick few weeks, cough,
		4-8-39	44	37.5	breath fast, pneumonia,
		4-15-39	51	22.3	diarrhea.
		4-25-39	61	9.0	
Bunny	Ayrshire	4-18-39	2	4.5*	Died 5-1-39
		4-25-39	19	19.8	Lung infection.
		5-1-39	15	35.0	Autopsy showed body fat very yellow. On pasture.
Valeria	Guernsey	4-24-39	2	30.0*	Died 4-29-39 Lung infection
Flo	Jersey	4-24-39	2	27.0*	Died 5-1-39 Indigestion.

*Yellow precipitate

No evidence of vitamin A deficiency was noted in any of the animals that died. Indications point to a contagious

malady of some sort in that all animals died about the same time. Blood carotene was as high or higher than normal for all animals except Radio Sybil where a very low value was apparent five days before death occurred.

Table 12 shows the blood carotene values of calves that were maintained in a pasture lot for some time before being moved into the barn. As a general rule the calves remained with their dam for about four days so that they received colostrum milk. From then on the calves obtained milk from Holstein nurse cows that were also on pasture. A few of the calves were taken from their dams and moved directly into the barn. These were not included in Group I because the dams were on pasture previous to parturition. Through an oversight the definite dates on which several of the calves were moved into the barn were not recorded.

In the entire group of calves the blood carotene was decidedly higher than for calves of corresponding ages in Group I on the regular calf ration. For example the Guernsey, Ellen, reached a level of 701.7 micrograms per 100 c.c. of plasma at the age of fifty-six days which is comparable to values found in mature cows on pasture. In general, the Guernseys were higher than the Jerseys.

The high level can be explained by two and possibly three reasons. It would be expected that the blood carotene would show an immediate rise as soon as an animal started to consume grass. This would partially explain the high values reached in calves such as Ellen, Peggy, or Tiddledly who had

Table 12 Effect of Pasture on Blood Carotene in Young Calves

Date	Age in Days	Carotene	Date	Age in Days	Carotene
Nedra (Jersey)			Frances (Jersey)		
4-10-39	13	35.7	5-10-39	6	38.5
4-24-39	19	89.0	5-23-39	19	78.4
5-1-39	26	30.8	Moved to barn about 5-23-39		
5-10-39	35	31.0	6-1-39	28	11.0
5-19-39	44	63.3	6-9-39	36	26.0
Moved to barn about 5-21-39			6-15-39	42	29.6
5-23-39	48	207.2	6-29-39	56	34.0
5-27-39	52	219.8	7-3-39	61	27.8
6-5-39	61	95.8	Peggy (Jersey)		
6-9-39	65	64.5			
6-17-39	73	38.8	Outside Continuously		
6-27-39	83	46.5	5-21-39	0	26.3*
Tiddledy (Jersey)			5-23-39	2	64.7
			6-5-39	15	23.3
4-10-39	2	27.3	6-9-39	19	38.8
4-12-39	10	55.7	6-19-39	29	75.5
4-24-39	18	25.3	6-27-39	37	151.5
5-1-39	25	60.0	Tibby (Jersey)		
5-10-39	32	42.5			
5-19-39	41	146.1	Outside Continuously		
Moved to barn about 5-21-39			6-23-39	1	72.7*
5-25-39	45	266.7	6-27-39	5	38.3*
5-27-39	49	285.7	Sybil (Jersey)		
6-1-39	54	222.2			
6-5-39	58	221.3	Moved to barn 6-10-39		
6-9-39	62	127.6	6-11-39	5	35.3*
6-17-39	70	109.3	6-15-39	7	28.9
6-27-39	80	39.9	6-23-39	15	31.0
			7-1-39	24	18.8

(Continued)

Table 12 Continued

Date	Age in Days	Carotene	Date	Age in Days	Carotene
Fortune (Jersey)			Beauty (Guernsey)		
Moved to barn before 6-7-39			5-21-39	1	44.5*
6-7-39	8	111.1	5-23-39	3	91.3
6-13-39	14	90.1	6-3-39	14	53.5
6-17-39	18	66.3	6-5-39	16	62.5
6-21-39	22	38.8	Moved to barn about 6-5-39		
7-1-39	33	38.8	6-9-39	20	40.8
7-3-39	35	30.0	6-15-39	26	30.3
Ellen (Guernsey)			6-23-39	34	19.8
			7-1-39	43	12.5
4-18-39	4	32.5	Beulah (Guernsey)		
4-25-39	11	253.2			
4-29-39	15	166.6	6-3-39	7	27.8
5-1-39	17	142.9	6-9-39	13	55.4
5-10-39	26	90.9	6-19-39	23	150.4
5-19-39	35	166.6	Moved to barn 6-24-39		
5-23-39	39	449.4	6-25-39	29	370.3
6-3-39	50	571.4	6-29-39	33	232.5
6-9-39	56	701.7	Juliana (Guernsey)		
Moved to barn 6-10-39					
6-11-39	58	500.0	6-9-39	7	70.2
6-13-39	60	444.0	6-19-39	17	50.0
6-15-39	62	287.8	Moved to barn 6-24-39		
6-17-39	64	303.0	6-25-39	23	144.4
6-19-39	66	209.4	7-3-39	32	213.9
6-21-39	68	168.1			
6-23-39	70	168.1			
6-25-39	72	168.1			
7-1-39	79	76.9			
7-3-39	81	73.8			

*Yellow precipitate.

Carotene in Micrograms per 100 c.c. Plasma

undoubtedly reached an age where they could consume grass before being moved into the barn. However, high values were also apparent in calves before the animals were old enough to eat any grass. Undoubtedly this was due to a relatively high carotene value of the milk from the nurse cows or from the colostrum milk. A record of blood carotene was obtained for only one calf on the day of birth. The value was relatively low, 56.3 micrograms per 100 c.c. of plasma in comparison to 64.7 micrograms when two days old. Calves Libby and Beauty showed a blood carotene value of 72.7 and 64.6 micrograms, respectively, when one day old.

Of interest in the change in blood carotene when animals were moved into the barn and put on the regular calf ration. A fairly complete record is available for the Harmony, Milton, previously maintained on barley a very high blood carotene level. Determinations were made every other day until it was thought that the blood carotene had reached a stationary level. When five days were skipped and two more determinations were made. The maximum blood carotene value was on June 9 the day before she was moved into the barn. The next two determinations June 11 and June 13, were lower than the maximum but still high, 600.0 and 444.0 micrograms carotene respectively. From then until June 21 the trend was rapidly downward until a value of 160.1 micrograms was maintained for three determinations. It was assumed that the lower limit had been reached but determinations on July 1 and 5 of 70.9 and 75.0 micrograms of carotene respectively, disproved this

assumption.

All of the other calves transferred from pasture to barn behaved in a similar manner to Ellen, although, most of them seemed to maintain a high level of blood carotene for a longer length of time after being removed from pasture. In all cases animals showed a striking decline in blood carotene after a few days of barn feeding.

CONCLUSIONS

Blood carotene levels in barn fed calves rose gradually as the animals advanced in age. At corresponding ages the blood carotene levels of Guernsey calves were usually more than double those of Holsteins, Jerseys, or Ayrshires.

In general, newborn calves showed low blood carotene values. However when young calves were kept with nurse cows on pasture, blood carotene rose in just a few days to values comparable to those of barn fed animals several months older.

When animals, either cows or calves, were changed from a dry ration to pasture increases were usually noted in blood carotene the very next day. Conversely, when animals were changed from pasture to barn feeding blood carotene values declined, although, not as rapidly as they had previously risen.

A commercial carotene supplement did not increase the blood carotene as much or as rapidly as pasture. This may have been due to a higher carotene intake for the animals on pasture.

Cows on a mungbean silage ration were considerably higher in blood carotene than cows on a ration containing alfalfa hay as the roughage.

Daily supplements of cod liver oil seemed to lower blood carotene values about one half when compared to animals of corresponding breed and age fed the same ration without cod liver oil.

No symptoms of vitamin A deficiency were noted in any of the animals.

BIBLIOGRAPHY

(1) Atkeson, F. W., Hughes, J. S., Kunerth, B. L., Peterson, W. J., and Kramer, M. Recovery of carotene and vitamin A from butter when cows were fed unlimited quantities of green rye. J. of Nut. 14:621, 1937.

(2) Bechdel, S. I., Eckles, C. H., and Palmer, L. S. The vitamin B requirements of the calf. J. Dair. Sci. 9:409, 1926.

(3) Clausen, S. W., and McCoord, A. B. The determination of carotene and xanthophyll by a single distribution between liquid phases. J. Biol. Chem. 113:89, 1936.

(4) Conner, C. L. The quantitative estimation of carotin in blood and tissues. J. Biol. Chem. 77:619, 1928.

(5) Dann, W. J. The transmission of vitamin A from parents to young in mammals. Biochem. J. 26:1072, 1932.

(6) Gillman, A. E. and El Ridi, M. S. Carotinoids and vitamin A in cow's blood serum. Biochem. J. 29:2464, 1935.

(7) Guilbert, H. R. and Hart, G. H. Storage of vitamin A in cattle. J. of Nut. 8:25, 1934.

(8) Guilbert, H. R. and Hart, G. H. Minimum vitamin A requirements with particular reference to cattle. J. of Nut. 10:409, 1935.

(9) Halverson, J. O. and Sherwood, F. W. Investigations in the feeding of cottonseed meal to cattle. N. Car. Ag. Ex. Sta. Tech. Bull. 39, 1930.

(10) Hegsted, D. M., Porter, J. W., and Peterson, W. H. Determination of carotene in silage. Ind. Eng. Chem. Anal. Ed. 11:256, 1939.

(11) Jones, I. R., Eckles, C. H., and Palmer, L. S. The role of vitamin A in the nutrition of calves. J. of Dairy Sc., 9:119, 1926.

(12) Mackinney, G. Leaf Carotenes. J. Biol. Chem. 111:75, 1935.

(13) Madsen, L. L., and Davis, R. E. Spectrophotometric examination of cattle blood for carotene and vitamin A. Amer. Soc. An. Prod. Proc. 31:327, 1938.

(14) Maynard, L. A. Animal Nutrition. McGraw-Hill Book Company, Inc., 1937.

(15) Moore, L. A. Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and nyctalopia in calves. J. Nut. 17:443, 1939.

(16) Moore, T. Vitamin A and carotene. VI. The conversion of carotene to vitamin A in vivo. Biochem. J. 24:692, 1930.

(17) Palmer, L. S. Carotinoids and Related Pigments. The Chemical Catalog Company, Inc., 1922.

(18) Phillips, P. H., Rupel, I. W., Oleson, J. J., and Bohstedt, G. The effects of a typical blindness-producing ration upon the vitamin K and C content of calf blood. Amer. Soc. of An. Prod. Proc. 31:320, 1938.

(19) Semb, J., Baumann, C. A., and Steenbock, H. Fat soluble vitamins. The carotene and vitamin A content of colostrum. J. Biol. Chem. 107:697, 1934.

(20) Steenbock, H., and Boutwell, P. W. The comparative nutritive value of white and yellow maizes. J. Biol. Chem. 41:81, 1920.

(21) Witnah, C. H., Peterson, W. J., Atkeson, F. W., and Cave, H. W. Carotene balance and blood-carotene levels in heifers and lactating dairy cows and their relation to the production of off-flavor milk. J. Ag. Res. 58:543, 1939.

Typed by
Mary Carolyn Hall